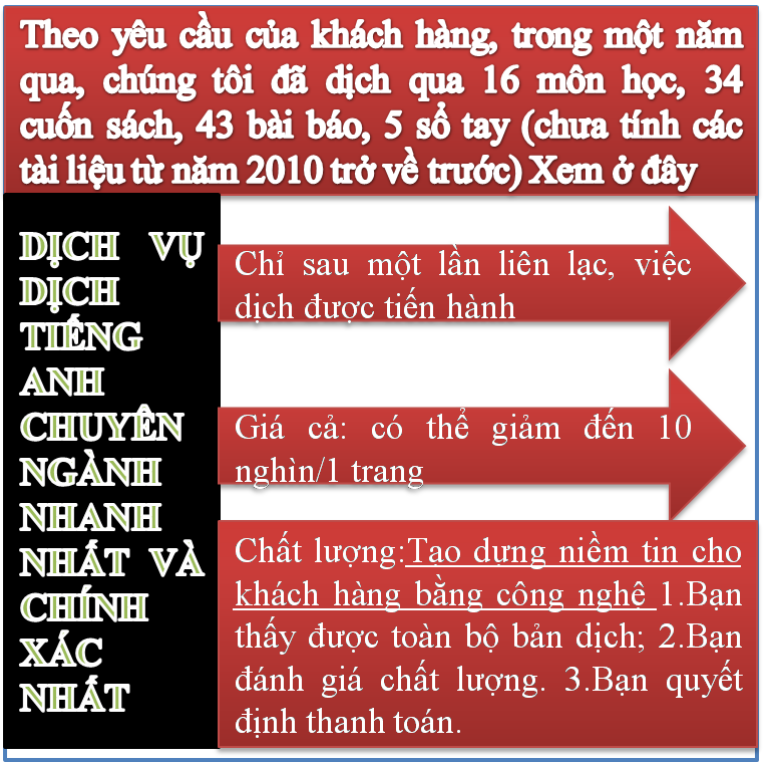

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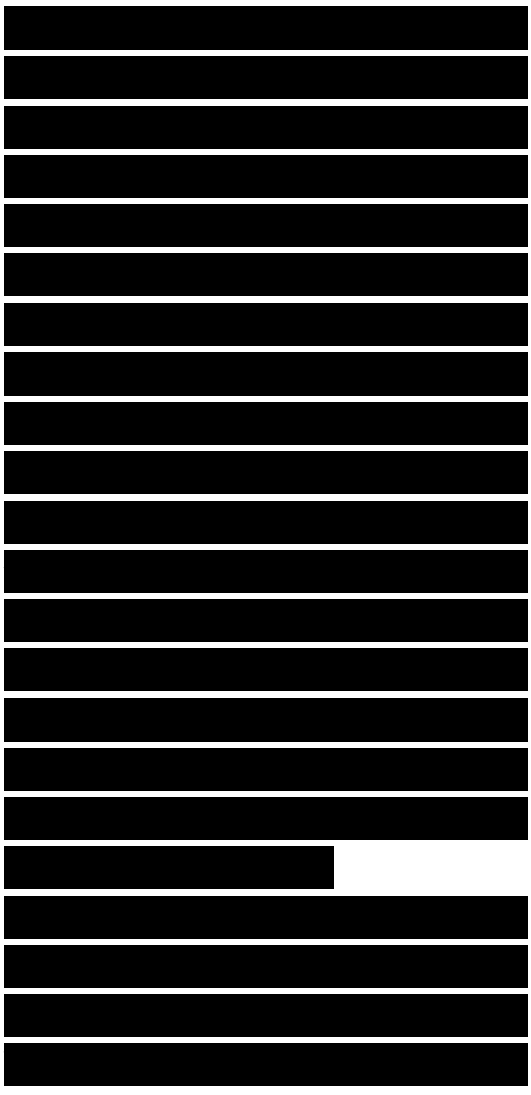
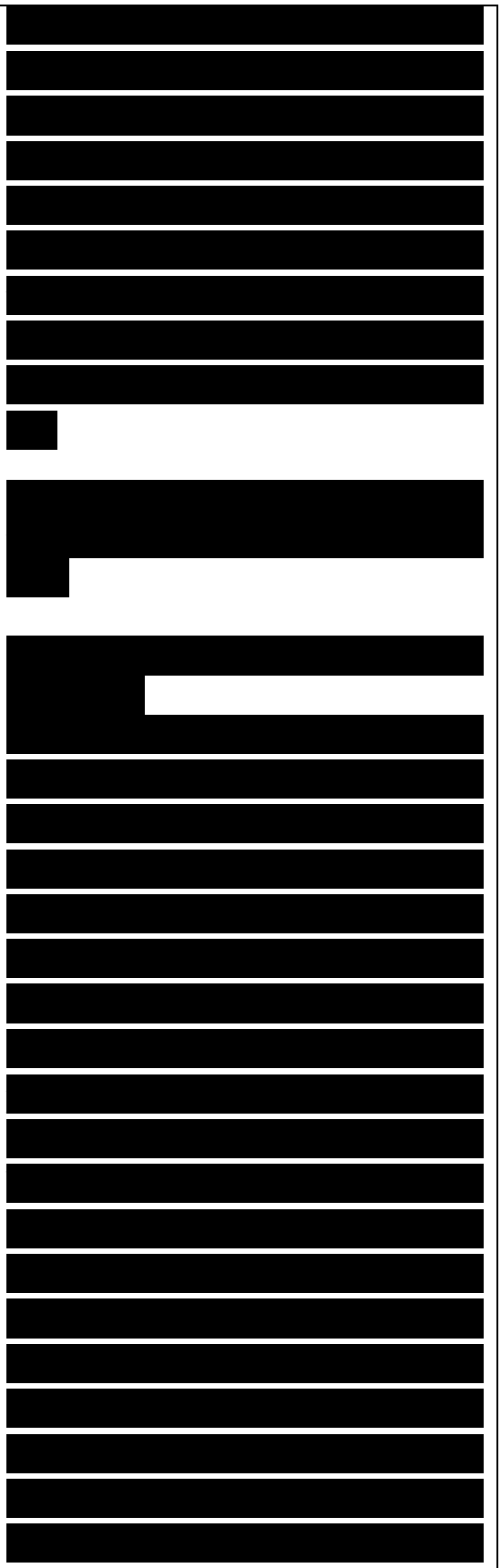
Inorganic materials have been largely used to give stability to MNPs but mostly to produce multifunctional materials. Gold has been used for example to produce multifunctional (with optical and magnetic properties) and biocompatible materials such as hybrid nanorings, spherical MNPs decorated with Au NPs on the surface and core-shell structures.85-87 Silica is another favorable surface coating or host material for the inclusion of MNPs because of its biological compatibility and optical transparency which makes possible to introduce or conjugate to the same silica matrix molecules or particles with optical properties such as dyes.88-90 Gadolinium is a promising coating agent for MNPs because it is a popular positive contrast agent for MRI. In particular, the growth of a silica shell can be exploited to functionalize MNPs with gadolinium giving rise to materials combining T1 positive and T2 negative contrasting efficiency.91,92	Việc sử dụng rộng rãi các chất vô cơ không chỉ để tạo độ ổn định cho các MNP mà mục đích chủ yếu là tạo các vật liệu đa năng. Ví dụ, người ta đã sử dụng vàng để tạo ra những vật liệu đa năng (có cả tính chất quang và từ) và tương thích sinh học chẳng hạn như các vòng nano lai hóa, các MNP hình cầu được gắn các hạt nano vàng trên bề mặt và các cấu trúc lõi-vỏ. 85-87 Silic điôxit là một dạng lớp phủ bề mặt được ưu chuộng khác hoặc vật liệu chủ để pha tạp các MNP do sự tương thích sinh học và tính trong suốt quang học tạo điều kiện thuận lợi để đưa vào hoặc liên hợp với các phân tử và hạt của nền Silic điôxit đó những tính chất quang học chẳng hạn như nhuộm.88-90 Gadolini là một tác nhân phủ bề mặt nhiều tiềm năng cho các MNP bởi vì nó là chất cản quang dương phổ biến trong MRI. Đặc biệt, người ta đã phát triển một lớp vỏ silic điôxit để chức hóa các MNP cùng với gadolini cho ra những vật liệu kết hợp hiệu ứng cản quang dương T1 và âm T2 .91, 92
3.3. Strategies for bioconjugation	3.3. Các phương pháp liên hợp sinh học (bioconjugation) Bioconjugation: liên kết cộng hóa

	trị giữa các phân tử sinh học, ở đây tạm dịch là liên hợp sinh học Strategy: có thể dịch là chiến lược
There is a wide variety of procedures for the adequate bio- functionalization of MNPs which will yield to biocomposites with the appropriate features for biomedical applications. This paragraph deals with the strategies commonly followed to bind or encapsulate biomolecules to MNPs. The main issue here is how to build up such biomagnetic structures by keeping the activity and properties of their constituents. In this context, functionalization of MNPs with monoclonal antibodies (mAbs) represents an interesting example. These Y-shaped proteins used by the immune system to indentify foreign objects have their most reactive amine groups in the antigen binding site (Fab domain). One of the most popular strategies involves the covalent attachment of the mAb through their most reactive amine groups, but it can lead to random orientation of the mAb resulting in a loss of recognition activity.93,94 Several strategies have been proposed to avoid random	Có một loạt quy trình thích hợp để chức hóa sinh học các MNP cho ra các composite sinh học nhằm phục vụ các ứng dụng y sinh. Phần này đề cập đến các phương pháp phổ biến để liên kết hoặc đóng gói các phân tử sinh học vào các MNP. Ở đây chúng tôi sẽ trình bày phương pháp phát triển các cấu trúc từ sinh học như thế nhưng vẫn giữ lại hoạt tính và các tính chất của các thành phần của chúng. Trong bối cảnh này, chúng ta hãy xét một ví dụ lý thú, đó là việc chức hóa các MNP với các kháng thể đơn dòng (các mAb). Hệ miễn dịch sử dụng các protein hình chữ Y này để nhận diện các đối tượng ngoại lai, các protein này có các nhóm amin hoạt động mạnh nhất ở vị trí gắn kháng nguyên (miền Fab). Một trong những chiến lược phổ biến nhất là gắn cộng hóa trị mAbs thông qua các nhóm amin hoạt động mạnh nhất của chúng, nhưng điều này có thể dẫn đến sự định hướng ngẫu nhiên của mAb làm suy giảm hoạt tính nhận diện.93, 94 Người ta đã đề xuất một số phương pháp để tránh sự định

orientation. Mainly they imply the mAb modification through several steps of purification or specific immobilizing proteins.95,96 Recently, another approach has been published that takes advantage of unspecific reversible interactions between the mAb and the MNP in order to orient the mAb on the magnetic surface before being attached covalently in an irreversible way.97,98	hướng ngẫu nhiên. Những phương pháp này chủ yếu thay đổi mAbs qua một vài bước tinh chế hoặc cố định các protein một cách chọn lọc.95,96 Gần đây, người ta đã đưa ra phương pháp khác tận dụng các tương tác thuận nghịch không đặc hiệu giữa mAb và MNP để định hướng mAb trên bề mặt từ trước khi được gắn cộng hóa trị theo kiểu không thuận nghịch.97 , 98
Aptamers are nucleic acid ligands that bind to a specific target molecule. Their synthetic design is a common biochemical practice even though natural aptamers exist. They consist of DNA, RNA or short peptides. Their specific aptamer-protein interaction makes them ideal candidates to produce recognition and specific uptake of aptamer labelled MNPs by target cells. There are several strategies to attach aptamers to the magnetic surface. MNPs can be functionalized with aptamers by ethyl(dimethylaminopropyl) carbodiimide/ N-hydroxysuccinimide (EDC/NHS) chemistry if the MNPs have been previously coated with a molecule that provides carboxy groups to the	

