Evaluation of Magnetosensitive Cytostatic Concentration and Different Mechanisms of their Antitumor Effects

Dmytro V. Demash[1*], Natalia Yu. Lukianova[1] and Vasyl F. Chekhun[1]

[1]*Department of Mechanisms of Antitumor Therapy, R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine.*

Authors' contributions

This work was carried out in collaboration between all authors. Author VFC designed the structure of the review and wrote introduction. Authors DVD and NUL managed the literature searches and prepared the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

| Review Article |

ABSTRACT

The review covers different aspects of structural and functional features of magnetic nanoparticles. Especially those which are associated with their interaction with cells and cause development of oxidative stress, apoptosis disruption of DNA structure and cytoskeleton, changes in intracellular signal cascades etc. It is suggested that use of iron oxide nanoparticles for magnet-driven drug delivery in cancer therapy might be safe and promising because it can enhance antitumor effects of known cytostatic drugs.

1. INTRODUCTION

Magnetic materials based on iron, cobalt, nickel or their oxides are widely used in different fields of modern technologies [1]. Particularly, because small-sized magnetic nanoparticles (MNPs) demonstrate properties, which differ from those in macroscopic scale [2]. Nowadays MNPs are

---

Corresponding author: Email: ddemash@gmail.com;

mainly used in biomedicine as MRI contrasting agents and for tumor treatment using local hyperthermia with alternating magnetic field [2-4]. Another promising application of MNPs is their use for target delivery of cytostatics to the tumor region using external static magnetic field (SMF) [5,6].

2. PHYSICAL CHARACTERISTICS OF MNPS

The main specific features of MNPs which determine their magnetic moment are shape, size and surface properties [2]. It is known that interaction between magnetic moments of atoms of the same material causes development of the particular magnetic structure. Reduction of MNPs size might cause the situation when every particle would carry only one magnetic domain, resulting in its totally different properties compared to the entire material [7]. MNPs are often characterized by superparamagnetic properties due to their small size. It means that vectors of every single MNP magnetic moments would rotate in random direction only due to particle thermal motion, resulting in zero total magnetic moment of entire MNPs [8].

Use of external magnetic field causes alignment of magnetic moment vector directions and significant increase of total magnetic moment of the material. Such features and absence of residual magnetization after treatment by external SMF make possible stabilization of MNPs in colloid solution without their agglomeration [4].

It is known that many factors affect MNPs morphology and the main among them are conditions of synthesis reactions [9]. It is accepted that the shape of MNPs greatly influence on their biodistribution, but detailed mechanisms of this process are not understood in detail [10]. Mahmoudi et al. (2009) mentioned that rod-shaped and non-spherical particles are characterized by longer period of circulation in blood compared to spherical ones [11]. On the other hand, spherical magnetite and maghemite MNPs better conjugated cytostatic drugs and showed higher cytotoxic effects. Particularly, spherical MNPs were much more toxic than rod-shaped MNPs or nanocrystals [12].

Size of MNPs often determines their circulation period in the bloodstream [11,13]. MNPs, smaller than 10 nm in diameter, are often removed from the organism through kidneys, while MNPs larger than 200 nm are usually concentrated in spleen or in phagocytes. Both cases lead to reduction of MNPs concentration in serum [14]. Thus, it is suggested that MNPs with 10-100 nm diameter are best for use in medical in biological studies because they have significant magnetic features, longer period of circulation in blood, do not accumulate in reticuloendothelial system cells [2,15]. It should be mentioned that small MNPs are able to penetrate through the blood vessel walls, what makes it possible to concentrate them in the tumor region. At the same time, MNPs, which are smaller than 2 nm in diameter, are not used in biology and medicine because they of their toxic effects on cell membranes and other cells organelles [10]. Thus, control of MNP's size during their synthesis is very important.

Surface charge of MNPs also determines their colloid stability [16]. MNPs with high positive or negative zeta-potential are stable in dispersion and do not form agglomerates for a long period of time. Surface charge also determines biodistribution of these MNPs in the organism and is an important parameter, which affects the process of their accumulation in target cells [17]. It was shown that positively charged MNPs better penetrated into malignant human breast cancer cells compared to ones with negative surface charge. The speed of MNP uptake by cells differed between studied cell lines and depended on their histogenesis [18].

It was shown that MNPs with hydrophobic properties easily adsorb on the protein surface with further uptake by macrophages, resulting in their elimination from serum and low period of circulation. On the other hand, particles with hydrophilic polymers on their surface (e.g. polyethyleneglycol) and with hydroxyl or amino groups avoid uptake by phagocytes, showing higher therapeutic effects because of longer circulation time [19,20]. So, modification of MNPs surface with different functional groups makes it possible to change their properties to make them suitable for wide spectrum of biomedical and industrial technologies.

Due to very small size of iron oxide particles which are often measured by nanometers, iron ions on their surface strongly influence on magnetic properties of nanoparticles. Degree of iron oxidation on the particle surface depends on environment conditions and presence of surface-active compounds [21]. It is known that degree of iron oxidation affect morphology of synthesized particles. For example, Fe^{3+} ions predominantly

form spherical MNPs, while divalent metals usually form nanorods [22,23]. So, one of the main goals for today is to develop standards for MNP synthesis with given parameters of shape, size and coatings for their effective use in medical and biological studies and in clinical practice.

