



## ERYTHROCYTE DRUG DELIVERY SYSTEM

Yogeshkumar A Biranwar\*, Kapil R Bare, Vaishali R Mahajan, Mangesh K Sapate, Dheeraj T Baviskar

Department of Pharmaceutics, Institute of Pharmaceutical Education, Boradi, Shirpur, Dist- Dhule, Maharashtra, India.

\*Corresponding author's E-mail: [yogesh.biranwar@gmail.com](mailto:yogesh.biranwar@gmail.com)

Accepted on: 01-03-2011; Finalized on: 01-05-2011.

### ABSTRACT

Carrier erythrocytes loaded by a drug or other therapeutic agents, controlled delivery of a wide variety of drugs and other bioactive agents owing to their remarkable degree of biocompatibility, biodegradability and a series of other potential advantages. Therapeutically significant peptides, proteins, nucleic acid-based biologicals, antigens and vaccines are among the recently focused pharmaceuticals for being delivered using carrier erythrocytes. Magnetic nanoparticles (MNPs) possess unique magnetic properties carriers of drug delivery. To better address specific clinical needs, MNPs with higher magnetic moments, non-fouling surfaces and increased functionalities are now being developed for applications in the detection, diagnosis and treatment of malignant tumours, cardiovascular disease and neurological disease. This review provides a background on applications of MNPs as magnetic resonance (MR) imaging contrast agents and as carriers for drug delivery and an overview of the recent developments in this area of research.

**Keywords:** Erythrocyte, Cellular carriers, Resealed erythrocytes, Magnetic nanopartical, drug delivery.

### INTRODUCTION

Erythrocyte was slow drug release or site-targeted delivery systems for a variety of bioactive agents from different fields of therapy have gained a remarkable degree of interest in recent years<sup>1-3</sup>. MNPs are a major class of nanoscale materials with the potential to revolutionize current clinical diagnostic and therapeutic techniques. Carriers for targeted drug delivery<sup>5,6</sup> with a wide range of applications in the detection, diagnosis and treatment of illnesses, such as cancer<sup>7</sup>, cardiovascular disease<sup>8</sup> and neurological disease<sup>9</sup>. MNPs in the form of superparamagnetic iron oxides (SPIO) have been actively investigated as MR imaging contrast agents for over two decades<sup>10</sup>. With applications, such as bowel contrast agents and liver/spleen imaging<sup>11,12</sup>, already on the market. SPIOs have led the way for MNPs into the clinic. Several forms of ultrasmall super paramagnetic iron oxides (USPIO) have undergone clinical trials with one of the most notable being Combidex® which is in late stage clinical trials for use in the detection of lymph node metastases<sup>13</sup>. Active targeting through the attachment of high affinity ligands<sup>14-16</sup> in the spirit of Ehrlich's "Magic Bullet"<sup>17</sup>. A significant challenge associated with the application of these MNP systems is their behaviour in vivo. The efficacy of many of these systems is often compromised due to recognition and clearance by the reticuloendothelial system (RES) prior to reaching target tissue, as well as by an inability to overcome biological barriers, such as the vascular endothelium or the blood brain barrier. The fate of these MNP upon intravenous administration is highly dependent on their size, morphology, charge and surface chemistry. These physicochemical properties of nanoparticles directly affect their subsequent pharmacokinetics and biodistribution<sup>18</sup>. To increase the effectiveness of MNPs several techniques including reducing size and grafting nonfouling polymers have been employed to improve

their "stealthiness" and increase their blood circulation time to maximize the likelihood of reaching targeted tissues<sup>19,20</sup>.