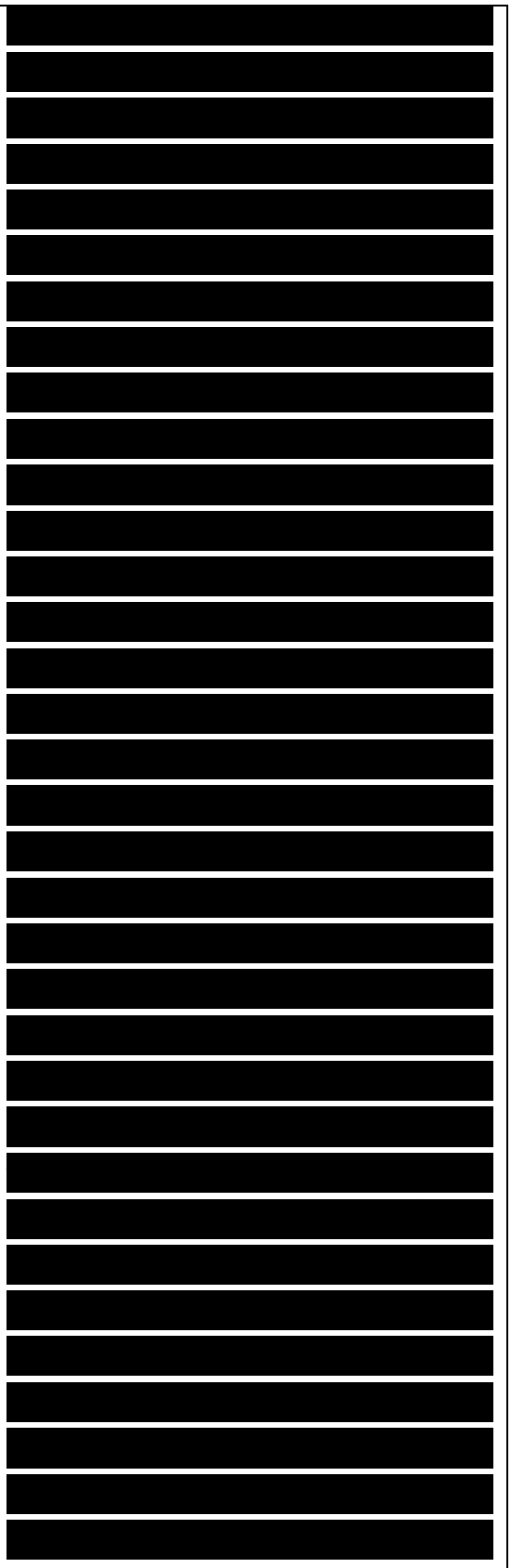
magnetic surface (e.g. PEGylation).99,100 Streptavidin coated MNPs can also be conjugated to aptamers that have been labelled with biotin101 and MNPs coated with Au NPs or Au shells can be functionalized with thiolated aptamers directly by mixing both constituents.102,103

Another example of magnetic biocomposites is the adenoviral vector tagged with MNPs. This hybrid system composed of MNPs and an adenovirus (Ad) has applications in simultaneous MRI and gene delivery. Such nanostructure can be fabricated for example by self-assembly of: (i) MNPs coated with a fluorinated surfactant combined with branched poly-ethylenimine (positively charged) and the Ad,104 (ii) biotiny- lated adenovirus and streptavidin conjugated MNPs,105 (iii) MNPs coated with N-hexanoyl chitosan (positively charged) and the Ad,106 (iv) organic matrix of



MNPs coated with Ad binding proteins and the adenovirus107 and (v) Au NPs decorated MNPs with the Ad (Au surface bind to cysteine and methimine residues of Ad surface proteins).108 It has been also possible to bind MNPs to an adenovirus by cross-linking of maleimide-modified adenovirus and thiol-functionalized $MnFe_2O_4$ MNPs.109 The main body of an adenovirus has around 90 nm of diameter, thus the number of MNPs per virus will vary depending on the MNP size from thousands to tens or less.104,109	[redacted]
MNPs are regarded as nanocarriers that may enhance the bioactivities of some drugs by delivering them directly into the area of the body where they have to act. Even if drugs are not considered as biomolecules, we include their conjugation with MNPs here, because they require the production of final biocompatible MNPs. In this paragraph we will focus only on two different anticancer drugs, paclitaxel (PTX) and doxorubicin (DOX). There are several problems associated	[redacted]

with their use as effective anticancer drugs: (i) low solubility in aqueous solutions, (ii) low bioavailability for selectively targeting cancer cells, and (iii) lack of an efficient method for their detection and tracking. In theory, these drawbacks could be solved by including the drugs in an appropriate carrier such as magnetic biocomposites. PTX is a mitotic inhibitor used for the treatment of breast, ovarian, lung, prostate, melanoma, as well as other type of solid tumors. It is administered by injection and it is an irritant, thus it can cause inflammation of the veins and tissue damage. Therefore, drug loading into a matrix is especially convenient. Core-shell structures (where the core is magnetic and the shell is a polymer) and biodegradable polymeric matrix containing both the MNPs and PTX have been proposed as carrier systems.110,111 PTX can be also bound on the surface of the MNP.112 For instance, Hua et al. have produced MNPs covalently labelled with PTX by modification of polyaniline. This hydrophilic polymer modified with succinic anhydride which



forms water-soluble self-doped poly[aniline-cosodium N-(1-one-butyric acid) aniline] was used to functionalize the MNPs previous to covalent immobilization of PTX on the surface.113 DOX is an anthracycline antibiotic, a family of drugs that works by intercalating DNA which includes among the most effective anticancer treatments. It has also side effects. DOX is a vesicant that can cause extensive tissue damage and blistering if it escapes from the vein. Most of the strategies to encapsulate DOXs are also based on the formation of a coating layer of amphiphilic polymer and the loading of this layer with the hydrophobic drug via hydrophobic interactions or covalent bonds. However, the composition of the magnetic nanostructure is generally selected based on the selected release mechanism (e.g. pH triggered release, enzymatic degradation, and thermic effects). As an example, pH-triggered DOX-releasing MNPs can be based on a change of affinity between DOX and the coating agent of the MNP upon a pH change (e.g. pyrene based polymers and DOX can bind to each other by p-p interactions at	[redacted]

neutral pH, but protonation of DOX under intracellular acidic conditions can cause its sudden release by decreasing this p-p interaction)114 or in a change of conformation of the polymer matrix upon pH variation (from swollen to shrunk state upon pH decreasing) (Fig. 4).115,116	
Many interesting applications of bioconjugated MNPs require the presence of DNA on the surface of the MNP. DNA is negatively charged and can be coated with small positively charged MNPs via electrostatic interactions by keeping its biological activity.117 In another approach, magnetic silica particles have demonstrated to attach successfully oligonucleotides upon functionalization of their surface with amino or thiol groups.89 Tat peptide coatings induce intracellular accumulation of MNPs, they can be attached on the magnetic surface via disulfide linkage.75 This biomolecule belongs to the family of cell-	

penetrating peptides and facilate the cellular uptake. MNPs can be coated with enzymes but also be used to detect them.118,119 In summary, there is a large list of strategies commonly used for the bioconjugation of MNPs that depends on the specific application of the magnetic biocomposite. Parameters such as stability, activity and orientation of the biomolecules within the magnetic biocomposite are key aspects in the fabrication of efficient systems useful in biological applications demanding high control of the particle-biomolecule interactions in a molecular level. Table 3 shows some of the biomolecules and the coating agents mentioned in this manuscript together with their features and applications.

4. Cellular and in vivo toxicity

As it has been mentioned, within the big family of different MNPs, some dextran-coated formulations have been already FDA- and EMA-approved as MRI contrast agents. In the future, the use of new types of MNPs in clinical trials is expected, but the factors that make MNPs

suitable for medical applications are not yet well-known. Some recent findings, such as the intracellular degradability of MNPs120 or the close correlation between the cellular localization and concentration of MNPs and their cytotoxic effect,121,122 have provided new insights in understanding the effect of MNPs in vitro. However studies such as the toxic effects of inhalation exposure to ferric oxide123 or the long term in vivo biotransformation of MNPs124 are of crucial interest for the expansion of diagnosis assays and therapies based on MNPs. Regardless of the intrinsic differences between the various MNPs, the size-factor itself appears to cause several adverse effects. As the superparamagnetic MNPs are in the same size of natural proteins, these MNPs can reach places where larger MNPs cannot enter. Furthermore, the confinement of MNPs in subcellular structures such as endosomes can lead to very high local concentrations which cannot be achieved by free ions. The shape of the MNP has also been demonstrated to influence the uptake of MNP by living cells.125 Thus, size, shape and physico-chemical

properties dictated by the coating agent of MNPs greatly determine the extent of cellular interactions.

4.1. Cytotoxicity end-points

MNPs such as nickel ferrites have shown potential toxicity affecting cell proliferation and viability.126’127 In contrast, some of the iron oxide MNPs are biocompatible when coated with specific surface modifiers.128 In both cases, there is a lack of information concerning the molecular mechanisms of toxicity. In this paragraph some of the most reported and discussed mechanisms which affect cell homeostasis will be discussed although they are still all a matter of debate.

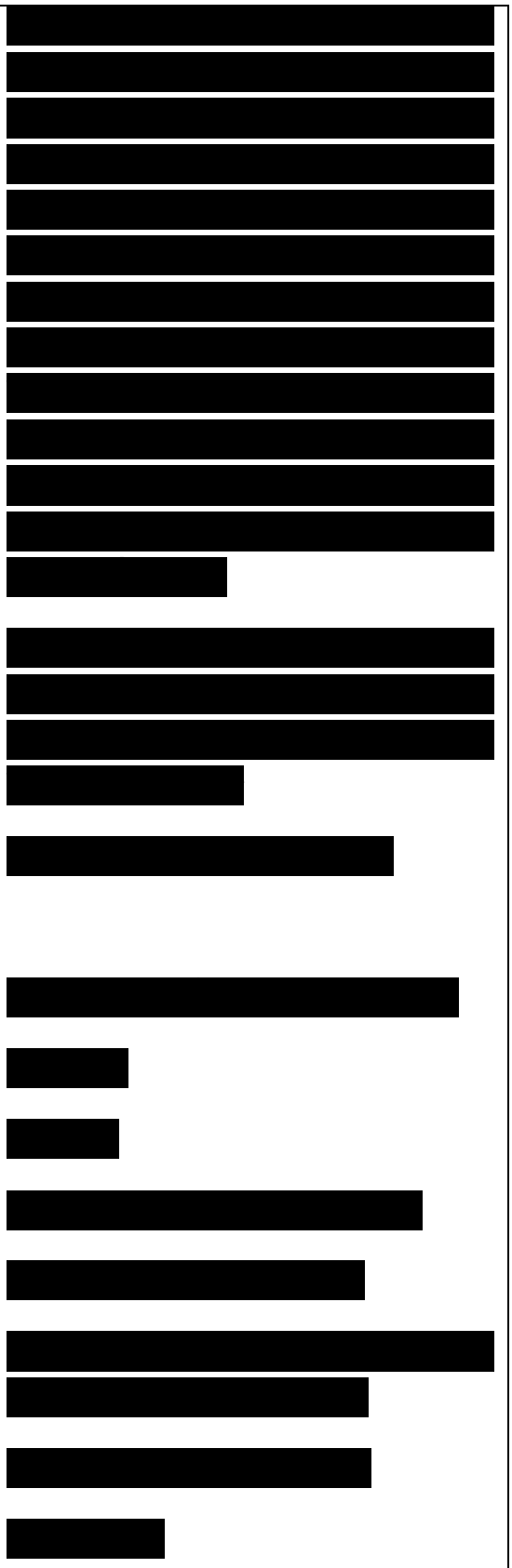
One of the frequent concerns related with MNP cell uptake is the generation of reactive oxygen species (ROS). These species can initially serve as a defense mechanism against invading foreign species or, alternatively, they can lead to the induction of apoptosis. Its

riskiness is cell type-dependent, but most of cells have defense mechanisms that buffer a certain amount of ROS making possible only transient high levels of these species.129 For MNPs, the induction of ROS is typically a transient effect that highly depends on the stability of the coating agent, in its nature (if it produces ROS or not), and in the concentration of MNPs that have been internalized by cells.130-133 In the case of nickel ferrite MNPs several studies have reported the induced toxic response in cells through ROS generation and recently its dependence on the concentration of MNPs has been pointed out.134 As transient higher ROS levels can sometimes be observed without any clear cytotoxic effects,135 the overall impact of elevated ROS levels associated with the presence of MNPs remains unclear. Due to the physical dimensions of MNPs, their intracellular accumulation can also affect the structure of the cellular cytoskeleton network.136 The interaction	[redacted]

between MNPs and the cytoskeleton can be direct if the MNPs have been internalized in the cytoplasm or indirect if the MNPs are localized in endosomes. MNPs with a wire shape125 or functionalized with special coating agents can be found in the cytosol, but most studies report on the typical endosomal localization. In this context, it is generally accepted that the coating of MNPs favors different types of cytoskeleton disorganization and the

Table 3 Examples of different coating agents and biomolecules used in the fabrication of magnetic biocomposites

Biomolecule/coating agent	
Adenoviral vectors	Antibodies
Aptamers	
Dextran	
DNA or RNA	Doxorubicin
Enzymes or proteins	
Folic acid	Nitrilotriacetic acid
Oligonucleotides	Paclitaxel
Polyethylene glycol	Silica
Tat peptide	



engulfed MNPs concentration influences the degree of disorganization.137 As the cytoskeleton is involved in many intracellular signaling pathways, it remains to be investigated whether the MNP-induced cytoskeletal disruption leads to secondary effects such as cell death, diminished proliferation or other mechanisms.	[redacted]
The complex intracellular signaling pathways can be altered not only due to cytoskeleton changes but through several mechanisms, such as: (1) genotoxic effects caused by high levels of ROS,138 (2) altered protein or gene expression due to the perinuclear localization of the MNPs which may hinder the functioning of the transcription and translation machinery,139 (3) altered protein or gene expression levels due to leaching of free metal ions,131 (4) altered activation status of proteins by interfering with stimulating factors such as cell-surface receptors140 or (5) altered gene expression levels in response to the cellular stress that the MNPs	[redacted]

induce.141 To date, the effect of MNPs on protein or gene expression levels has only scarcely been investigated and more data need to be generated in order to get a better idea to what extent MNPs can cause alterations to intracellular signaling pathways.