3. MAIN WAYS OF INTERACTION BETWEEN MNPS AND CELLS

The last available data show that the latest promising achievements in the field magnetosensitive target delivery are associated with use of multilevel nanocomposites and nanorobots [13]. These types of MNPs provide detection of specific microbiological objects in biological environment, target delivery of antitumor drugs to malignant cells, cancer diagnostics and therapy on the cell level, adsorption of tumor cell destruction products on the MNP surface after chemotherapy or hyperthermia and their further excretion from the organism [7,24-26]. Such nanocomposites can also be addressed to non-malignant cells for use in diagnostics or modification of their functional activity [27,28].

At the same time, only few MNP-based compositions with dextrane coating were approved by FDA and EMA for clinical use as MRI contrast agents [29]. In future we are waiting for first clinical tests of new types of MNPs, but factors which make them accessible for use in clinics are not properly studied yet. New results about mechanisms of MNPs intracellular degradation showed presence of tight connections between their localization in cells and cytotoxic effects, thus giving us some new ideas about impact of MNPs on cell metabolism *in vitro* [30,31].

It was shown that size plays the main role in processes of interaction of MNPs with cells, resulting in different negative side effects. MNPs have the same size scale as natural proteins, so they are able to penetrate into places, which are unreachable for bigger particles. Also accumulation of MNPs in subcellular structures, such as endosomes, might cause strong local increase of iron concentration [32,33]. It was also shown that the shape of MNPs and their coating put serious impact on their uptake by cells and determine features of their interaction [27].

It was demonstrated that nickel ferrite nanoparticles showed potential cytotoxicity and seriously affected cell proliferation and viability [34,35]. On the other hand, different groups of scientists synthesized biocompatible iron oxide MNPs with different surface coatings. It should be mentioned that there is lack of data about molecular mechanisms of MNPs toxicity, which explain the observed effects [30].

After cellular uptake by endocytosis MNPs often form a cluster in lysosomes, where they degrade in presence of hydrolytic ferments at low pH to iron ions according to classical cellular iron metabolism pathways. This was proved by our light and electron microscopy studies *in vitro* on MCF-7 human breast cancer cells. Also we showed that cisplatin-resistant cells accumulated MNPs more actively compared to w/t MCF-7 cells [32]. It should be mentioned that application of 150 mT SMF significantly accelerated accumulation of MNPs, resulting in higher numbers of nanoparticles detected inside the cells.

Accumulation of different MNPs in cells often causes activation of ROS generation, which might serve as a defense mechanism to neutralize xenobiotics, as well as apoptosis inducers [36,37]. The degree of toxic side effects depends on the type of studied cells, but many of them show activation of defense mechanisms able to neutralize low amounts of ROS, making only high concentrations of MNPs dangerous [23,38]. Induction of ROS generation by MNPs often depends on their coating composition as well as on MNPs concentration inside the cell [39]. For example, nickel ferrite MNPs were shown to induce toxic effects in cells by activation of ROS generation, which depended on high cellular concentration of nanoparticles [34]. The same results about activation of ROS generation by iron oxide MNPs, especially in presence of exogenous SMF, we obtained on MCF-7 and Ehrlich ascetic carcinoma cells *in vitro* and *in vivo*.

It is thought that iron oxide MNPs cause Fenton-type chemical reactions, which lead to active ROS generation. It was shown that naked magnetite nanoparticles had were characterized by severe cytotoxic effects [40]. At the same time, cytotoxic action of maghemite (fully oxidized iron oxide, Fe_2O_3) is not associated with Fenton reactions [7]. In general, mechanisms underlying ROS generation by MNPs are not fully understood, but there is a hypothesis that changes in structured electron configuration of the nanoparticle surface lead to development of

new electron donor or acceptor sites, resulting in ROS generation. MNP-induced ROS generation activates defense antioxidant systems in multi-stage process through transcription factor Nrf-2 [41], resulting in elevation of more than 200 phase II antioxidant proteins expression (haemoxygenase-1, superoxide dismutase, etc.). As the damage increases, defense systems are substituted by MAPK- and NF-kB-activated intracellular signal transduction pathways, leading to excretion of pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs), and, finally, to apoptosis [34,36,42]. Such activation of MAPK cascades was detected in cells of respiratory and gastrointestinal tracts, blood cells, skin and neurons. We also found some changes in expression of apoptosis regulator proteins (p53, Bcl-2 and Bax), as well as miRNA expression profile in MCF-7 cells with different sensitivity to cisplatin, which confirmed increase of cell number in apoptosis after their treatment with stabilized iron oxide MNPs [43]. It should be mentioned that complex of MNPs with cisplatin resulted in much more serious effects, which were amplified by SMF.

Recent studies show direct correlations between MNP size, shape and dispersion properties with cytotoxic effects and pro-inflammatory cell reaction [10]. For example, ROS control MMP activity via two different mechanisms: MAPK-induced overexpression of MMP genes and direct oxidation of thiol groups in pro-MMP molecules, resulting in their activation [44]. So, MMPs might serve as a messenger in the process of macrophage activation in presence of MNPs. It was shown that accumulation of chitozan-coated MNPs led to increase invasion potential of cells, caused by MMPs activation.

Size of MNPs and features of their intracellular accumulation also affect their interference with cytoskeleton elements [45]. Interaction between MNPs and cytoskeleton proteins might be direct (MNPs reached cytoplasm) or indirect (MNPs localized in endosomes). The last type of interaction is most commonly observed [46]. It is suggested that different coating types of MNPs cause different changes in cytoskeleton, while high concentration of uptaken particles also causes its disruption. It is also known that cytoskeleton takes part in many intracellular signal cascades, so, one of the main goals of studies is to found whether MNP-induced cytoskeleton disruptions are able to cause

secondary effects like cell death, changes in proliferative activity etc [7].