### CARRIER ERYTHROCYTES

Cellular carriers including erythrocytes, leukocytes, platelets, islets, hepatocytes and fibroblasts have all been suggested as potential carriers for drugs and other bioactive agents. They can be used to provide slow release of entrapped drugs in the circulatory system to deliver drugs to a specific site in the body as cellular transplants to provide missing enzymes and hormones (in enzyme or hormone replacement therapy) or as endogenous cells to synthesize and secrete molecules capable of affecting the metabolism and function of other cells. Since these carriers are actual endogenous cells they produce little or no antigenic response and upon aging or being damaged can be removed from the circulation by macrophages as a complete natural process. Another important feature of these carriers is that they can be stored at 4°C for several hours to several days before re-entry to the host body, depending on the storage medium and the entrapment method used.<sup>[18]</sup> Since erythrocytes have been receiving the greatest attention for their potential applications as drug delivering microspheres<sup>19-21</sup>, the discussion that follows will be limited to these carriers. These carriers offer some unique characteristics such as excellent biocompatibility and biodegradability as well as considerably long life-span in circulation<sup>22,23</sup>. Generally, 'carrier erythrocytes' can be prepared by withdrawal of some blood samples from the organism of interest, separation of erythrocytes from plasma and leukocytes, entrapment of the drug in the erythrocytes and finally resealing and reannealing of the resulting cellular carriers<sup>19</sup>. Upon re-injection to the same organism, these drug-loaded microspheres can serve as the intravenous slow-release carriers and/or targeted



drug delivery systems especially to target the drug to the RES<sup>20-22</sup>.

### ADVANTAGES OF ERYTHROCYTES IN DRUG DELIVERY

A remarkable degree of biocompatibility, particularly when the autologous cells are used for drug loading<sup>22</sup>. Complete biodegradability and the lack of toxic products resulting from the carrier biodegradation. Avoidance of any undesired immune responses against the encapsulated drug. Considerable protection of the organism against the toxic effects of then capsulated drug e.g., antineoplasms. Remarkably longer life-span of the carrier erythrocytes in circulation in comparison to the synthetic carriers. In the optimum condition of the loading procedure, the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes. An easily controllable life-span within a wide range from minutes to months. Desirable size range and the considerably uniform size and shape. Availability of knowledge, techniques, facilities for handling, transfusion and working with erythrocytes<sup>22,23</sup>. Loading a relatively high amount of drug in a small volume of erythrocytes which in turn, assures the dose sufficiency in clinical as well as animal studies using a limited volume of erythrocyte samples, possibility of decreasing drug side effects.

### DISADVANTAGES OF ERYTHROCYTES IN DRUG DELIVERY

The use of erythrocytes as carrier systems also covers some disadvantages, which can be summarized as follows. The major problem encountered in the use of biodegradable materials or natural cells as drug carriers is that they are removed *in vivo* by the RES as result of modification that occurred during loading procedure in cells. Although this expands the capability to drug targeting to RES, seriously limits their life-span as long-circulating drug carriers in circulation and in some cases, may pose toxicological problems. The rapid leakage of certain encapsulated substances from the loaded erythrocytes. The storage of the loaded erythrocytes is a further problem provided that there are viable cells and need to survive in circulation for a long time upon re-entry to the host body. Conditioning carrier cells in isotonic buffers containing all essential nutrients as well as in low temperatures, the addition of nucleosides or chelators, lyophilisation with glycerol or gel immobilization have all been exploited to overcome this problem. Possible contamination due to the origin of the blood, the equipment used and the loading environment. Rigorous controls are required accordingly for the collection and handling of the erythrocytes<sup>24</sup>.

### DELIVERY STRATEGIES

#### Intravenous slow drug release strategy

The normal life-span of an erythrocyte in systemic circulation is about 120 days. In the optimum conditions of the loading procedure (using more gentle methods for

loading), the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes. Erythrocytes have been used as circulating intravenous slow-release carriers for the delivery of antineoplasms, antiparasitics, antiretroviral agents, vitamins, steroids, antibiotics and cardiovascular drugs. A series of mechanisms have been proposed for drug release in circulation from carrier erythrocytes, including passive diffusion out of the loaded cells into circulation, specialized membrane-associated carriers, phagocytosis of the carrier cells by the macrophages of RES and the depletion of the drug into circulation,<sup>[23]</sup> accumulation of the drug in RES upon lysis of the carrier and slow release from this system into circulation, accumulation of the carrier erythrocytes in lymphatic nodes. Subcutaneous injection of the cells drug release upon haemolysis in this site and finally haemolysis in the injection sites.