The biodegradation of MNPs is the responsible mechanism for the generation of free ferric iron and further complete dissolution of the magnetic core. The different dissolution kinetics of MNPs has been observed to depend on the surface coating.142 Free ferric iron was found in some cases to induce high levels of ROS, apoptosis or inflammation and to alter the transferrin receptor.143 Another possible source of toxicity is the interaction of MNPs with biological molecules. Due to the charge of MNPs serum proteins are prone to bind the magnetic surface unless a protective MNP coating inhibits this process.144,145 Finally, the application of MNPs in hyperthermia or drug delivery brings new issues to be taken into account. Hyperthermia requires the application of an AMF that is

used to kill tumor cells,146 but without full control of this technique non-tumoral cells can be also damaged. Magnetically guided drug delivery or MRI employs a constant magnetic field gradient, thus no direct effect on cells are expected. However, the increased internalization of MNPs can induce toxic effects by exceeding the local toxic threshold of the MNPs147 or by affecting the relative localization of the endosomes inside the cells and therefore changing their normal intracellular routing and maturation.148

4.2. Potential in vivo toxicity

There are many MNPs manufactured for application as MRI contrast agents such as Feridex, Resovist, Endorem, Lumirem, Sirenem, etc.149 but some of them have been currently removed from the market.150 They are all based in magnetite composites and most of them are coated with dextran or carboxy dextran. The number of in vivo studies performed in humans so far is limited but in continuous

growth151-153 and is expected to bring more information about the potential toxic effects of MNPs. It is known that in the case of Feridex intravenous (i.v.) administration may cause severe back, groin, leg or other pain, or allergic reactions. Ferumoxtran-10 for example is also inducing side effects such as urticaria or nausea, all of which are mild and short in duration.154,155 It is thought that these mild side effects are due to the degradation and clearance of MNPs from circulation by the endogenous iron metabolic pathways. The clearance mechanisms in humans will be discussed in Section 6.6.

Long term studies in animals have not been yet been performed for most of the commercially available contrast agents based on MNPs. Therapeutic iron dextran products have been associated with the development of sarcomas at the intramuscular injection sites; the length of treatment or the length of time after injection until development of tumor is not known. The MNPs used as contrast agents with patients are iron oxides associated with dextran. Whether these MNPs have a

risk of tumorigenesis that is similar to that of iron dextran is not known. Therefore studies that deal with the long term influence of MNPs in the organism are highly required. In this context, Levy et al. have recently studied the long term in vivo biotransformation of iron oxide MNPs.124 A three- month magnetic follow-up of MNPs gave evidence of the degradation and loss of their superparamagnetic properties. They observed as well the relocation of iron species from liver to spleen in the organism.

5. Molecular detection with magnetic nanoparticles

In the language of nature, biological entities exploit high- affinity specific interactions between molecular pairs to achieve reciprocal recognition and trigger signaling processes. If one of the biomolecular entities is immobilized on MNPs, the resulting magnetic nanoconjugates can specifically bind to the biomolecular counterpart. There are two immediate consequences of such an effect. The first is that it is possible to control the localization of selected biological targets by applying

an external magnetic field gradient and, under certain conditions, isolate them. A second complementary application exploits the unique superparamagnetic character of these MNPs to interact with an external magnetic field inducing dephasing of spin-spin relaxation times (T2) of the surrounding water protons of the solvent, in which they are immersed. In this case, the extent of magnetic interference is dependent on the size of MNP assembly, which is in turn caused by molecular recognition events. Based on these concepts, several applications using biofunctionalized MNPs, including protein and DNA separation, molecular biosensing and pathogen detection and sequestration, have been explored.

5.1. Protein and DNA separation

Isolation, purification and controlled manipulation of peptides and proteins represent a need of paramount importance in biotechnology and in life sciences. Conventional protocols may involve electrophoresis, ultrafiltration, precipitation and chromatography.156 Among the available methods, affinity chromatography is

often considered the choice of election in terms of efficiency and selectivity. However, the use of liquid chromatography is limited to pre-treated solutions. Inhomo- geneous matter such as protein production mixtures are incompatible with the particulate-free conditions required for a correct usage of commercial columns. Magnetic separation exploiting MNPs represents an attractive alternative method for the selective and reliable capture of specific proteins, DNA and entire cells, as it makes use of cheap materials and does not necessitate time-consuming sample preparation.157-159 The basic principle of magnetic separation is very simple (Fig. 5). MNPs bearing an immobilized affinity tag, or ion-exchange groups, or hydrophobic ligands, are mixed with the mixture containing the desired molecules. Samples may be crude cell lysates, whole blood, plasma, urine, or any biological fluid or fermentation broth. After a suitable incubation time, in which the affinity species are allowed to tightly bind to the ligands anchored to the MNPs, the complexes are isolated by magnetic decantation and the

contaminants washed out. Finally, the purified target molecules are recovered by displacement from the MNPs by proper elution procedures. At present, the most thoroughly investigated affinity tag-based approach for magnetic separation of proteins makes use of MNPs functionalized with ligands bearing Ni2+-chelating species, such as nitrilotriacetic acid (NTA), which allows for the selective sequestration of (6 x His)-tagged proteins with highly conserved folding down to picomolar concentrations.160,161 His-tagged proteins cover the surface of MNPs selectively and quickly, reducing nonspecific adsorption of undesired entities, which represents a major drawback of commercial microbeads.162 As it is likely that the multivalent action of the chelating agent plays an important role in enhancing the binding selectivity of His-tagged proteins at low concentration, the choice of the anchoring strategy as well as the chelating ligand might significantly affect the binding capacity and reusability of biofunctional MNPs without loosing efficiency.163,164 New advances made in the	[redacted] Lysate: dịch thủy phân, hợp chất giun giải tế bào Hiện nay, các phương pháp dựa trên tag ái lực được nghiên cứu kỹ lưỡng nhất để tách từ các protein sử dụng các MNP được chức hóa với các phối tử mang gốc Ni 2 +-chelating, như acid nitrilotriacetic (NTA), cho phép việc cô lập chọn lọc các protein có gắn (6 x His) với nồng độ bảo tồn cao đến bậc pico mol.160 , 161 Protein gắn His bao phủ bề mặt của các MNP có chọn lọc và nhanh chóng, làm giảm hấp phụ không đặc hiệu các đối tượng không mong muốn, thể hiện một nhược điểm lớn của các microbead thương mại.162 Vì rất có thể tác động đa hóa trị của các chất tạo càng đóng một vai trò quan trọng trong việc tăng cường tính liên kết có chọn lọc của protein gắn His ở nồng độ thấp, việc chọn phương pháp gắn cũng như phối tử tạo càng có thể ảnh hưởng đáng kể đến khả năng liên kết và khả năng dùng lại của các MNP chức sinh học mà không mất mát hiệu suất. 163.164 Những tiến bộ mới trong tách / chiết phân tử sinh học bằng các MNP cho thấy rằng công nghệ này sẽ phổ biến và

separation/extraction of biomolecules by MNPs suggest that this technology could be general and versatile. Similar approaches can be envisaged for alternative affinity tags, which selectively bind with different biological targets if proper anchors and ligands are used. For example, it is possible to utilize MNPs functionalized with specific peptides, including protein A or G, having strong affinities for the Fc portion of human IgG Abs to achieve a tight and reversible capture useful for Ab sorting.165,166	linh hoạt. Chúng ta cũng có thể sử dụng cách này cho các thẻ ái lực khác, chúng liên kết có chọn lọc với các mục tiêu sinh học khác nhau nếu sử dụng neo hoặc phối tử thích hợp. Ví dụ, chúng ta có thể sử dụng các MNP được chức hóa với peptide chuyên biệt, bao gồm protein A hoặc G, có ái lực mạnh đối với phần Fc của IgG Abs người để bắt chắc chắn và có thể đảo ngược phục vụ cho việc phân loại Ab .165, 166

Protein separation with MNPs is advantageous compared with conventional affinity chromatography for several reasons. The purification process is simple, rapid, cheap and scalable.

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Small amounts of materials are necessary for the separation process thanks to the high surface-to-volume ratio of nanosized sequestrants. Moreover, magnetic separation does not require dedicated equipment, such as centrifuges, filters or liquid chromatography systems, and no sample concentration is needed after elution. It is worth mentioning that automated systems for the separation of proteins or nucleic acids are now available.157 DNA or RNA can be isolated and concentrated using selected oligonucleotides grafted on MNPs, which allow the capture of complementary strands.167 When using labelled primers in a polymerase chain reaction (PCR), the amplicons can be isolated, concentrated, and even used in an automated assay for sequencing. Nucleic acids deriving from bacterial and mammalian cells have been captured and purified by magnetic beads using a microfluidic chip in nanolitre volumes of untreated samples. This method allowed the automated extraction of amounts of mRNA from a single mammalian cell.168 One of the problems often encountered by researchers when using cationic

extractors, including amine-functionalized MNPs, to purify nucleic acids resides in the low release of captured genetic materials. Indeed, the efficiency is generally modest using phosphate buffer as a competitor and even much lower with other ions. Tanaka et al. found that using deoxy-nucleotide triphosphates in place of phosphate buffer can increase significantly the desorption efficiency thus improving the chances of successful PCR analyses.169

5.2. Biosensing with magnetic nanoswitches

The unique optical, electronic, and magnetic properties of several metal and metal oxide NPs functionalized with affinity ligands combined with agglomerative phenomena induced by specific interactions occurring at their surface has led to the development of highly sensitive NP-based biosensors. In particular, gold NPs and semiconductor NPs (so-called quantum dots) have been largely exploited for colorimetric and fluorescence- based detection of oligonucleotides, proteases, Abs and other molecular species.170-176 The major disadvantage in biosensing assays based on optical responses resides in the necessity of reducing the sample

turbidity or background signals from the biological extracts. A novel class of nanosensors has thus been developed by exploiting the peculiar magnetic properties of MNPs. Magnetic relaxation nanoswitches have been first proposed by the group of Weissleder in a series of seminal works, which demonstrated the efficacy of this new nano-biosensor for the accurate and sensitive detection of a broad range of biological species, including DNA, proteins, pathogens, and processes such as enzymatic function.177-182 These magnetic relaxation switches consisted of 3-5 nm iron oxide MNPs coated with a 10 nm thick dextran layer that is stabilized by crosslinking (CLIOs) and functionalized with amino groups, useful to covalently anchor the affinity ligands.183 Such nanoswitches are able to undergo reversible assembly in the presence of a specific molecule that is selectively recognized by the affinity ligands immobilized on the MNPs, resulting in a change in transverse magnetic relaxivity (R2 = 1/T2) of water protons adjacent to the floating nanodipole. According to the outer-sphere diffusion theory, when clusters of MNPs are sufficiently small, e.g., within a few hundred nanometres, the

Fig. 6 Schematic drawing showing the principle ofsuperparamagnetic nanosensors also called ‘‘magnetic relaxation switches’’. The scheme illustrates the detectable effect on T2 caused by MNP aggregation sequence in response to the presence of target analyte (blue cross) and its removal by adding a competing inhibitor (red square).

effect of assembly is to reduce the average T2 value. In contrast, when large agglomerates are formed (with size range of several micrometres), T2 is instead increased compared with that of individual MNPs dispersed in the same fluid or matrix.184,185 Both strategies have been demonstrated useful depending on the experimental requirements,186 magnetic relaxation nanosensor assays being designed to form reversible nanoassemblies upon MNP interaction with specific analytes in solution both in a forward (clustering) or reverse (declustering) setup (Fig. 6).

By this approach the concentration of analytes, such as glucose and calcium in solution has been quantitatively determined.187,188 In particular, a combination of CLIO-glucose and concavalin-A enabled the measurement of glucose concentration across a semi-permeable membrane over the clinically

meaningful range, suggesting a future potential clinical utilization in an implantable biosensor. In principle, the same concept could be applied to simultaneous analysis of multiple metabolites in a continuous and noninvasive way by MRI in vivo.189	[redacted]
By using functional MNPs endowed with higher inherent magnetic susceptibilities, several other pathological biomarkers have been detected with similar experimental setups, including autoantibodies, toxins and human plasma glyco- In a conceptual evolution of this approach, sets of primary and metastatic cancer cells could be detected, characterized and distinguished from normal cells using a library of magnetic glyco-NPs.192	[redacted]
Recently, a chip-based diagnostic magnetic resonance (DMR) system for multiplexed, quantitative and rapid analysis of unprocessed biological samples has been developed.193 The source of the signal is the molecular interaction amplification caused by assemblies of MNPs with enhanced magnetisation.181 The potential of this device has been demonstrated by monitoring the presence and amount of proteins in parallel and by detecting bacteria and analyzing them at a molecular level with high sensitivity. Miniaturized DMR has several	[redacted]

advantages over the reported conventional bulk experiments: (1) only microlitre volumes of the sample are required, with a remarkable increase in detection sensitivity; (2) multiplexed measurements can be easily performed enabling a rapid screening of analytes even in opaque biological media provided that they are transparent to magnetic fields; (3) the magnetic field is generated using a small, portable magnet with more homogeneous radio-frequency magnetic fields and less electrical resistance compared with conventional relaxometers; (4) DMR microsystem can be produced as disposable units. All these features suggest a potential of DMR technology as a friendly handheld diagnostic device.