It is known that regulation of many cellular functions, including cell growth, motility, and differentiation is highly dependent on cellular adhesion properties. Research data show that cellular adhesion properties could be interfered by MNPs, but results of these studies are controversial. For example, ZnO-containing composite caused significant reduction of astrocyte adhesion properties after 4 hours of incubation, and after 72 hours of incubation this index was almost 2 times lower compared to control. This might be a result of changes in adhesion receptors expression on cell surface induced by nanomaterials [47,48]. We showed that MNPs can significantly change adhesion properties and colony-forming activity of MCF-7 cells, resulting in reduction of their invasion properties and proliferation activity [15].

In another study endotheliocytes were co-cultivated with non-toxic iron-oxide MNPs concentrations for 24 hours and showed no changes in their adhesion properties. Authors did not found significant changes in cell morphology and MNPs aggregation features [40].

Intracellular signal pathways might be changed not only due to cytoskeleton disruptions under MNP impact, but also because of different other mechanisms, such as: (1) genotoxic effects, caused by high ROS levels, (2) changes in gene and protein expression as a result of disruptions in transcription and translation processes, (3) changes of gene or protein expression pattern due to increase of metal ion levels, (4) changes in protein activation by preventing their interaction with stimulating factors, e.g. cell surface receptors, (5) changes of gene expression profile as an answer on stress reaction caused by MNPs [26,27,45,49].

Genotoxic effects of MNPs are usually associated with induction of free radicals generation, including reactive oxygen and nitrogen species [23,50], cytoskeleton damage, and also with ability of some nanomaterials to penetrate into cell nucleus and interact with DNA [32]. Experiments on isolated cells showed that nanoparticles of different origin were able to damage DNA by themselves or by induction of oxidative stress and inflammation. Even if MNPs did not enter the nucleus, they accumulated in cells and still were able to interact with DNA during mitosis, when nuclear membrane integrity

was disrupted, causing DNA aberrations, oxidative stress, and inhibition of DNA replication and transcription [51]. We showed that iron oxide MNPs by themselves were able to cause genotoxic effects, which were significantly amplified with use of MNP- cisplatin nanocomposite and SMF [32].

Experimental studies also show, that Fe_3O_4 MNPs, conjugated with cisplatin, decreased proliferation rate of cisplatin-resistant SKOV3/DDP ovarian cancer cells and 7402 hepatoma cells and led them to apoptosis of [52].

Biodegradation of MNPs is the mechanism, which results in formation of free trivalent iron ions after solution of the MNP core [30]. As it was mentioned, kinetics of MNP solution depend on their surface coating. Accumulation of free trivalent iron ions might in some cases result in generation of high levels of ROS, inducing apoptosis or inflammation.

Another possible mechanism of MNP toxicity is their interaction with biological molecules. They are able to aggregate with serum proteins due to their charge if their coating is unable to prevent this process [53]. The use of MNPs for local hyperthermia or for target drug delivery cause new problems, which also should be taken into account. Hyperthermia needs alternating magnetic fields, which are used to increase MNPs temperature and kill tumor cells. It is known that alternating magnetic fields can also damage healthy tissues, which are situated near the tumor burden. Magnet-driven target delivery of drugs or MRI contrasting with SMF, is thought not to cause direct effects on cells [25], but we already showed *in vitro* that even 150 mT SMF alone starting from 3 hours of continuous impact was able to cause significant changes in MCF-7 and Ehrlich ascetic carcinoma cells, resulting in ROS generation, genotoxic effects, changes in mitochondria activity and in accumulation of MNPs in cells.

In the last case toxic effects might be associated with active income of MNPs into cells and with changes in localization of endosomes inside the cell and their malfunctioning [24]. So, endocytosis is the main mechanism of MNPs uptake by cells (Fig. 1). Then nanopartcles degrade in lysosomes to iron ions, resulting in generation of ROS due to Fenton-type reactions or accumulate in cytoplasm and nucleus, causing changes in cytoskeleton, mitochondria and genotoxic effects. Accumulation of these

changes usually lead to cell death due to apoptosis.

4. PERSPECTIVES OF MNPS USE FOR TARGET DELIVERY OF ANTITUMOR DRUGS WITH MAGNETIC FIELD

Antitumo*r* therapy is often based on use of chemotherapeutic drugs, which are highly cytotoxic, but have low specificity against their biological target [54]. Usually they cause severe systemic damage to the organism, resulting in well-known side effects due to interaction between antitumor drugs and healthy tissues [55].

The idea of using SMF (SMF implants or external SMF) as a vector to increase drug accumulation in tumor region first appeared in early 1980s. Widder et al. [56] performed first preclinical studies with use of magnetic microspheres, covered by albumin, which contained doxorubicin, for treatment of transplanted rat tumors.

Magnetite (Fe_3O_4) is often used as a basis for MNPs for biomedical use because of its chemical and magnetic stability and low cytotoxicity. Such MNPs are covered by organic (polyethyleneglycol, dextran, chitosan, polyethyleneimine, phospholipids) or inorganic (SiO_2) polymer coatings for stabilization of their dispersion in water solution [22,23]. Today many different complexes of MNPs with antitumor agents adsorbed, conjugated on the surface or loaded inside were developed. Particularly, scientists synthesized nanocompositions with doxorubicin, daunorubicin, tamoxifen, cisplatin, gemcitabin, mitoxantron, cerfradine, fludarabin, non-steroid anti-inflammatory drugs, ametopterin, mitomicyn, adriamicyn, ferments, toxines, folic acid, growth factors, radionuclides [23,57,58].