#### Magnetic nanoparticles

##### *Magnetic properties*

The penetration of magnetic fields through human tissue and the ability to remotely detect or manipulate magnetic materials have been investigated for use in medicine for centuries<sup>25</sup>. One of the more recent and significant applications of these properties has been in MRI as a non-invasive imaging modality capable of providing high resolution anatomical images. The potential of current clinical medical imaging can be greatly expanded through the use of MNPs to improve differentiation of malignant and healthy tissue. In addition upon location of a malignancy or lesion, external magnetic fields can then be controlled to direct particle accumulations to deliver therapeutics. To better understand the advantages of MNPs as MRI contrast agents, we briefly review some of the fundamental concepts of magnetism and the properties of MNPs. More thorough and detailed discussion of this topic can be found in the literature<sup>26</sup>. Ferri- or ferromagnetic materials, such as MNPs, become a single magnetic domain and therefore maintain one large magnetic moment. Superparamagnetism is a favourable property of small particles, the reduction of particle size is not without some consequences. As particle sizes decrease, surface-to-volume ratios increase resulting in pronounced surface effects.

##### *Iron oxide nanoparticles*

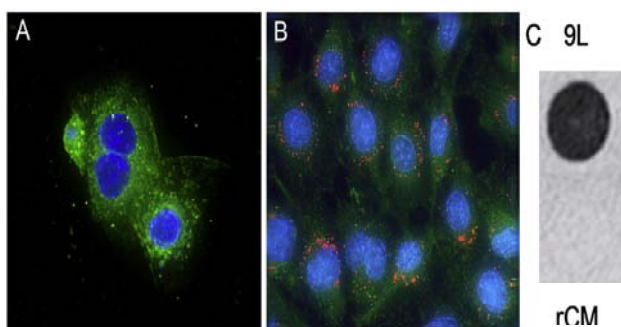
Colloidal iron oxide nanoparticles, such as SPIO and USPIO, the most extensively investigated MNPs for biomedical applications due to their excellent biocompatibility and ease of synthesis. Typically composed of nano crystalline magnetite ( $\text{Fe}_3\text{O}_4$ ) or maghemite ( $\gamma\text{Fe}_2\text{O}_3$ ) protected with a polymeric coating, these ferrite nanoparticles possess a spinel crystal structure with oxygen ions forming a close-packed cubic lattice and iron ions located at interstices. In the case of  $\text{Fe}_3\text{O}_4$ , magnetisation arises from electron hopping between the  $\text{Fe}_2^+$  and  $\text{Fe}_3^+$  ions that coexist at the octahedral sites. In addition to magnetic properties, the favourable biocompatibility and biodegradability of these



MNPs have contributed greatly to their widespread use in biomedical applications. Upon metabolism iron ions are added to the body's iron stores and eventually incorporated by erythrocytes as haemoglobin allowing for their safe use *in vivo*<sup>27</sup>.

### Metallic nanoparticles

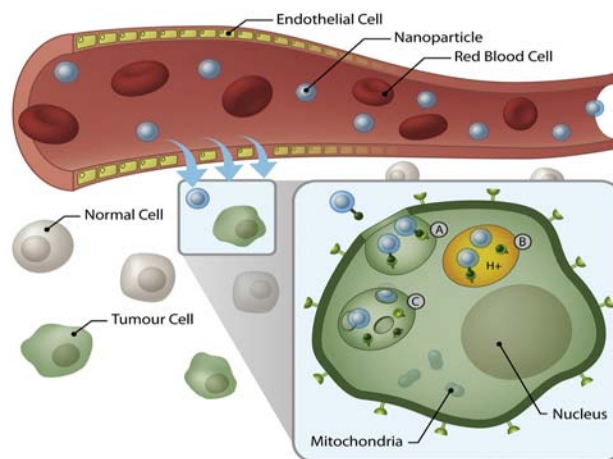
Metallic MNPs made of iron, cobalt, or nickel are often overlooked for biological applications due to their chemical instability. Readily forming oxides in the presence of water and oxygen, these metallic MNPs are typically protected by coatings, such as gold or silica, to form a core-shell structure. Despite complex synthesis processes, research continues on these metallic nanoparticles due to the unique advantages some of these MNPs can offer. For example, iron nanoparticles possess relatively high magnetization and are able to maintain Superparamagnetism at larger particle sizes compared to their oxide counterparts<sup>28</sup>. Veisheh et al. constructed a multi model agent composed of PEG-coated iron oxide nanoparticle conjugated to both Cy5.5 and a targeting agent, chlorotoxin to improve specificity and internalization of nanoparticles into 9L glioma cell (fig.1).