5.3. Bacteria detection and sequestration by magnetic capture

Magnetic separation, and particularly immunomagnetic separation using MNPs coated with Abs against surface antigens of cells, has been exploited for separation of eukaryotic cells.194 Recently, the same approach has been used for the detection and isolation of bacteria from biological samples (Fig. 7). Once the microorganism has been selectively captured and concentrated, the identification can be accomplished

by conventional methods.195-197 In a pioneering study in 1988, Lund et al. isolated the K88 (F4) fimbrial antigen responsible for colonization of piglet intestines using magnetic beads functionalized with a specific mAb and examined the extracts

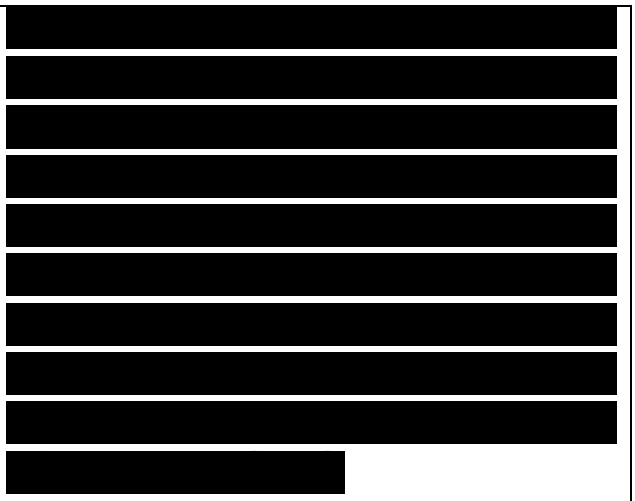


Fig. 7 Schematic drawing showing the principle of cellular detection and sequestration by tagged MNPs.

by fluorescence microscopy. Magnetic beads coated with anti-K88 mAb were also used by Hornes et al. for immuno-magnetic separation (IMS) of enterotoxigenic E. coli strains from pigs with diarrhea.199

In general, bacteria at low concentrations are hard to detect with a conventional analytical method in complex biological mixtures. However, it is expected that nanotechnology will improve sensitivity, selectivity and analytical time-efficiency in clinical diagnosis and environmental monitoring. Gu et al. developed a vancomycin-conjugated FePt MNP system (FePt@Van) to capture and detect Gram-positive bacteria at ultralow concentrations.200 Polyvalent vancomycin tightly binds to the D-Ala-D-Ala dipeptide, which is a major constituent of the microbial capsule,

enabling magnetic capture and enrichment of bacteria. The declared detection limit of this method was 4 colony-forming units (cfu) per mL, on the same level of the best assays based on polymerase chain reaction. FePt@Van MNPs were also used to isolate and detect Gram-negative bacteria such as E. coli.201 Combining FePt@Van with fluorescent dyes allowed for the detection of bacteria in blood samples.202	[redacted]
El-Boubbou et al. developed silica-coated magnetic glyco-NPs that could detect E. coli strains in 5 minutes, concomitantly enabling up to 88% removal from the sample exploiting the bacterial interaction with mammalian cell surface carbohy- drates.203 MNPs functionalized with a single-domain Ab proved to be highly efficient and selective in targeting and capturing Staphylococcus aureus cells in a mixed cell population.204 The authors suggest that the superior specificity obtained with these nanocomplexes can be ascribed to the unique targeting selectivity of multimerized VH Ab small domains. In another study, a surrogate of Mycobacterium tuberculosis was detected in native biological samples, such as sputum, in less than 30 min analysis with a sensitivity of 20 cfu.182 The authors used MNPs with high inherent susceptibility to target the pathogens in	[redacted]

a selective manner. The detection signal was amplified by concentrating the specimen in a microfluidic chamber and measured by a miniaturized NMR system. More recently, magnetite- and cobalt- based MNPs functionalized with poly(hexamethylene biguanide) were demonstrated to be able to bind tightly to lipid A, a glyco- lipid constituent of endotoxins, and DNA from E. coli, allowing for the sequestration of bacterial strains and inhibiting the cell growth and viability at concentration levels below 10 mg ml-1.205

6. Contrast agents for magnetic resonance

In vivo molecular imaging has been identified by the National Cancer Institute of the United States of America as an extraordinary opportunity for studying diseases non-invasively at the molecular level.206 The aim is to visualize molecular characteristics of physiological or pathological processes in living organisms before they manifest in the form of anatomic changes without invasive procedures. During the past, worldwide many working groups showed the feasibility to image molecules in vivo by different scanning modalities. MRI offers several advantages such as lack of irradiation,

possibility to generate 3D images, excellent spatial resolution with optimal contrast within soft tissues, and a very good signal-to-noise ratio. In this paragraph, we present the current advances in the development of new generation contrast agents for MRI and their applications.

6.1. Molecular imaging with targeted contrast agents

Paramagnetic (e.g. gadolinium (Gd)-, europium (Eu)-, neodynium (Nd)-, and manganese (Mn)-containing materials) and super- paramagnetic (iron oxide in the form of maghemite (g-Fe2O3) and/or magnetite (Fe3O4)) compounds can be used as MRI contrast materials.14 With respect to molecular imaging, iron oxide based MNPs have been favored because iron oxide induces a stronger contrast. Moreover, Gd-contrast materials may induce severe adverse effects with lethal outcome that have been observed in patients with compromised renal function and subsequent deposition in different organs/tissues and release of Gd3+ which is called nephrogenic systemic fibrosis (NSF).

The first and major prerequisite of targeted contrast agents is the identification of cells and/or disease and/or function- specific biomarkers. Ideally, the biomarkers should be solely and abundantly expressed on the desired cell types. Further¬more, disease-

specific biomarkers should be clearly different from healthy status. Biomarkers for targeted contrast agents are cell surface receptors (i.e. transferrin receptor, folate receptor, avb3 integrin, Her2/neu), phospholipids of the outer leaflet of the cell membrane (i.e. phosphatidylserine (PS)), and enzymes (See Section 6.3).206-214 Targeted MNPs are composed of at least two components: (1) the magnetic iron oxide represents the imaging or sensing component and (2) the attached molecule represents the targeting or affinity component. MNPs without targeting component are engulfed by monocytes/macrophages. Thereby, they can be used to image monocytes/macrophages and their phagocytic capacity in vivo (Fig. 8).

In most cases the used MNPs are completely artificial products, but naturally occurring MNPs such as lipoproteins can be also used.215 Recently, it has been shown that both loading lipoprotein NPs with signaling substances (e.g. MNPs, Au-NPs) and functionalisation of the surface are possible, which transform them into molecular probes for the specific recognition of molecular targets.215 Several studies demonstrated

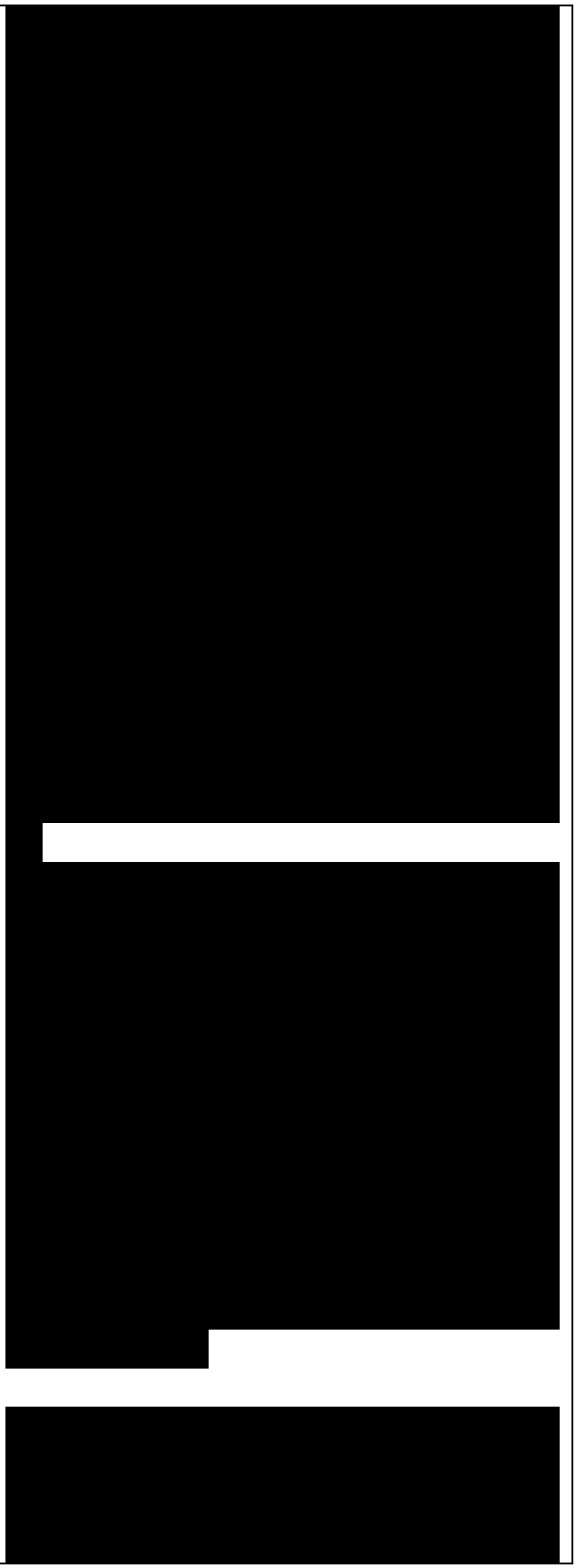


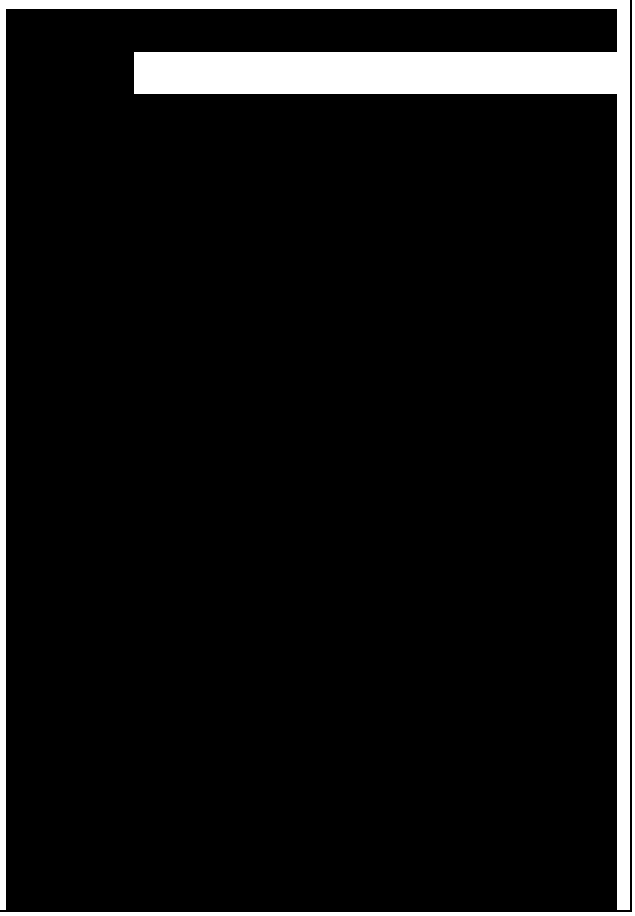
Fig. 8 Target-specific detection of two different breast cancer types (SKBR-3 and KB) by anti-Her2/neu-MNPs (A-D). (A) and (B) are tradional black-white

MRI, and (C) and (D) show color maps. (A) and (C) MRIs show the pre-contrast, and (B) and (D) display the post¬contrast (data taken from Chen et al.).209 Both post-contrast MRI- images show that SKBR-3 breast cancer cells express the Her2/ neu-receptor, while KB cells do not.

the feasibility to specifically image molecules on cells in living organisms. The major drawback of this approach is the need of specific mAbs for each molecule, cell type or cell function. Moreover, the desired molecules are specific species.