The main advantage of target drug delivery by MNPs and SMF is increase of local cytostatic concentration in the tumor region with use of lower doses of drugs [57]. One of the complex problems in this approach is small size of MNPs. On the one hand, this prolongs their circulation after injection, increasing chances of successful drug delivery to the tumor with use of external SMF. On the other hand, small diameter of MNPs is a limiting factor for use of SMF [58], because magnetic force value depends on MNP magnetic moment and magnetic field gradient. This force is proportional to the MNP volume, meaning that 10 times decrease of linear MNPs

dimensions would result in 1000 times decrease of force value. It was shown that minimal diameter for agglomerates with phospholipid coating which were effectively driven by SMF was 40 nm, for MNPs with polymer coating - 70 nm. Such difference is observed because of bigger volume of magnetite in MNPs, covered with phospholipids [59,60].

Motion of MNPs in matrix or in fluid depends on many factors (magnetic field gradient, temperature, viscosity of the substance, velocity of fluid flow and interaction of MNPs with fluid components, size and shape of MNPs). Nowadays dynamics of MNP motion in bloodstream is actively studied [61]. Magnetic field gradient is needed for accumulation of MNPs in particular region, because in homogenous fields the value of magnetic forces applied to MNPs would be equal to zero. Magnetic field gradient must be strong enough to overcome bloodstream, so the closer magnet would be to the vessel wall, the better the resulting effect would be [12,62]. It should be mentioned that MNPs would also accumulate in tissues which lie between target and SMF source. So, external magnets are likely to be used when target region is located near the body surface. Together with our colleagues we developed different systems of static magnets, allowing us to create a high-gradient magnetic field in the tumor zone (induction near pole 0.6 T; gradient 40 T/m), which made us possible to effectively accumulate MNPs in rat tumor tissue (Guerin carcinoma, Walker-256 carcinosarcoma) and achieve better therapeutic effects without elevation of their general toxicity [5].

Scientists showed significant increase of dextrane-coated MNPs penetration rate through artificial three-layer membrane under 0.410 T external SMF [63]. In other study Lamkowsky et al. found that brain astrocytes accumulate iron oxide MNPs covered with dimercaptosuccinate and this process depended on duration, temperature and MNP concentration. MNP accumulation rate proportionally increased with magnification of external SMF induction, resulting in growth of cellular iron content from 10 nmol/mg of protein after 4 hours of incubation at 37°C to 12000 nmol/mg of protein [64]. The mentioned data suggest that use of external SMF enforces interactions of iron MNPs with cell membranes as well as their uptake by astrocytes *in vitro*.
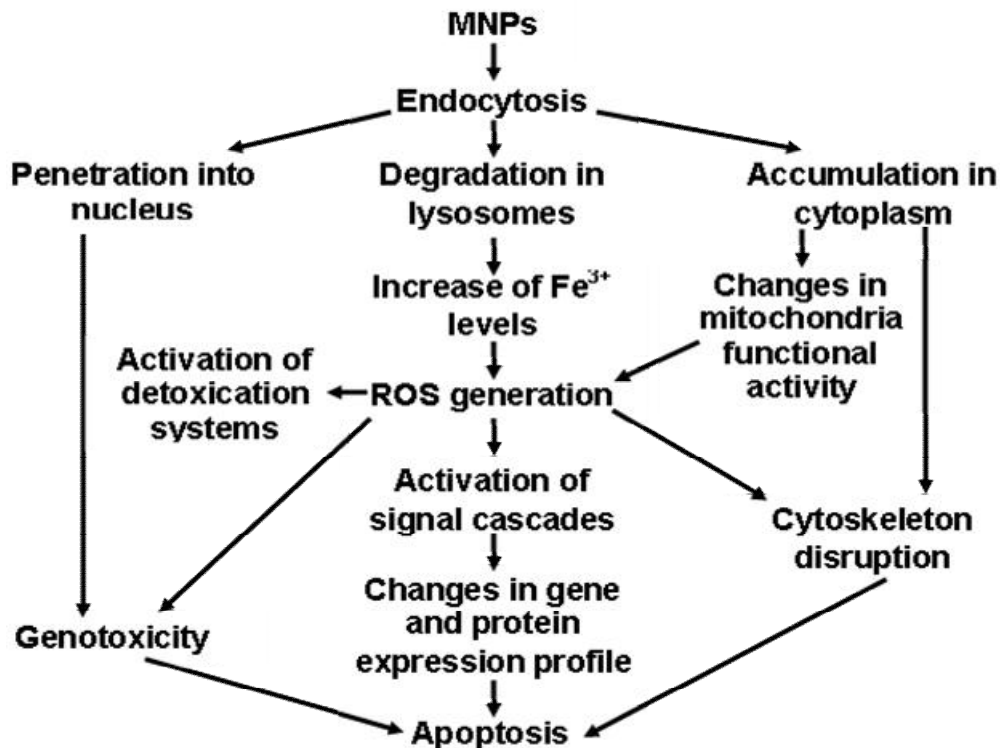

Fig. 1. Possible mechanisms of MNPs cellular effects

We found that MNPs and nanocomposite of MNPs and cisplatin alone and in combination with SMF caused elevation of tumor cells membranes fluidity and permeability in both sensitive and resistant to cisplatin MCF-7 cells. Particularly we noticed changes in lipid composition of cell membranes, elevation of amount of phospholipids, cholesterol, its ethers and "phosphatydylcholine / sphingomyelin" ratio [43].