**Figure 1:** Confocal fluorescent images of cells incubated with chlorotoxin-targeted iron oxide nanoparticles conjugated to Cy5.5. (A) rat cardiomyocytes (rCM) representing normal cells. (B) 9L glioma cells. (C) MR phantom image of 9L (top) and rCM (bottom) cells cultured with the chlorotoxin-targeted nanoparticles (4.7 t, spin echo pulse sequence, TR 3000 ms, TE 30 ms)<sup>16</sup>.

### Active targeting

One promising approach toward increasing the local accumulation of MNPs in diseased tissue, known as active targeting or specific targeting, is by the conjugation of targeting molecules that possess high affinity toward unique molecular signatures found on malignant cells<sup>29</sup>. Often augmented by the EPR effect, these receptor-ligand or antigen-antibody interactions provide an effective strategy to improve the residence time in malignant tissues, such as tumors (fig.2). Targeting ligands, such as proteins<sup>30,31</sup>, peptides, aptamers<sup>32-34</sup> and small molecules<sup>[35]</sup> have been investigated to increase the site specific accumulation of MNPs. In some cases, specific binding can also facilitate internalization of the nanoparticle by receptor-mediated endocytosis.



**Figure 2:** Illustration of tissue specific delivery of MNPs through active targeting facilitated by “leaky” vasculature. (a) internalization of nanoparticles by (a) receptor-mediated endocytosis and formation of an endosome. (b) endosomal acidification by proton pumps results in elevated osmotic pressure, swelling and (c) rupture of the endosome allowing for release of the nanoparticle and conjugated therapeutic agents.

## APPLICATIONS

### Cancer imaging

MNPs have been examined extensively as MRI contrast agents to improve the detection, diagnosis and therapeutic management of solid tumors. Currently, clinical imaging of liver tumors and metastases through RES-mediated uptake of SPIOs has been capable of distinguishing lesions as small as 2–3 mm<sup>36</sup>. In addition, USPIOs have been shown to be effective in identification of lymphnode metastases with a diameter of 5–10 mm under MRI. This non-invasive approach has broad implications as identification of lymphatic dissemination is an essential component of staging and determining the approaches to treatment of diseases such as prostate, breast and colon cancers<sup>37</sup>. Another clinical application of USPIO MNPs under evaluation is their use in improving the delineation of brain tumor boundaries and quantify tumor volumes<sup>38,39</sup>. Current approaches utilizing gadolinium chelate-based contrast agents are typically limited by edema surrounding tumor and diffusion of these small molecules from the tumor vasculature. In comparison, MNP-based contrast agents offer prolonged delineation of tumor margins due to enhanced cellular internalisation and slower clearance from the tumor site<sup>38,40</sup>. Although it has been shown that these USPIOs will not replace gadolinium chelates, they have been demonstrated to be helpful in distinguishing neoplastic tissue from areas of radiation necrosis. The next generation of active targeting MNPs currently being investigated have the potential to offer significantly improved tumor detection and localization by exploiting the unique molecular signatures of these diseases<sup>41</sup>. For example, we have recently demonstrated the specific accumulation of CTX-targeted iron oxide nanoparticles in 9L glioma flank xenografts resulting in more thorough

contrast enhancement of tumors in comparison to nontargeted control nanoparticles<sup>42</sup>.