6.2. Multimodal magnetic resonance imaging probes

Since different imaging modalities provide complementary information bi- or multimodal imaging probes have been designed.83,216 So far, two different classes of multimodal MRI probes have been synthesized: (1) magneto-optical probes, and (2) magneto-radioactive probes. Most of the multimodal MRI studies deal with MNPs conjugated with organic fluorophores, because they cover several advantages like high anatomical resolution of MRI, and high sensitivity provided by the optical component that is comparable with radioactive tracers but lacks all the disadvantages that are associated with the latter.121 The optical component can be detected by a broad range of in vivo (Fig. 9) and in

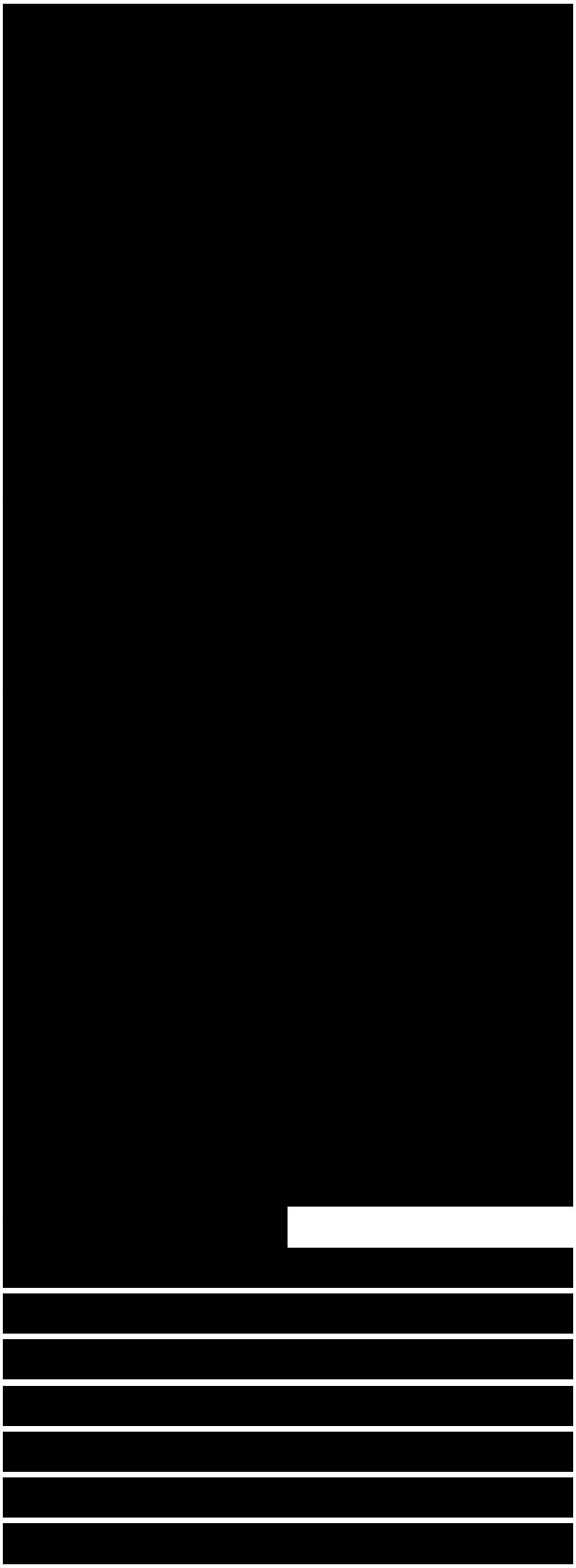


vitro techniques such as optical scanners (e.g. fluorescence mediated tomography, fluorescence reflectance tomography, optical coherence tomography) as well as fluorescence microscopy, laser scanning (confocal) microscopy, flow cytometry, spectro¬photometry, intravital microscopy, intravascular noninvasive near-infrared (NIRF) imaging, clinical endoscopy, and detec¬tion during surgery.217

Since near-infrared (NIR) fluorescence (700-1000 nm) avoids interferences with background fluorescence of mole¬cules of living organisms, and thereby provides an excellent contrast between desired target and background tissues, NIR fluorophores should be favoured for imaging living organisms in real time. Several chemical compounds and materials fulfil the criteria of NIR fluorophores: (1) organic fluorochromes (e.g. fluorescent cyanine dyes such as Cy5.5), (2) fluorescent semiconductor quantum dots (QDs), (3) lanthanides, and (4) gold NPs.103,212,216,218-223 Organic fluorochromes are widely available and can be easily coupled to the shell of MNPs to build up Cy 5.5-MNPs for example. As these compounds suffer from fast photobleaching, QDs have been introduced due to their improved photo-stability. QDs are NPs composed of semiconductors such as CdSe, CdTe, ZnS, or InGaAs212,216,224 having narrow, symmetric emission spectra with long, excited- state lifetimes220 and possessing high extinction coefficients combined with a quantum

yield comparable to fluorescent dyes.220 Like MNPs, QDs can be coated with polymeric materials to allow subsequent functionalization with the above listed molecules.225 Due to their toxic components nowadays their use is still limited to in vitro experiments and animal studies, though QDs from more biocompatible materials are currently being developed.220,226,227 Au NPs are ideal for molecular imaging studies because they reduce cellular toxicity, and have a bright NIR fluorescence emission of 700-900 nm.228-231 Gold MNPs have been used for both in vivo and in vitro applications including in vivo imaging of small animal models and protein purification system using novel peptide tags.216,223 Moreover, sensitive biosensing gold MNPs combining magnetic relaxation switch diagnosis and colorimetric detection of human a-thrombin have been produced to be specifically activated by matrix metalloproteinases expressed in tumors and to be sensitive fluorescent biosensors for detection of DNA hybridation.103,177,232,233

Lanthanides are both paramagnetic and fluorescent substances. Europium(III) oxide (Eu2O3) shows intense red fluorescence after excitation with UV-light. NPs have been synthesized in Co : Nd : Fe2O3/Eu : Gd2O3 core-shell geometry.219 On the other hand, it is possible to produce lanthanide-doped MNPs that contain 5 mol% of Eu exhibiting nearly identical physical superparamagnetic behaviour than the



pure MNPs, and additionally have attractive optical properties combined with high photostability, a narrow emission band, and a broad absorption band for longterm multilabelling studies.221

Although positron emission tomography-magnetic resonance (PET-MR) hybrid scanners are now increasingly established in several radiological clinics and scientific units, routine radiotracers such as fluordeoxyglucose18 have been used unmodified, as conjugation to MNPs has to the best of our knowledge not yet been done. However, recently dual modality tumor imaging MNPs have been described, namely RGD-conjugated radiolabelled MNPs.211

6.3. In vivo MRI of enzyme activity

Enzymes are essential molecular players in many physiological and pathological events, and can be used as biomarkers for different processes such as apoptosis, atherosclerotic plaques, inflammatory reactions, cancer and metastasis, to name a few. Detection of in vivo enzyme activity is achieved either directly by detection of the active form of an enzyme (e.g. by mAbs directed against the active enzyme molecule) or indirectly by detection of enzymatic induced changes of specific

substrate molecules. Currently, enzyme-sensitive MR contrast agents use the latter principle. They are functionalized or made of mAbs with affinity to enzyme modified substrates. Upon enzyme activation the probes are specifically modified, and these induced molecular changes relate to MR signal changes. The mAb-based approach has been demonstrated to image cell surface ADP-ribosyltrans-ferse 2 upon lymphoma cells.232 Protein ADP-ribosylation was successfully determined with R2 and R2 relaxometry on a clinical 3T MR scanner after application of both specific anti-ADP- ribosyl-mAbs and secondary SPIO conjugated mAbs. Smart MR probes, also called nanosensors, for depiction of enzyme activity are mainly Gd-complexes. Only some approaches demonstrate the feasibility of superparamagnetic nanosensors. The principle of enzyme sensitive iron oxide nanosensors is a special design of complex molecules that either agglomerate or disagglomerate upon enzymatic activity which subsequently leads to a corresponding switch in the spin-spin relaxation time (T2) (Fig. 10). They are	[redacted]

called magnetic relaxation switches (MRS, cf. Section 5.2) and they are designed to for example measure enzyme activity of proteases, methylases, and restriction endonucleases.177’178’233’234 A peptide substrate with a protease recognition sequence flanked by two biotin molecules has been designed that bind to CLIO-avidin (CLIO-A) MNPs and forms a superparamagnetic nanosensor.234 In the presence of a specific protease, the peptide linker substrate is cleaved, and the CLIO-A nanoassembly disagglomerates. By using this approach, magnetic nanoassemblies responsive to trypsin, renin, and matrix metalloprotease-2 (MMP2) activity have been developed and tested.234 Another example of this technology is a MNP containing a biotinylated caspase-3- specific peptide substrate that was incubated with a second MNP to form a caspase-3-sensitive magnetic nanoassembly.178 The used peptide substrate is specifically recognized by caspase-3, thus serving as an assay for this enzyme. The caspase-3-mediated

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High Relaxivity

■W

Low Relaxivity

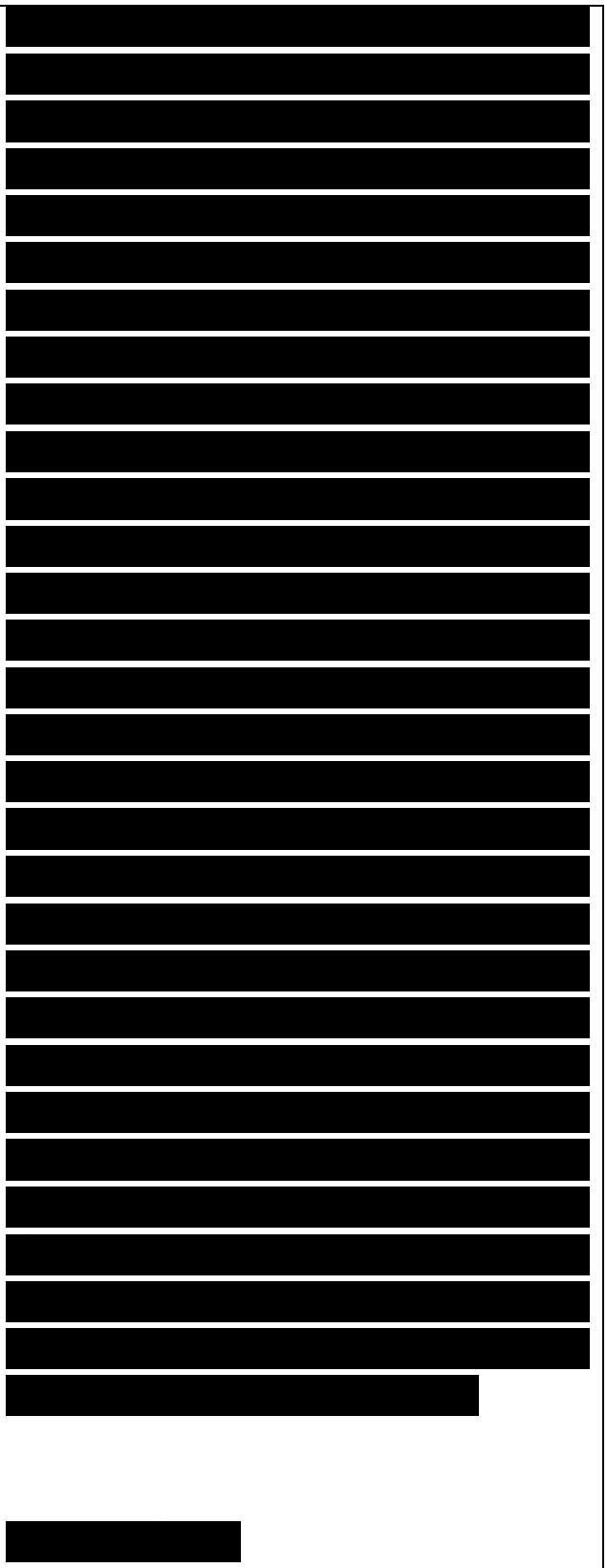

Fig. 10 Principle of detection of enzyme activity in vivo. The enzymatic activity produces changes in the stability of the MNPs which induce changes in the spin-spin relaxation time (T2) and therefore in the relaxivity R2 (= 1/T2) of the protons around the MNPs.

Fig. 11 Principle of preparation steps of DCs/DC-vaccine for MR tracking studies in vivo. Note that the magnetic labelling procedure can be either done before DCs are triggered with antigens or afterwards.

reaction was associated with a dose-dependent increase in the T2 relaxation time with kinetics similar to those reported with fluorogenic substrates. In a similar fashion activity of restriction endonucleases such as BamHI that cleaves doublestranded oligonucleotides linked by two MNPs has been shown to cause a designed nanoassembly to switch to a dispersed state and produce an increase in T2.177 Two MNPs (P1 and P2) were designed that hybridized to each other and form a BamHI recognition site. T2 decreased when P1 and P2 were mixed together since oligonucleotides on these two NPs hybridize and form a BamHI-sensitive nanoassembly. Incubation with BamHI resulted in an increase of T2. Other endonucleases such as EcoRI, HindIII, and DpnI did not influence T2 when

incubated with the BamHI-sensitive nanosensor.

Although the above described MRS systems are very exciting particles their introduction for in vivo MR measurements has not yet been done. The main challenge to image enzyme activity in vivo is the fact that in most cases enzymes act within the cytoplasm or within cellular organelles (e.g. mitochondria, nuclei), and only some types are localized on the surface of cells (ecto-enzymes) or are secreted into the interstitium. Both targeting of the desired cell types and delivery of these complex MNPs into the cytoplasm should be solved in the future to enable measurements of enzyme activity in vivo as well.

6.4. In vivo tracking of labelled dendritic cells

Dendritic cells (DCs) derive from bone marrow hematopoietic cells and can be generated in vitro from either autologous CD34+ progenitors or monocytes.235 DCs present powerful antigens that play important roles in a huge number of immune responses including anti-cancer reactions.236 Cancer vaccines DCs are of special clinical interest because they enhance the antitumor immune responses due to their capacity to process and present tumor associated antigen (TAA), and

subsequently to migrate into secondary lymphoid compartments.235,237,238 In therapeutic approaches DCs are loaded with TAA supplied as whole tumor cell extracts, synthetic peptides, purified whole TAA protein content or by genetic transfection of TAA expressing DNA or, mRNA.235 Pulsing of DC based vaccines with TAA induced their maturation. Afterwards mDCs migrate to secondary lymphoid tissues and present TAA to specific T-cell clones (Fig. 11). This event initializes TAA-specific T-cell responses that might result in tumor cell death.