Gautier et al. observed that MNP-doxorubicin complex, covered by polyethyleneglycol, was a better drug delivery agent compared to naked MNPs [65]. Prijic et al. studied features of MNPs uptake by cells of different genesis under the impact of different types of SMFs. It was shown, that use of magnets made of neodymium, iron and boron alloys significantly increased intensity of MNPs uptake by cells, especially – by malignant ones. Also in was shown that accumulation of MNPs inside transformed cells depended on duration of external SMF action [24].

In vivo (on Guerin carcinoma) we observed enhancement of cisplatin and MNP-cisplatin nanocomposite cytotoxic action by SMF. We found significant increase of necrosis and apoptosis rates in tumor tissue. Many Guerin carcinoma cells showed changes in normal structure of cypolasm organelles and had iron oxide nanoparticles in cytoplasm or nuclei. Aggregates of iron oxide MNPs were the biggest after the combined impact of MNP-cisplatin nanocomposite and SMF. Another feature of SMF impact was damage blood vessels endothelium, which resulting in elevation of their permeability, thus also increasing the antitumor effect of the chemotherapeutic drugs [5].

5. CONCLUSION

So, during last years many promising MNP models were developed and they proved their efficacy *in vitro* and *in vivo*. On the other hand, effective nanocomposite of MNPs with cytostatics, officially approved for clinical trials, was not synthesized yet. The only officially accepted field of MNPs application in clinics was their use as MRI contrast agents during diagnostics [29]. Thus, development of new models of magnet-driven target delivery of drugs and detailed studies of interactions of MNPs and SMF with cells and organism, are still actual and need further active efforts from scientists.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mao R, Wang J, Pei J, Wu S, Feng J, Lin Y, Cai X. Pharmacokinetics and applications of magnetic nanoparticles. Current drug metabolism. 2013;14(8):872-8. PMid:24016109.
Available:http://dx.doi.org/10.2174/138920021131400109

2. Dürr S, Janko C, Lyer S, Tripal P, Schwarz M, Zaloga J, et al. Magnetic nanoparticles for cancer therapy. Nanotechnology Reviews. 2013;2(4):395-409.
Available:http://dx.doi.org/10.1515/ntrev-2013-0011

3. Gallo J, Long NJ, Aboagye EO. Magnetic nanoparticles as contrast agents in the diagnosis and treatment of cancer. Chemical Society Reviews. 2013;42(19):7816-33.
Available:http://dx.doi.org/10.1039/c3cs60149h PMid:23788179

4. Kucheryavy P, He J, John VT, Maharjan P, Spinu L, Goloverda GZ, et al. Superparamagnetic iron oxide nanoparticles with variable size and an iron oxidation state as prospective imaging agents. Langmuir. 2013;29(2):710-6. PMid:23249219 PMCid:PMC3666865.
Available:http://dx.doi.org/10.1021/la3037007

5. Chekhun VF, Naleskina LA, Polishchuk LZ, Todor IM, Demash DV, Lukianova NY. Evaluation of biological effects of nanosystems of directed transport in experiments *in vivo* and their application possibilities in anti-tumor therapy. Advances in Nanoparticles. 2013;2:145-52.
Available:http://dx.doi.org/10.4236/anp.2013.22023

6. Mok H, Zhang M. Superparamagnetic iron oxide nanoparticle-based delivery systems for biotherapeutics. Expert Opinion on Drug Delivery. 2013;10(1):73-87. PMid:23199200 PMCid:PMC4167583.
Available:http://dx.doi.org/10.1517/17425247.2013.747507

7. Melanie A, Jerome R, Armand M, Jerome L, Corinne C, Wiesner MR. Impacts and

physico-chemical behavior of inorganic nanoparticles in: Environment. In Nanomaterials: A Danger or a Promise? London: Springer; 2013.

8. Verma J, Lal S, Van Noorden CJ. Nanoparticles for hyperthermic therapy: Synthesis strategies and applications in glioblastoma. International journal of nanomedicine. 2014;9:2863-70. PMid: 24959075. PMCid:PMC4061173.

9. Zhang L, Dou YH, Gu HC. Sterically induced shape control of magnetite nanoparticles. Journal of Crystal Growth. 2006;296(2):221-6.
Available:http://dx.doi.org/10.1016/j.jcrysgr o.2006.08.010

10. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual review of biomedical engineering, 2012;14:1-16. PMid:22524388.
Available:http://dx.doi.org/10.1146/annurev -bioeng-071811-150124

11. Huang X, Li L, Liu T, Hao N, Liu H, Chen D, Tang F. The shape effect of mesoporous silica nanoparticles on biodistribution clearance and biocompatibility *in vivo*. ACS Nano. 2011;5(7):5390-9. PMid:21634407.
Available:http://dx.doi.org/10.1021/nn2003 65a

12. Laurent S, Saei AA, Behzadi S, Panahifar A, Mahmoudi M. Superparamagnetic iron oxide nanoparticles for delivery of therapeutic agents: Opportunities and challenges. Expert opinion on drug delivery. 2014;(0):1-22.