### Cardiovascular disease imaging

MNPs have been proposed as MRI contrast agents for several injury, atherosclerosis and other vascular disease<sup>43,44</sup>. The uptake of MNPs by macrophages, which have been shown to be a marker of unstable atheromatous plaques has been exploited to clinical applications in cardiovascular medicine including myocardial visualize these lesion prone arterial sites. Clinical studies have demonstrated that MR imaging using USPIOs may be useful in evaluating the risk of acute ischaemic events<sup>45,46</sup>. Recently, Kelly et al. have identified 30 families of new peptides that bind to atherosclerotic lesions, through in vivo phage display<sup>47</sup>. From this study the adhesion molecule VCAM-1 was identified as a target for endothelial and macrophage cells responsible for atherosclerosis. Utilizing a VCAM-1 targeted peptide sequence, the group demonstrated specific binding of MNPs and MRI contrast enhancement of early lesions in juvenile mice as well as resected human carotid artery plaques<sup>48</sup>.

### DRUG OF THE FUTURE

The term biopharmaceutical is most commonly used to refer to all therapeutic, prophylactic and in vivo diagnostic agents produced using live organisms or their functional components. New technologies (recombinant DNA and monoclonal antibody/hybridoma) and 'old' technologies (fermentation, non-recombinant cell culture derived proteins, vaccines and other products from live organisms including blood/plasma products)<sup>49</sup>. 160 biopharmaceuticals have now gained medical approval and several hundred in the pipeline. Most biopharmaceuticals are protein-based, although two nucleic acid-based products are now on the US/European market from DNA-based products to antibodies, vaccines to therapeutics, administered by every route known to medicine, formulation and delivery system play a critical role in the ultimate success of biopharmaceutical products<sup>50</sup>.

### CONCLUSION

The development of MNPs has been greatly accelerated in the past decade by advances in nanotechnology, molecular cell biology and small-animal imaging instrumentation. MNPs of various formulations have been developed to diagnose and treat diseases for which conventional therapy has shown limited efficacy. In particular, the use of MNPs as MRI contrast agents and drug carriers has drawn enormous attention, as it holds great potential of providing new opportunities for early cancer detection and targeted therapies. This technology will not only minimize invasive procedures, but also reduce side effects to healthy tissues, which are two primary concerns in conventional cancer therapies. The advancement of carrier erythrocytes through their five

decades of development has covered a wide variety of drugs and other bioactive agents. This is mainly due to their remarkable degree of biocompatibility, biodegradability, bioavailability and ease of preparation and use, the most important among other advantages. The controlled and/or targeted release of biopharmaceuticals is among the mostly attractive applications of erythrocyte carriers in drug delivery in the recent decade. In this context, a series of successful studies have been carried out to extend the benefits of these cellular carriers to peptide/protein, gene/oligonucleotide and vaccine delivery. Most of the studies in this area are in the *in vitro* phase and the ongoing projects worldwide remain to step into preclinical and then clinical studies to prove the capabilities of this promising delivery system.