Although DC-based immunotherapy has been successfully used in several studies to treat skin, breast, prostate, and neuronal cancer for example,235,239-241 some patients do not respond, and only a maximum of 3% of ex vivo generated DCs reach the lymph nodes.242,243 Still a lot of work needs to

Fig. 12 Overview of methods to magnetically label DCs for MR tracking studies in vivo. Upper part (above of the dotted line) shows the different MNPs that have been used to magnetically label DCs, and the lower part (under the dotted line) shows the different delivery

mechanisms to accumulate MNPs within DCs.	[redacted]
be done to optimize DC-based vaccination. Both the exact delivery in vivo and the migration of mDCs to lymph nodes are critical steps that are necessary for a therapeutic success. Unfortunately, established methods to ensure DC migration are invasive.244 Therefore, the possibility to non-invasively monitor DC-cancer vaccines in real time in vivo would be of great scientific and clinical impact. Since cellular MRI offers the possibility to non-invasively visualize in vivo cell delivery and real-time cell tracking, several groups transformed this approach for DC-based immunotherapy. Currently, no stan¬dardised labelling protocols exist: DCs have been labelled with different MNPs (e.g. SPIONs, micro-sized particles of iron oxide called MPIOs, or multifunctional polymer NPs containing ovalbumin protein/IgG, MNPs), and fluorophores (e.g. fluorescein isothiocyanate, indocyanine green) and different loading methods (Fig. 12).245-247 Complex MNPs have been designed to both trigger DCs with antigens and monitor them by MRI and/ or other imaging modalities. MNP accumulation has been achieved by simple cell culture when using phagocytosing iDCs or enhanced by receptor mediated endocytosis via the CD11c- or Fcg-receptor, addition of transfection agents (TAs) such as protamine sulfate, polylysine in concert	[redacted]

with mDCs (Fig. 12).233,247-251

MPIOs have a diameter of at least 1 mm and cover higher iron contents per particle than conventional MNPs.252 This fact improved their detection by MRI.244 But on the other hand MPIOs induced dramatic changes in the phenotype and morphology of DCs, while ultrasmall SPIONs led to remarkably inefficient labelling (0.59 ± 0.02 pg Fe per cell) that was below the detection threshold for cellular MRI.250,253 MNP DC labelling has been shown to efficiently load DCs without affecting cellular morphology and functional maturation with minimal or no effect on viability.233,248-250,254,255
However, a closer insight demonstrated a dose-per-cell-dependent decrease of the viability, and an increase of apoptotic cells especially when higher iron doses of 400 mg ml-1 have been added to cell cultures.244,251,255 The same was true for the velocity: magnetic DCs showed efficient migration that was slightly decreased in parallel to increasing iron doses.251

An iron content of 6 to 78 pg Fe per cell allowed the depiction of DCs in vivo by clinical and small animal MR scanners at magnetic field strength ranging from 1.5 T up to 11.7 t.233,244,248-251,254-256 The number of in vivo detectable magnetically labelled cells ranges from

1.0 x 105 cells up to 1.0 x 106 cells, and 100 cells mm-3 at 3 T or 50 cells mm-3 at 7 T with an iron content of 25 pg Fe per cell, respectively.250,254,256 MR-based DCs tracking in vivo enabled monitoring of the delivery of the vaccine, trafficking of DCs to lymph nodes and other lymphoid tissues (Fig. 13 and 14).233,247,255,256

Most DC-tracking studies by MRI in vivo have been performed in small animals such as mice,233,247,250,255,256 and to the best of our knowledge only few studies have been published that present data obtained with patients.254,257 The reason for this phenomenon is unclear, but there is evidence for the assumption that the labelling protocols are not standardized so far, and the migration of the DCs is limited. For example, iron quantification of magnetically labelled cells is currently performed by atomic absorption spectrometry (AAS), a method that is seldom established in clinical units. Recently, on the basis of absorption spectrophotometry, a user-friendly and inexpensive method has been described to overcome these difficulties.258 Other researchers published an optimized labelling protocol with short incubation time and low concen¬tration of SPIONs.256 Further experimental studies are warranted to step-by-step improve this cellular treatment regimen with the assistance of MR-based tracking of DC-vaccines, and/or imple¬ment more sophisticated applications (e.g. rapamycin inhibition on lymphoid

homing and tolerogenic function of nanoparticle- labeled DCs247 or targeted delivery of nanovaccine MNPs to DCs in vivo)245 in clinical routine.

6.5. Monitoring stem cell migration

Stem cells transplants are expected to have tremendous potential for the treatment of many degenerative diseases because of their capability to perform multiple cell cycle divisions and of their differentiation efficiency.259,260 Several clinical trials are ongoing with different types of stem cells. Mesenchymal stem cells are used for reparation of damaged tissue, regeneration of bone defects,261,262 spinal cord injury,263 stroke,264 and myocardial infarction,265 while neural stem cells

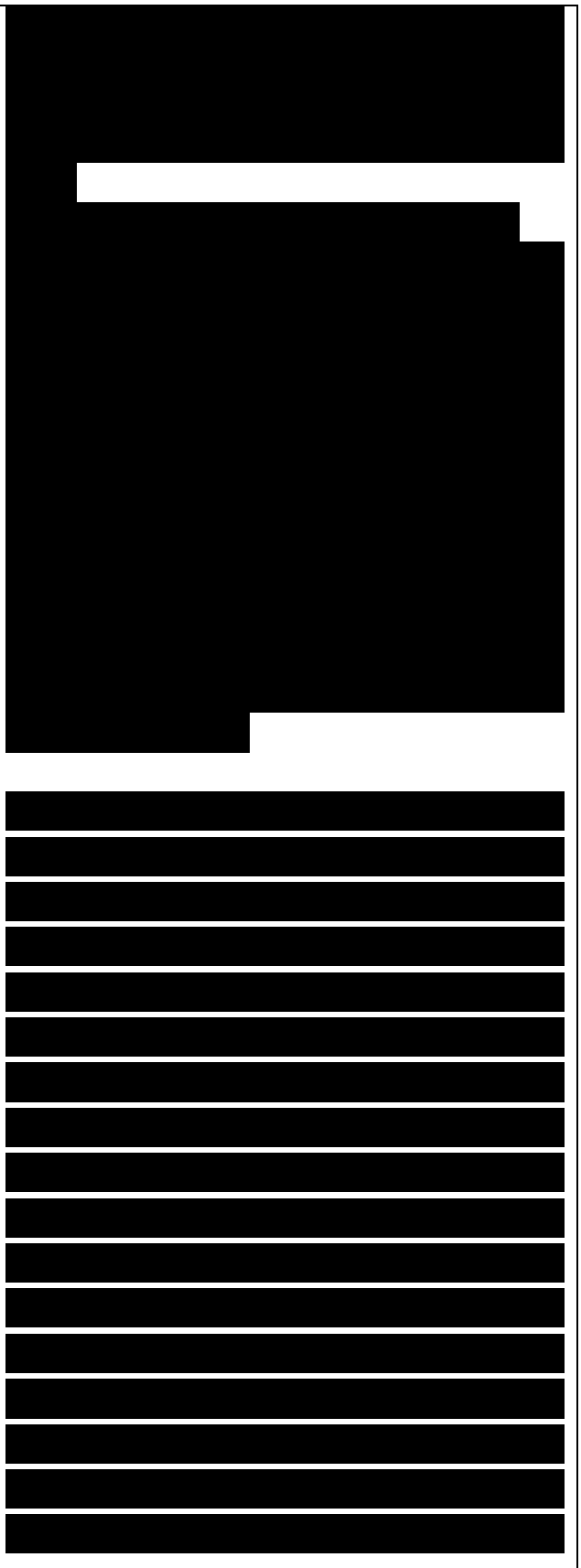

Fig. 13 (A, B) Example for MR-guided exact DC-vaccination delivery in vivo. (A) MRI before vaccination; the inguinal lymph node to be injected is indicated with a black arrow. (B) MRI after injection showing that the dendritic cells were not accurately delivered into the inguinal lymph node (black arrow) but in the vicinity, in the subcutaneous fat (white arrow) (images taken from de Vries et al.).254 (C, D) Migration of DCs is detectable by cellular MRI. Coronal 3D-FIESTA images (200 x 200 x 200 mm) showing the popliteal lymph nodes from one representative mouse, 2 days after injection with (a) 1 x 106 MPIO-labelled DCs or (b) 1 x 106 unlabelled DCs (images taken from the study of Rohani et al.).244

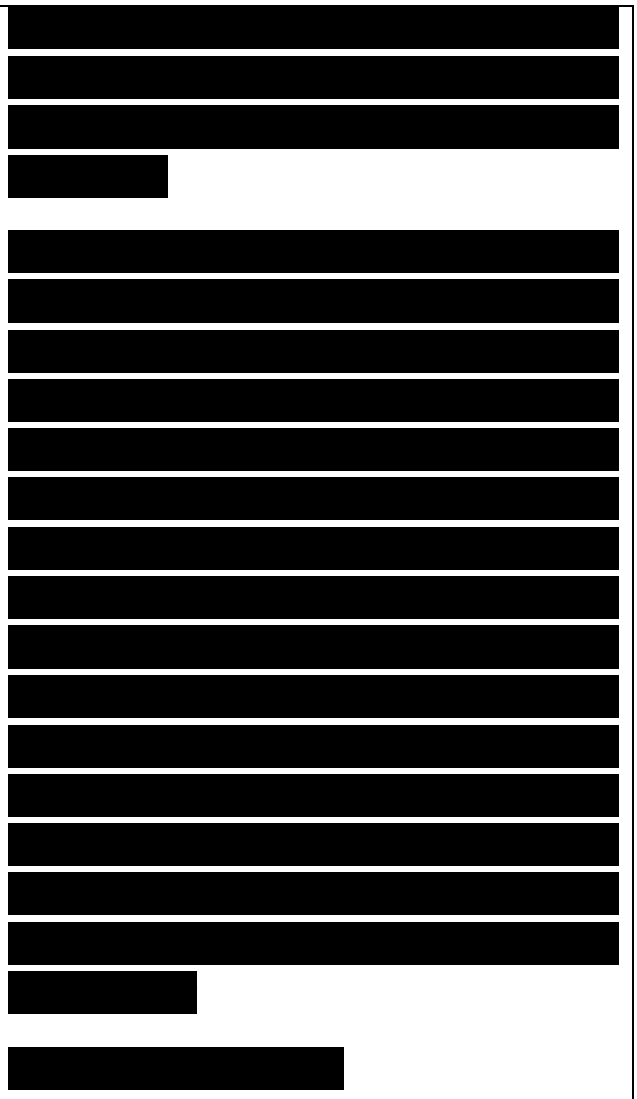

Fig. 14 Donor DC traffic to secondary lymphoid organs after local injection and retention of SPIONs (i.m. injection of lucSPIOCD11c cells in the right proximal leg 1 h after bone marrow transplant (BMT) (C57B/63BALB/c)). Trafficking is monitored by bioluminescence imaging (BLI) on the indicated days. (cLN) Cervical lymph node, (aLN) axillary lymph node, (iLN) inguinal lymph node, (mLN) mesenteric lymph node (images taken from the study of Reichardt et al.)247 Copyright 2008. The American Association of Immunologist, Inc.

are investigated for the neural lineages generation of the nervous system.266 On this basis, one important issue is to identify and track the stem cells after their injection in the body, to monitor their motility and to follow the localization and their expansion thereafter. Among the available in vivo imaging techniques useful for stem cell monitoring, MRI is particularly promising since it can provide high spatial resolution images without compromising the patient's care.267-269 T2 relaxivity MRI contrast agents

based on iron oxides offer a powerful labeling for the in vivo visualization of the stem cells. As for DCs, MNP sizes for utilization in stem cell labeling can vary from ultrasmall, within 35 nm diameter,270 to micron-sized.271 To this aim, the MNPs can be coated by different polymers, including polyethylene glycol,272 silica,273 dextran,274 and polystyrene,275 to increase the stability of the suspension and thus avoiding the cell toxicity caused by the formation of large agglomerates. These chemical- physical characteristics affect labelling efficiency of MNPs, which determines the interaction between MNPs and cells.276 The typical MNP uptake follows an endocytosis pathway that can be induced by mere incubation of the suspension of MNPs in the cell medium,277 which, in turn, can be improved by application of an external magnetic field.278 The addition of adjuvants, such as transfection agents,130 or MNP functionalisation with Abs exploiting a ligand-receptor specific interaction,279 could be of help with some cell types. In alternative, it is possible to induce a temporary permeability of membrane by electroporation280 or ultrasound pulses.281

Several MNP based contrast agents were successfully applied to in preclinical trials.282 The feasibility of MRI tracking after injection of MNP-labelled stem cells for the treatment of cardiovascular diseases offers not only a potential regeneration of heart tissue,

but also allows us to follow the long-term migration cell without impairment of myocardial function and without altering their cardiac differentiation.283-286

The in vivo cellular imaging after neurotransplantation for the treatment of acute and chronic central nervous system diseases, such as demyelination and lysosomal storage disorders, acute spinal cord injury, Parkinson's and Huntington's diseases and multiple sclerosis, serves several purposes, including tracking cell migration and integration, postoperative visualization of stem cells localization, and monitoring graft conservation.287-296 Although several clinical trials have been approved by the FDA at the present time,254'297-299 there are a number of constraints and limitations that remain unsolved: the stem cells uninterrupted proliferation after transplantation cause the dilution of the MNPs as labelling agents at the expense of the long-term tracking and in some cases the cells divide asymmetrically, leading to an unequal distribution of the MNPs. Furthermore this kind of labelling prevents the discrimination between live and dead marked cells.300

6.6. Clearance mechanisms in humans

Clearance mechanisms of MNPs in humans have been studied with MRI.