13. Shimanovskii NL. Targeted transport of drugs by iron oxide nanoparticles. Russian Journal of General Chemistry. 2014;84(2):391-406.
Available:http://dx.doi.org/10.1134/S10703 63214020418

14. Chouly C, Pouliquen D, Lucet I, Jeune JJ, Jallet P. Development of superparamagnetic nanoparticles for MRI: Effect of particle size charge and surface nature on biodistribution. Journal of microencapsulation. 1996;13(3):245-55. PMid:8860681.
Available:http://dx.doi.org/10.3109/026520 49609026013

15. Chekhun VF, Naleskina LA, Polishchuk LZ, Todor IM, Demash DV, Lukianova NYu.

Evaluation of biological effects of nanosystems of directed transportin experiments *in vivo* and their application possibilities in anti-tumor therapy. Advances in Nanoparticles. 2013;(2):145-53.
Available:http://dx.doi.org/10.4236/anp.201 3.22023

16. Ayala V, Herrera AP, Latorre-Esteves M, Torres-Lugo M, Rinaldi C. Effect of surface charge on the colloidal stability and *in vitro* uptake of carboxymethyl dextran-coated iron oxide nanoparticles. Journal of Nanoparticle Research. 2013;15(8):1-14. PMid:24470787 PMCid:PMC3901157.
Available:http://dx.doi.org/10.1007/s11051- 013-1874-0

17. He C, Hu Y, Yin L, Tang C, Yin C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials. 2010;31(13):3657-66. PMid:20138662.
Available:http://dx.doi.org/10.1016/j.biomat erials.2010.01.065

18. Gupta AK, Gupta M. Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. Biomaterials. 2005;26(13): 1565-73. PMid:15522758.
Available:http://dx.doi.org/10.1016/j.biomat erials.2004.05.022

19. Walkey CD, Olsen JB, Guo H, Emili A, Chan WC. Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. Journal of the American Chemical Society. 2012;134(4):2139-2147. PMid:22191645.
Available:http://dx.doi.org/10.1021/ja20843 38

20. Weissleder R, Nahrendorf M, Pittet MJ. Imaging macrophages with nanoparticles. Nature Materials. 2014;13(2):125-38. PMid:24452356.
Available:http://dx.doi.org/10.1038/nmat37 80

21. Ganguly A, Kundu R, Ramanujachary KV, Lofland SE, Das D, Vasanthacharya NY, et al. Role of carboxylate ion and metal oxidation state on the morphology and magnetic properties of nanostructured metal carboxylates and their decomposition products. Journal of Chemical Sciences. 2008;120(6):521-8.

Available:http://dx.doi.org/10.1007/s12039-008-0081-5

22. Lee N, Hyeon T. Designed synthesis of uniformly sized iron oxide nanoparticles for efficient magnetic resonance imaging contrast agents. Chemical Society Reviews. 2012;41(7):2575-89.

    PMid:22138852.
    Available:http://dx.doi.org/10.1039/c1cs15248c

23. Rauch J, Kolch W, Laurent S, Mahmoudi M. Big signals from small particles: Regulation of cell signaling pathways by nanoparticles. Chemical Reviews. 2013;113(5):3391-406. PMid:23428231.

    Available:http://dx.doi.org/10.1021/cr3002627

24. Prijic S, Sersa G. Magnetic nanoparticles as targeted delivery systems in oncology. Radiology and Oncology. 2011;45(1):1-16. PMid:22933928 PMCid:PMC3423716.

    Available:http://dx.doi.org/10.2478/v10019-011-0001-z

25. Habib AH, Ondeck CL, Chaudhary P, Bockstaller MR, McHenry ME. Evaluation of iron-cobalt/ferrite core-shell nanoparticles for cancer thermotherapy. Journal of Applied Physics. 2008;103(7):07A307.

26. Schaffer TE. Nanomechanics of molecules and living cells with scanning ion conductance microscopy. Analytical chemistry. 2013;85(15):6988-94. PMid:23692368.
    Available:http://dx.doi.org/10.1021/ac400686k

27. Kievit FM, Zhang M. Surface engineering of iron oxide nanoparticles for targeted cancer therapy. Accounts of chemical research. 2011;44(10):853-62. PMid:21528865 PMCid:PMC3192288.

    Available:http://dx.doi.org/10.1021/ar200027

28. Rosen JE, Chan L, Shieh DB, Gu FX. Iron oxide nanoparticles for targeted cancer imaging and diagnostics. Nanomedicine: Nanotechnology Biology and Medicine. 2012;8(3):275-90. PMid:21930108.

    Available:http://dx.doi.org/10.1016/j.nano.2011.08.017

29. Eifler AC, Thaxton CS. Nanoparticle therapeutics: FDA approval clinical trials regulatory pathways and case study. In: Biomedical Nanotechnology. Humana Press; 2011.
    Available:http://dx.doi.org/10.1007/978-1-61779-052-2_21

30. Kunzmann A, Andersson B, Thurnherr T, Krug H, Scheynius A, Fadeel B. Toxicology of engineered nanomaterials: Focus on biocompatibility biodistribution and biodegradation. Biochimica et Biophysica Acta (BBA)-General Subjects. 2011;1810(3):361-73. PMid:20435096.

    Available:http://dx.doi.org/10.1016/j.bbagen.2010.04.007

31. Laskar A, Ghosh M, Khattak SI, Li W, Yuan XM. Degradation of superparamagnetic iron oxide nanoparticle-induced ferritin by lysosomal cathepsins and related immune response. Nanomedicine. 2012;7(5):705-17. PMid:22500704.
    Available:http://dx.doi.org/10.2217/nnm.11.148

32. Chekhun VF, Yurchenko OV, Naleskina LA, Demash DV, Lukianova NY, Lozovska YV. *In vitro* modification of cisplatin cytotoxicity with magnetic fluid. Experimental Oncology. 2013;35(1):15-9. PMid:23528310.