### REFERENCES

1. Hamidi M, Tajerzadeh H. Carrier erythrocytes an overview. *Drug Delivery*, 10, 2003, 9–20.
2. Rossi L, Serafini S, Piergie F, Antonelli A, Cerasi A, Fraternali A, *et al.* Erythrocyte-based drug delivery. *Expert Opin. Drug Deliv*, 2, 2005, 311-322.
3. Magnani M, Rossi L, Fraternali A, Bianchi M, Antonelli A, Crinelli R, *et al.* Erythrocyte-mediated delivery of drugs, peptide and modified oligonucleotides. *Gene Ther*, 11, 2002, 749-51.
4. Corot C, Robert P, Idee JM, Port M. Recent advances in iron oxide nanocrystal technology for medical imaging. *Advanced Drug Delivery Reviews*, 58, 2006, 1471-1504.
5. Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of Magnetic nanoparticles in biomedicine, *Journal of Physics. D. Applied Physics*, 36, 2003, R167-R181.
6. Dobson J. Magnetic nanoparticles for drug delivery. *Drug Development Research*, 67, 2006, 55-60.
7. Ferrari M. Cancer nanotechnology: opportunities and challenges, *Nature Reviews. Cancer*, 5, 2005, 161-171.
8. Wickline SA, Neubauer AM, Winter PM, Caruthers SD, Lanza GM. Molecular imaging and therapy of atherosclerosis with targeted nanoparticles. *Journal of Magnetic Resonance Imaging*, 25, 2007, 667-680.
9. Corot C, Petry KG, Trivedi R, Saleh A, Jonkmanns C, Le Bas JF, *et al.* Macrophage imaging in central nervous system and in carotid atherosclerotic plaque using ultrasmall superparamagnetic iron oxide in magnetic resonance imaging. *Investigative Radiology*, 39, 2004, 619–625.
10. Weissleder R, Bogdanov A, Neuwelt EA, Papisov M. Long-circulating iron oxides for MR imaging. *Advanced Drug Delivery Reviews*, 16, 1995, 321-334.
11. Wang YX, Hussain SM, Krestin GP. Superparamagnetic iron oxide contrast agents physicochemical characteristics and applications in MR imaging. *European Radiology*, 11, 2001, 2319-2331.
12. Bonnemain B. Superparamagnetic agents in magnetic resonance imaging: Physicochemical characteristics and clinical applications - a review. *Journal of Drug Targeting*, 6, 1998, 167-174.
13. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, *et al.* Noninvasive detection of clinically occult lymph-nodemetastases in prostate