The MNPs that have been used for this purpose were ionic ferucarbotran, and non-ionic ferumoxides or AMI-25, with hydrodynamic diameter of 62 and 150 nm, respectively. They were rapidly cleared after intravenous injection by professional macrophages. Their blood half-life was 6 minutes.301 Macrophages engulfed these MNPs via phagocytosis. Afterwards MNPs could be found within lysosomes. This kind of particle aggregation induced a signal enhancement as explained above (see Fig. 10). Due to this fact macrophages could be imaged by MRI. In other words, macrophage-rich organs and tissues such as liver (Kupffer cells), spleen, bone marrow, and inflammatory areas with increased macrophages like atherosclerotic plaques were hypointensive on T2/T2 weighted MRI after active engulfment of MNPs. Peak concentrations of iron were found in the liver after 2 hours and in the spleen after 4 hours; afterwards MNPs were slowly cleared from these organs with half-life of 3-4 days.301 In lysosomes MNPs were enzymatically degraded and free iron were subsequently released into the metabolic iron pool of the organism. Macrophages incorporate ferucarbotran into lysosomal vesicles containing a-glucosidase, which were capable of degrading the carboxy- dextran shell of the ferucarbotran particles.143 Serum iron and ferritin levels increased.302 Some of the MNPs remained intact and were exocytosed by the cells, so that neighbouring macrophages could phagocytose them.

	[redacted]

7. Magnetic nanoparticles as drug delivery systems Most pharmacological approaches to cancer therapy are based on chemotherapeutic substances, which generally exhibit high cytotoxic activities but poor specificity for the intended biological target. This practice mostly results in a systemic distribution of the cytotoxic agents leading to the occurrence of well documented side effects associated with chemotherapy caused by the undesired interaction of antitumor drugs with healthy tissues.303,304 The idea of exploiting magnetic guidance, making use of an implanted permanent magnet or an externally applied field, to increase the accumulation of drugs to diseased sites dates back to the late 1970s. The first preclinical experiments using magnetic albumin microspheres loaded with doxorubicin for cancer treatment in rats were reported by Widder et al.305 Since then, several improved MNP models have been developed, particularly for cancer therapy. However, despite very promising results in preclinical investigations, the first clinical trials have shown poor effective response and thus no magnetic nanocarriers have been clinically approved yet.306,307	rất độc hại đối với tế bào nhưng tính chọn lọc mục tiêu sinh học lại kém dẫn đến chất gây độc tế bào các hiệu ứng phụ do tương tác không mong muốn của các thuốc chống ung thư với mô khỏe mạnh sự dẫn truyền bằng từ cấy nam châm vĩnh cửu hoặc sử dụng từ trường bên ngoài đã trình bày từ với nghiên cứu các phần tử mang từ
Besides magnetic force delivery, two alternative “physiological” routes can be followed by MNPs, which are common to all kinds of nanoparticulates. The passive targeting route takes advantage of the biological function of the reticuloendothelial system (RES), a cell	chúng ta cũng có thể dùng hai quy trình “sinh lý học” khác để điều khiển các MNP, đây là những quy trình phổ biến đối với tất cả các loại hạt nano Quy trình hướng mục tiêu

family of the immune system comprising circulating monocytes, bone marrow progenitors and tissue macrophages, which is deputed to the first clearance activity in mammalian organisms.308 Once unprotected MNPs are immersed in the blood stream, an array of plasma proteins called opsonins, including immunoglobulins, complement proteins, fibronectin and other species, recognize them as an invading agent and immediately adsorb on their surface. The parameters affecting the extent of opsonization are essentially related to the physical properties of the MNP surface, including size, shape, charge and state of agglomeration. Large objects are rapidly cleared and highly charged NPs have a tendency to attract opsonins.309	họ luân chuyển hệ tạo tham gia vào hoạt động làm sạch đầu tiên các được nhúng bổ sung chất quá trình hóa điện tích bị đào thải nhanh điện tích cao
Subsequently, MNPs coated by these plasma proteins are rapidly endocytosed by the RES cells, resulting in their removal from circulation and accumulation in organs with high phagocytic activity, such as liver and spleen. Size is a key parameter in NP clearance, MNPs smaller than 4 mm accumulate in the liver (70-90%) and spleen (3-10%) quickly. NPs larger than 250 nm are usually filtered to the spleen; NPs in the range 10-100 nm are mainly phagocytosed through liver cells,310 while NPs below 10-15 nm can be cleared by a renal route.311 Therefore the optimal particle size for drug delivery treatments ranges between 10 to 100 nm, as these will have the longest blood	Các quá trình dẫn truyền thuốc

circulation time (Fig. 15). It has been suggested that the particle shape can also play a role. Anisotropic MNPs with high aspect ratio have demonstrated enhanced blood circulation compared to spherical MNPs in vivo.312 In the absence of MNP protecting shells, MNP distribution in the above-mentioned organs is accomplished within a few minutes, depending on the size of the MNPs.313 Hence, passive nanocarriers can be used to deliver drugs for the treatment of hepatic diseases, such as liver metastases,314 and to favor the internalization of antibiotics by phagocytic cells of the RES for the treatment of intracellular infections.315	đóng vai trò nhất định Người ta thấy rằng bất có tỷ số hướng lớn có thể sự tác nhân mang nano dẫn truyền của thực bào
Magnetically assisted targeting of MNPs will have the advantage of increasing the local concentration of the administered drug, while the overall dose is reduced (Fig. 15). Controlled transport is crucial for delivery but it is challenging because of the small MNP size. On one hand, long circulation time after the MNP injection is desirable to give the MNPs more chances to be held by the magnetic field close to the target area. On the other hand, in this application the minimum diameter for successful MNP capture by a magnet is a limiting factor. A single MNP in a magnetic field gradient will experience a force that depends on the magnetic moment and on the field gradient around it. This force is proportional to the volume of the MNP and, therefore, decreasing the size by a factor of 10 decreases the magnetic force	Tính hướng mục tiêu được hỗ trợ bằng từ các giảm liều lượng tổng cộng đối với quá trình truyền dẫn người ta luôn muốn để chúng có cơ hội được từ trường giữ gần khu vực mục tiêu nhiều hơn trong ứng dụng này, chúng ta cần đường kính cực tiểu để bắt MNP thành công, đó cũng là một yếu tố hạn chế chịu tác dụng của 10 lần

by 1000.316,317 For example, individual Fe3O4 MNPs with a core diameter less than 20 nm cannot be captured permanently by a HGMS (high-gradient magnetic separation) column. The minimum agglomerate size for permanent capture was calculated to be 40 nm for phospholipid-coated MNPs and 70 nm for polymer- coated MNPs. The difference is attributed to the higher volume fraction of magnetite in the phospholipid agglomer- ates.318 The movement of MNPs inside a matrix or fluid depends directly on a multitude of factors such as the external magnetic field gradient, the temperature and the viscosity of the medium, the fluid flow, the interaction between MNPs and fluid components, and the size and shape of the MNPs. The dynamics of MNP transport in vivo through a vein or artery to an area of interest are far from being fully understood, but there are nowadays several studies in this direction.316,319 Firstly, to hold the MNPs in the area that one wants to target, field gradients are required, as MNPs will experience no force in a homogeneous field. For this reason, rare-earth magnets are generally used. The field gradient has to be high enough to overcome the blood flow strength that keeps moving the MNPs in the vessels, and for that purpose, the closer to the magnet surface, the better.	dẫn đến giảm lực từ 1000 lần từng cao kết tụ quá trình bắt vĩnh viễn là các phủ đối với các phủ phần magnetite có thể tích lớn hơn các khối kết tụ các nền vào các Tính chất động học vẫn chưa được hiểu thấu đáo chúng ta cần các gradient trường các chịu tác dụng của lực trong trường đồng nhất người ta thường dùng các nam châm đất hiếm giữ cho các MNP di chuyển trong các mạch càng gần bề mặt nam châm hơn càng tốt hơn
The tissue between the target and the magnet source will also accumulate the MNPs, therefore, external magnets can be used for targets close to the body	

surface. However, internal magnets will be needed for deeper targets.

In contrast with the passive delivery route, active targeting has the advantage of improving the accumulation of chemo- therapeutics at the tumor site, but requires multiple synthetic steps to tailor the chemical properties of MNPs in order to achieve a suitably bioengineered magnetic nanocarrier. In principle, it is always necessary to stabilize the MNP dispersion in the aqueous environment. Thus, coating the MNPs with a polymer shell, including organic (PEG,73,322 dextran,323 chitosan,324 polyethyleneimine,325 and phospholipids)326 or inorganic (silica),327 is usually the first step. Whatever the stabilizer, the next requirement is to reduce significantly the possible interactions with opsonins and with the RES, which is usually accomplished by conjugating the MNPs with an appropriate protein-repellent molecular species, such as PEG. The resulting ‘‘stealth’’ MNPs are able to circulate in the blood for a long period of time without being cleared.328 The final step consists in functionalizing such long-circulating MNPs with targeting ligands having high selectivity for specific cancer cell receptors.329 The full-armed magnetic drug delivery nanosystem is obtained by loading a cytotoxic cargo at some stage of the above synthetic steps. A wide variety of antitumor agents has been loaded inside or external to the polymer coating, either by physical adsorption or by covalent

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conjugation (cf.Section 3.3). These include chemotherapeutics (DOX,330-332 danorubicin,333 tamoxifen,334 cisplatin and gemcitabine,335 PTX,336 mitoxantrone,337 cefradine,338 ammonium glycyrrhizinate,339 fludarabine,340 pingyangmycin,341 nonsteroidal anti-inflammatory pharmaceutics,342 amethopterin,343 mitomycin,344 diclofenac sodium,345 and adriamycin),346 enzymes,347 toxins,348 genes,349 folic acid (FA),322 Abs,350 growth factors,351 and radionucleo-tides.352,353 In the three next paragraphs, we summarize some recent achievements using MNPs for targeted drug delivery based on these concepts.

(tham khảo phần 3.3)

trong kỹ thuật dẫn truyền thuốc đúng mục tiêu

Các hạt

7.1. Long-circulating nanoparticles exploiting the ‘‘enhanced permeation and retention’’ effect

In order to avoid rapid clearance from the body by RES while concomitantly retaining high surface area and activity, the surface of MNPs needs to be protected. Among the various solutions investigated so far, PEG has demonstrated to confer the best performances to the organic/inorganic nanohybrids in terms of stability, solubility, biocompatibility and capability to shield the surface charge.78 PEGylation strategies may involve direct MNP synthesis using PEG precursors or graft copolymers as solvent/

chất lai hóa nano

complexant,354,355 or, alternatively, surface conjugation with PEG molecules modified with suitable anchoring ligands endowed with high affinity for iron oxide. The most used are siloxanes,322,356 phosphates,16 and catechol derivatives.357 PEG molecules have also been tailored to enhance their tumor localization and to promote the controlled release of therapeutic agents.358 The solubility increases as a function of PEG molecular weight from 500 to 5000 Da. However, the improved solubility results in a decrease of magnetic susceptibility and in an increase in hydrodynamic size.	hóa tiền chất gắn kết mạnh các dẫn xuất siloxanes Các điều chỉnh trọng lượng tăng
The ‘‘stealth’’ character of PEGylated MNPs confers them a long-term circulation capability in the blood vessels circumventing the possible immune response, opsonin interaction and clearance by the RES.358 To achieve the best bioinvisibility properties, the molecular weight of PEG should be in the range of 1500-5000 Da. As a result, MNPs can flow throughout the blood for a time long enough to allow them to passively penetrate through the fenestrations, which are typically in the range of 200-600 nm, of leaky vasculature in correspondence to the tumor tissue.359 The selectivity of	 PEG hóa hệ

targeting is essentially due to the absence of such fenestration in healthy tissues. The diseased vascular condition that favors this passive selective delivery process is usually termed ‘‘enhanced permeation and retention’’ (EPR) effect and is associated to a defective vascular architecture, impaired lymphatic drainage and extensive angiogenesis. It is worth noting that the release of drugs from passively diffusing PEGylated nanocarriers through peripheral tumor tissue by exploiting the EPR effect has produced some clinically relevant results. However, there have been contradicting data concerning the real effectiveness of introducing targeting molecules in these nanocomplexes.360 The diffusion process mediated by the EPR effect is dependent on the biophysical properties of the MNPs. Therefore, the chemical and physical characteristics of engineered MNPs should be carefully optimized, even in the absence of specific targeting ligands.361 Recently, PEGylated iron oxide MNPs have been used to associate selective transport of DOX in vivo with simultaneous MRI tumor localization, demonstrating sustained drug release and dose-dependent antiproliferative effects in vitro.362 Moreover, clever strategies for ‘‘intelligent’’ drug release have been attempted by using PEG-containing stimuli-responsive block copolymers for the coating of MNPs.363	gắn liền với PEG hóa mang lại một số kết quả lâm sàng khả quan có những dữ liệu trái ngược nhau này PEG hóa thể chống tăng sinh tế bào ống nghiệm