33. Serda RE, Mack A, van de Ven AL, Ferrati S, Dunner K, Godin B, et al. Logic-embedded vectors for intracellular partitioning endosomal escape and exocytosis of nanoparticles. Small. 2010;6(23):2691-700. PMid:20957619

    PMCid:PMC2997879.

    Available:http://dx.doi.org/10.1002/smll.201000727

34. Ahamed M, Akhtar MJ, Siddiqui MA, Ahmad J Musarrat J, Al-Khedhairy AA, et al. Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. Toxicology. 2011;283(2):101-8. PMid:21382431.

    Available:http://dx.doi.org/10.1016/j.tox.2011.02.010

35. Siddiqui MA, Ahamed M, Ahmad J, Majeed Khan MA, Musarrat J, Al-Khedhairy A, et al. Nickel oxide nanoparticles induce cytotoxicity oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin. Food and Chemical Toxicology. 2012;50(3):641-7. PMid:22273695.

Available:http://dx.doi.org/10.1016/j.fct.2012.01.017

36. Ahmad J, Ahamed M, Akhtar MJ, Alrokayan SA, Siddiqui MA, Musarrat J, et al. Apoptosis induction by silica nanoparticles mediated through reactive oxygen species in human liver cell line Hep G2. Toxicology and applied pharmacology. 2012;259(2):160-8. PMid:22245848.

    Available:http://dx.doi.org/10.1016/j.taap.2011.12.020

37. Huang G, Chen H, Dong Y, Luo X, Yu H, Moore Z, et al. Superparamagnetic iron oxide nanoparticles: Amplifying ROS stress to improve anticancer drug efficacy. Theranostics. 2013;3(2):116-21. PMid:23423156 PMCid:PMC3575592.

    Available:http://dx.doi.org/10.7150/thno.5411

38. Khan MI, Mohammad A, Patil G, Naqvi SAH, Chauhan LKS, Ahmad I. Induction of ROS mitochondrial damage and autophagy in lung epithelial cancer cells by iron oxide nanoparticles. Biomaterials. 2012;33(5):1477-88. PMid:22098780.

    Available:http://dx.doi.org/10.1016/j.biomaterials.2011.10.080

39. Singh RK, Kim TH, Patel KD, Knowles JC, Kim HW. Biocompatible magnetite nanoparticles with varying silica-coating layer for use in biomedicine: Physicochemical and magnetic properties and cellular compatibility. Journal of Biomedical Materials Research Part A. 2012;100(7):1734-42. PMid:22447364.

    Available:http://dx.doi.org/10.1002/jbm.a.34140

40. Buyukhatipoglu K, Clyne AM. Superparamagnetic iron oxide nanoparticles change endothelial cell morphology and mechanics via reactive oxygen species formation. Journal of Biomedical Materials Research Part A. 2011;96(1):186-95. PMid:21105167.

    Available:http://dx.doi.org/10.1002/jbm.a.32972

41. Wang J, Li N, Zheng L, Wang S, Wang Y, Zhao X, et al. P38-Nrf-2 signaling pathway of oxidative stress in mice caused by nanoparticulate TiO2. Biological trace element research. 2011;140(2):186-97.

    PMid:20422311.
    Available:http://dx.doi.org/10.1007/s12011-010-8687-0

42. Lim D, Roh JY, Eom HJ, Choi JY, Hyun J, Choi J. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode Caenorhabditis elegans. Environmental Toxicology and Chemistry. 2012;31(3):585-92. PMid:22128035.

    Available:http://dx.doi.org/10.1002/etc.1706

43. Lukianova N, Demash D, Borikun T, Chekhun S, Shvets Y. Manifestation of key molecular genetic markers in pharmacocorrection of endogenous iron metabolism in MCF-7 and MCF-7/DDP human breast cancer cells. CellBio. 2013;2(4):217-27.
    Available:http://dx.doi.org/10.4236/cellbio.2013.24025

44. Ansari C, Tikhomirov GA, Hong SH, Falconer RA, Loadman PM, Gill JH, et al. Development of novel tumor-targeted theranostic nanoparticles activated by membrane-type matrix metalloproteinases for combined cancer magnetic resonance imaging and therapy. Small. 2014;10(3):566-75. PMid:24038954.

    Available:http://dx.doi.org/10.1002/smll.201301456

45. Soenen SJ, Himmelreich U, Nuytten N, De Cuyper M. Cytotoxic effects of iron oxide nanoparticles and implications for safety in cell labelling. Biomaterials. 2011;32(1):195-205. PMid:20863560.

    Available:http://dx.doi.org/10.1016/j.biomaterials.2010.08.075

46. Andreas K, Georgieva R, Ladwig M, Mueller S, Notter M, Sittinger M, et al. Highly efficient magnetic stem cell labeling with citrate-coated superparamagnetic iron oxide nanoparticles for MRI tracking. Biomaterials. 2012;33(18):4515-25.

    PMid:22445482.
    Available:http://dx.doi.org/10.1016/j.biomaterials.2012.02.064

47. Heng BC, Zhao X, Xiong S, Ng KW, Boey FYC, Loo JSC. Cytotoxicity of zinc oxide (ZnO) nanoparticles is influenced by cell density and culture format. Archives of Toxicology. 2011;85(6):695-704.

    PMid:20938647.
    Available:http://dx.doi.org/10.1007/s00204-010-0608-7

48. Moos PJ, Olszewski K, Honeggar M, Cassidy P, Leachman S, Woessner D, et al. Responses of human cells to ZnO nanoparticles: A gene transcription study. Metallomics. 2011;3(11):1199-211. PMid:21769377 PMCid:PMC3881269.