- cancer. *New England Journal of Medicine*, 348, 2003, 2491-2499.
14. Torchilin VP. Multifunctional nanocarriers. *Advanced Drug Delivery Reviews*, 58 2006, 1532-1555.
  15. Zhang Y, Kohler N, Zhang MQ. Surface modification of Superparamagnetic magnetite nanoparticles and their intracellular uptake. *Biomaterials*, 23, 2002, 1553-1561.
  16. Veiseh O, Sun C, Gunn J, Kohler N, Gabikian P, Lee D, *et al.* Optical and MRI multifunctional nanoprobe for targeting gliomas. *Nano Letters*, 5, 2005, 1003-1008.
  17. Winau F, Westphal O, Winau R, Ehrlich P. In search of the magic bullet *Microbes and Infection*, 6, 2004, 786-789.
  18. Banker GS, Rhodes CT. *Modern Pharmaceutics*, 4th Edition. Marcel Dekker Inc. New York, 2002, 560.
  19. Ropars C, Chassaing M, Nicoulau C. *Advances in the Biosciences*, Pergamon Press. Oxford, 1987, 67.
  20. Lewis DA, Alpar HO. Therapeutic possibilities of drugs encapsulated in erythrocytes. *Int. J. Pharm*, 22, 1984, 137-46.
  21. Zimmermann U. Cellular drug-carrier systems and their possible targeting, in: Goldberg (Ed.) EP, *Targeted Drugs*, John Wiley & Sons. New York, 1983, 153-200.
  22. Jaitely V, Kanaujia P, Venkatesan N, Jain S, Vyas SP. Resealed erythrocytes: drug carrier potentials and biomedical applications. *Indian Drugs*, 33, 1996, 589-594.
  23. Talwar N, Jain NK. Erythrocytes as carrier of primaquin preparation: characterization and evaluation. *Control Release*, 20, 1992, 133-142.
  24. Pitt E, Lewis DA, Offord R. The use of corticosteroids encapsulated in erythrocytes in the treatment of adjuvant induced arthritis in the rat. *Biochem. Pharmacol*, 132 1983, 3355-3358.
  25. Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of magnetic nanoparticles in biomedicine. *Journal of Physics. D. Applied Physics*, 2003, 36, R167-181.
  26. Dobson J. Magnetic nanoparticles for drug delivery. *Drug Development Research*, 67, 2006, 55-60.
  27. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nature Reviews. Cancer*, 5, 2005, 161-71.
  28. Wickline SA, Neubauer AM, Winter PM, Caruthers SD, Lanza GM. Molecular imaging and therapy of atherosclerosis with targeted nanoparticles. *Journal of Magnetic Resonance Imaging*, 25, 2007, 667-680.
  29. Corot C, Petry KG, Trivedi R, Saleh A, Jonkmann C, Le Bas JF, *et al.* Macrophage imaging in central nervous system and in carotid atherosclerotic plaque using ultrasmall superparamagnetic iron oxide in magnetic resonance imaging. *Investigative Radiology*, 39, 2004, 619-625.
  30. Tartaj P, Morales MD, Veintemillas-Verdaguer S, Gonzalez-Carreno T, Serna CJ. The preparation of magnetic nanoparticles for applications in biomedicine. *Journal of Physics D. Applied Physics*, 36, 2003, R182-197.
  31. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials*, 26, 2005, 3995-4021.
  32. McNeil SE. Nanotechnology for the biologist. *Journal of Leukocyte Biology*, 78, 2005, 585-594.
  33. Frullano L, Meade TJ. Multimodal MRI contrast agents. *Journal of Biological Inorganic Chemistry*, 12, 2007, 939-949.
  34. Weissleder R, Bogdanov A, Neuwelt EA, Papisov M. Long-circulating iron oxides for MR imaging. *Advanced Drug Delivery Reviews*, 16, 1995, 321-334.
  35. Wang YX, Hussain SM, Krestin GP. Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. *European Radiology*, 11, 2001, 2319-2331.
  36. Bonnemain B. Superparamagnetic agents in magnetic resonance imaging: Physicochemical characteristics and clinical applications - a review. *Journal of Drug Targeting*, 6, 1998, 167-174.
  37. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH. Noninvasive detection of clinically occult lymphnode metastases in prostate cancer. *New England Journal of Medicine*, 348, 2003, 2491-2499.
  38. Senyei A, Widder K, Czerlinski G. Magnetic guidance of drug-carrying microspheres. *Journal Of Applied Physics*, 49, 1978, 3578-3583.
  39. Neuberger T, Schopf B, Hofmann H, Hofmann M, Rechenberg B. Superparamagnetic nanoparticles for biomedical applications: possibilities and Limitations of a new drug delivery system. *Journal of Magnetism and Magnetic Materials*, 293, 2005, 483-496.
  40. Torchilin VP. Multifunctional nanocarriers. *Advanced Drug Delivery Reviews*, 58 2006, 1532-1555.
  41. Zhang Y, Kohler N, Zhang MQ. Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. *Biomaterials*, 23, 2002, 1553-1561.
  42. Veiseh O, Sun C, Gunn J, Kohler N, Gabikian P, Lee D, *et al.* Optical and MRI multifunctional nanoprobe for targeting gliomas. *Nano Letters*, 5, 2005, 1003-1008.
  43. Winau F, Westphal O, Winau R, Paul Ehrlich - In search of the magic bullet. *Microbes and Infection*, 6, 2004, 786-789.
  44. 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*, 13, 1996, 245-255.
  45. Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S, *et al.* 'Stealthiness' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids and Surfaces. Biointerfaces*, 18, 2000, 301-313.
  46. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacological Reviews*, 53, 2001, 283-318.
  47. Suresh S. Biomechanics and biophysics of cancer cells. *Acta Biomaterialia*, 4, 2007, 413-438.
  48. Nahrendorf M, Jaffer FA, Kelly KA, Sosnovik DE, Aikawa E, Libby P, *et al.* Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis. *Circulation*, 117, 2006, 1504-1511.
  49. Rader RA. What is biopharmaceutical? Part 1. *Bioexecutive International* 2005, 60-65.
  50. Arkinson EM, Klum W. Formulation strategies for biopharmaceuticals. *Ensuring success to market, drugs*, 4, 2001, 557-560.

\*\*\*\*\*