    Available:http://dx.doi.org/10.1039/c1mt00061f

49. Singh N, Jenkins GJ, Nelson BC, Marquis BJ, Maffeis TG, Brown AP, et al. The role of iron redox state in the genotoxicity of ultrafine superparamagnetic iron oxide nanoparticles. Biomaterials. 2012;33(1):163-70. PMid:22027595.

    Available:http://dx.doi.org/10.1016/j.biomaterials.2011.09.087

50. Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. Metal-based nanoparticles and their toxicity assessment. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2010;2(5):544-68.

    PMid:20681021.
    Available:http://dx.doi.org/10.1002/wnan.103

51. Liu G, Gao J, Ai H, Chen X, Applications and potential toxicity of magnetic iron oxide nanoparticles. Small. 2013;9(9-10):1533-45. PMid:23019129.

    Available:http://dx.doi.org/10.1002/smll.201201531

52. Hong SC, Lee JH, Lee J, Kim HY, Park JY, Cho J, et al. Subtle cytotoxicity and genotoxicity differences in superparamagnetic iron oxide nanoparticles coated with various functional groups. International Journal of Nanomedicine. 2011;6:3219-25.

    PMid:22238510PMCid:PMC3254266.

53. Lartigue L, Wilhelm C, Servais J, Factor C, Dencausse A, Bacri JC. Nanomagnetic sensing of blood plasma protein interactions with iron oxide nanoparticles: Impact on macrophage uptake. Acs Nano. 2012;6(3):2665-78. PMid:22324868.

    Available:http://dx.doi.org/10.1021/nn300060u

54. Chabner BA, Longo DL. Cancer chemotherapy and biotherapy: Principles and practice. Lippincott Williams & Wilkins; 2011.

55. Skeel RT, Khleif SN, editors. Handbook of cancer chemotherapy. Philadelphia: Lippincott Williams & Wilkins; 2011. PMid:21244843 PMCid:PMC3021674

56. Widder KJ, Morris RM, Poore GA, Howard DP, Senyei AE. Selective targeting of magnetic albumin microspheres containing low-dose doxorubicin: total remission in Yoshida sarcoma-bearing rats. European Journal of Cancer and Clinical Oncology. 1983;19(1):135-9.

    Available:http://dx.doi.org/10.1016/0277-5379(83)90408-X

57. Knežević NŽ, Slowing II, Lin VSY. Tuning the release of anticancer drugs from magnetic iron oxide/mesoporous silica core/shell nanoparticles. Chem PlusChem. 2012;77(1):48-55.

    Available:http://dx.doi.org/10.1002/cplu.201100026

58. Schleich N, Sibret P, Danhier P, Ucakar B, Laurent S, Muller RN, et al. Dual anticancer drug/superparamagnetic iron oxide-loaded PLGA-based nanoparticles for cancer therapy and magnetic resonance imaging. International journal of pharmaceutics. 2013;447(1):94-101.

    PMid:23485340.
    Available:http://dx.doi.org/10.1016/j.ijpharm.2013.02.042

59. Santoyo Salazar J, Perez L, de Abril O, Truong Phuoc L, Ihiawakrim D, Vazquez M, et al. Magnetic iron oxide nanoparticles in 10− 40 nm range: Composition in terms of magnetite/maghemite ratio and effect on the magnetic properties. Chemistry of Materials. 2011;23(6):1379-86.

    Available:http://dx.doi.org/10.1021/cm103188a

60. Cho K, Wang X, Nie S, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clinical Cancer Research. 2008;14(5):1310-6.

    Available:http://dx.doi.org/10.1158/1078-0432.CCR-07-1441 PMid:18316549

61. Laurent S, Dutz S, Häfeli UO, Mahmoudi M. Magnetic fluid hyperthermia: Focus on superparamagnetic iron oxide nanoparticles. Advances in Colloid and Interface Science. 2011;166(1):8-23. PMid:21601820.

62. Sun Z, Worden M, Wroczynskyj Y, Yathindranath V, van Lierop J, Hegmann T, et al. Magnetic field enhanced convective diffusion of iron oxide nanoparticles in an osmotically disrupted

cell culture model of the blood–brain barrier. International Journal of Nanomedicine. 2014;9:3013-9.

PMid:25018630 PMCid:PMC4073976.

Available:http://dx.doi.org/10.2147/IJN.S62260

63. Wahajuddin SA. Superparamagnetic iron oxide nanoparticles: Magnetic nanoplatforms as drug carriers. International Journal of Nanomedicine. 2012;7;3445-52. PMid:22848170

 PMCid:PMC3405876.
Available:http://dx.doi.org/10.2147/IJN.S30320

64. Lamkowsky MC, Geppert M, Schmidt MM, Dringen R. Magnetic field-induced acceleration of the accumulation of magnetic iron oxide nanoparticles by cultured brain astrocytes. Journal of Biomedical Materials Research Part A. 2012;100(2):323-34. PMid:22065542.

Available:http://dx.doi.org/10.1002/jbm.a.33263

65. Gautier J, Allard-Vannier E, Munnier E, Souce M, Chourpa I. Recent advances in theranostic nanocarriers of doxorubicin based on iron oxide and gold nanoparticles. Journal of Controlled Release. 2013;169(1):48-61.

PMid:23567046.
Available:http://dx.doi.org/10.1016/j.jconrel.2013.03.018