7.2. Targeted delivery of cytotoxic agents In general, when isolated MNPs extravasate out of the vasculature at the tumor site, they usually exhibit poor retention unless their surface has been functionalized with specific cell targeting molecules, which, in turn, can trigger receptor- mediated endocytosis, resulting in higher intracellular drug concentration and increased cytotoxicity.360,364,365 The exploitation of the unique multifaceted properties of MNPs has led to the development of a new concept of ‘‘nanotheranostics’’, which refers to the simultaneous capability of MNPs to serve both as diagnostic and as therapeutic agents in the purpose of treatment of cancer and inflammatory diseases.366 MNPs functionalized with cytosine-guanine (CG) rich duplex containing prostate-specific membrane antigen showed selective drug delivery efficacy in a LNCaP xenograft mouse model.367 In another study, an anti-HER2 Ab-conjugated, pH-sensitive MNP system has been developed for the intelligent release of DOX inside HER2-overexpressing breast cancer cells.368 Multifunctional MNP clusters encapsulated in an amphiphilic block copolymer or in a silica@gold nanoshell	[redacted] để [redacted] Dẫn truyền [redacted] tế bào chuyên biệt kích hoạt các endocytosis do receptor làm trung gian cao hơn [redacted] duplex giàu [redacted] có khả năng dẫn truyền thuốc hiệu quả và có tính chọn lọc [redacted] với anti-HER2 Ab

functionalized with suitable mAbs were used for MRI-guided Ab therapy or NIR illumination-based gold nanoshell-triggered hyper-thermic treatment of different tumors, respectively.369,370 A new bioengineered iron oxide MNP, presenting the anti-HER2 Ab in an optimal orientation to maximize the binding with HER2 receptors in breast cancer cells, proved to be highly efficient in providing MRI and fluorescence images of the tumor mass and in strongly reducing HER2 expression in tumor tissue in vivo, which could be promising for neoadjuvant therapy of breast cancer.371 FA is also largely utilized as an effective tumor targeting agent conjugated to composite multifunctional MNPs. FA-functionalized mesoporous silica MNPs containing iron oxide NPs and DOX allowed for simultaneous imaging and improved antitumor drug delivery in MCF7 and HeLa cells,372 while FA-modified MNPs bearing b-cyclodextrin encapsulating drug molecules, could release the payload by applying a controlled high-frequency magnetic field.373 In a conceptually similar approach, Ruiz-Hernandez et al. exploited the local temperature enhancement produced by the heat generated by application of an AMF on doublestranded DNA fragments capping the pores of mesoporous silica MNPs, thus enabling the free release of chemotherapeutic cargo.89 Furthermore, iron oxide MNPs conjugated with an Ab selective for EGFR receptor deletion mutant (EGFRvIII) present on human glioblastoma multiforme (GBM) cells

biểu hiện
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được đóng gói
(có cả tính
chất ưa nước và kỵ nước)
silic điôxit
dẫn hướng bằng
nhiệt
kích hoạt vỏ nano vàng dựa trên chiếu
ánh sáng hồng ngoại gần áp dụng trên
cho theo hướng tối ưu
hỗ trợ
biến tính
bằng mang b-cyclodextrin đóng gói

were used for MRI-guided therapeutic targeting GBM, after convection-enhanced delivery (CED),374 allowing for the effective intra- tumoral and peritumoral distribution of MNPs in the brain.375 The importance of this proof-of-concept experiment is that it demonstrates a significant dispersion of the MNPs over days after the infusion, which may lead to the therapeutic effect against the primary mass and to the concomitant targeting of residual peripheral metastases.	các phân tử thuốc vào Với cách tiếp cận tương tự về mặt khái niệm như trên và các cộng sự khi áp các sợi silic điôxit có khả năng chọn lọc mất đoạn (xóa gen) các GBM nhằm mục tiêu trị liệu dẫn hướng bằng MRI (dẫn truyền có hiệu quả xung quanh khối u kiểm chứng minh chứng cho điều này
7.3. Magnetic field-assisted drug transport and magnetofection for gene therapy	Vận chuyển thuốc bằng từ trường và sử dụng từ tính trong liệu pháp gen

Magnetic targeting has been recently introduced in nanomedicine as an innovative approach for the targeted delivery.376 The basic principle of this technique is that MNPs loaded with the drug of interest are guided to a specific body tissue or organ by application of an external magnetic field gradient, achieving a high drug concentration in correspondence to the diseased area.377	[redacted] nạp cùng với thuốc dẫn đường [redacted] gradient từ trường ngoài để nồng độ thuốc đi vào khu vực bệnh cao nhất [redacted]
This method is mainly used successfully in cancer treatments. The magnetic field-assisted transport of cytotoxic agents associated with MNPs to tumor cells enhances the therapeutic efficacy of tumor treatment allowing for the reduction of administered dosage and minimization of side effects.81,113,378 Recently, in a very interesting approach MNPs have been also used to boost the oncolytic adenovirus potency. MNPs were associated to specific virus to improve their uptake by cancer cells by applying a magnetic force.104 Moreover, a significant enhancement of the natural immune response to tumor cells was achieved using MNPs for magnetically guided in vivo delivery of interferon gamma for cancer immunotherapy.379	[redacted] đến [redacted] tiêm [redacted] trong tiếp cận khá lí thú, [redacted] quá trình dẫn truyền trong được dẫn hướng bằng từ trong [redacted]
The feasibility of the magnetic field-assisted targeting approach and its	[redacted]

therapeutic potential in vitro as well as in vivo is studied and applied also in other therapeutics contexts. For instance, Chorny et al. used a PTX-loaded MNP formulation for the treatment of stent restenosis.111 In other interesting studies, MRI-guided magnetic delivery of multifunctional MNPs to the brain enabled crossing the blood brain barrier reducing the systemic toxicity.380 In the last few years, because of the importance of nucleic acid delivery to cells to make them produce a desired protein or to shut down the expression of endogenous genes,104,381 magnetofection is rapidly evolving as a novel and efficient gene delivery technique based on a magnetic force exerted upon gene vectors linked to, or encapsulated inside, MNPs to direct the genes to the target cells in vitro, as well as to a target tissue or organ in vivo.382-388 This research area opens new possibilities because through the development of coupled siRNA- and microRNA-MNPs it is possible to localize and efficiently deliver genes inside the cells with a direct cell function interference and tremendous research, diagnostic and therapeutic applications.389	nạp tái phát hẹp dẫn truyền từ dựa trên MRI của các MNP đa năng vách ngăn hệ thống phát triển nhanh dưới dạng các kỹ thuật dẫn truyền gen hiệu quả và mới lạ gắn với các MNP kết hợp dẫn truyền gen có hiệu quả bên trong tế bào bằng cách can thiệp trực tiếp vào chức năng tế bào các ứng dụng
8. Magnetic nanoparticles as heat	Các với vai trò là tác

mediators for hyperthermia	nhân điều hòa trong chứng thân nhiệt cao
8.1. Principles and preclinical investigations The concept of hyperthermia dates back to more than 4000 years ago when heating was already mentioned as a potential treatment for some diseases in the advanced cultures of the old Egypt. Nowadays, hyperthermia has received renewed attention due to the recent advances, which suggest a potential application in cancer therapy. In particular, the use of MNPs as heat mediators looks promising in the development of novel thermotherapy treatments, especially in combination with conventional cancer therapies, including surgery, radio and chemotherapy.31,328 The pioneering work of Gordon et al. in 1979 paved the way for the intracellular application using dextran MNPs and a high-frequency magnetic field.390	và chính điều này đã gợi ra dưới dạng các tác nhân điều hòa nhiệt và các cộng sự
The hyperthermia procedure, based on heat generation within cancer cells, takes advantage of the higher sensitivity of the tumor cells to temperature compared with normal tissues.391,392 The heating is obtained through the Brown losses of the MNPs induced by an AMF, to which MNPs are subjected.393 Depending on the extent of local heat production two	tính chất Người ta thu nhiệt thông qua các tổn hao Brown của các MNP được cảm ứng bởi một AMF, các MNP lệ thuộc vào AMF này

kinds of heating treatments have been defined: (1) hyperthermic effect refers to cell apoptosis triggered by controlled heating in the range 41-46 °C, high enough to modify several structural and enzymatic functions of cell proteins; (2) thermoablation event occurs as a consequence of cell carbonization as temperature is raised above 46-48 °C (usually up to 56 °C).394 The thermotherapy efficiency has been successfully applied to different cancer types, including breast,395-397 brain,398prostate cancers146,399,400 and melanoma.401,402 The efficiency of magnetic heating essentially depends on the size and magnetic susceptibility of the MNPs.403 To reduce systemic and side effects on the normal tissue, the generated heating has to be confined to the tumor area and a temperature control is required. Many efforts have been spent to reach these critical objectives. Mild hyperthermia in combination with other traditional cancer treatments, like radiotherapy and/or chemotherapy, has provided a substantial therapeutic improvement. Several studies have shown a reduction in tumor size when a combination with other therapies is applied.404-406

Exploiting the passive migration to the tumor region achieved by the EPR effect, magnetite cationic liposomes have been

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envisaged as a promising tool for several types of tumors because of their high accumulation favored by the positive charge of the nanocomplex.407-409 The surface modification and functionalization of the MNPs with biological ligands like proteins or Abs for active targeting allowed the accumulation in correspondence to the tumor in a good percentage of the total intravenously injected amount,410,411 and the binding with organic fluorophores or fluorescent NPs (QDs), exhibited simultaneous cancer diagnosis and treatment.412 In order to minimize the toxicity induced in the body by the chemically synthesized MNPs and minimize the administered amount of inorganic material, concomitantly improving the response to applied AMF, Alphandery et al. developed an innovative bioproduction approach to the preparation of colloidal mediators by using extracted chains of magnetosomes, which exhibited a specific absorption rate remarkably higher than the chemically synthesized MNPs.413

8.2. Heat shock-induced antitumor immunity

The therapeutic outcome of hyperthermia treatment is not only due to the direct effect of cell heating but also to the activation of an immune response which results in a reduction both of the primary tumor mass and also the metastatic lesions.400 Heat treatment itself

enhances the antitumor effect through the stimulation of the innate immune response. A temperature of approximately 42 °C is enough to activate natural killer cells, which are potent tumor-lytic agents when activated. Kubes et al. have shown that a high number of activated monocytes with increased cytotoxic effector function is recruited into B16-F10 melanoma-bearing mice after mild local microwave hyperthermia.402,414 The mechanism for the recognition of tumor cell antigens by the host immune system involves the release of the content of dying tumor cells, including heat shock proteins (HSPs). HSPs are responsible for the activation of neighboring monocytes to produce proinflammatory cytokines and recruit antigen-presenting cells.415,416 This stimulation of innate immune system triggered by hyperthermia continued for an extended period of time and the treated animals completely rejected new tumor cell invasion as a metastasis model.417,418	[redacted] và các cộng sự [redacted] Các [redacted] trình diện kháng nguyên [redacted] loại bỏ hiện tượng xâm lấn của tế bào khối u do di căn [redacted]
8.3. Clinical trials in humans As a consequence of the robust results achieved with MNP- based hyperthermia treatment of cancer animal models and in view of a comprehensive knowledge of	Với tư cách [redacted] từ [redacted] cho các mô hình ung thư

the molecular mechanisms, this therapy is now being established in clinical routine leading to an industrial development.419 Hyperthermia treatment has been approved by the FDA for use alone or in combination with radiation therapy in the palliative management of certain solid surface and subsurface malignant tumors (i.e., melanoma, squamous- or basal-cell carcinoma, adenocarcinoma, or sarcoma) that are progressive or recurrent despite conventional therapy. Clinical studies using combined hyperthermia and radiation therapy have shown that 83.7% of patients had some tumor mass decrease, of which 37.4% had a complete tumor regression while 24.5% exhibited a >50% tumor reduction. There are at least three operating companies that develop techniques that generate heat by MNPs exposed to an AMF.	ở động vật các tăng thân dưới bề mặt trong khi
Sirtex Medical's targeted hyperthermia research program treats the majority of liver cancer patients that do not have localized tumors with small magnetic micro-spheres (ThermoSpheres). Targeted hyperthermia therapy, used in combination with targeted radiotherapy using SIR-Spheres microspheres, should improve even further the efficacy of the SIR-Spheres microspheres.420 SIR-Spheres microspheres contain resin-based microparticles impregnated with yttrium-90, a radio isotope commonly used to treat patients with liver cancer. Aspen MediSys is developing MNPs,	sử dụng các hình cầu micro các hình cầu micro Các hình cầu micro các hạt có kích thước micro bằng resin

which act as cellular ablation devices that operate at a size scale typical for drug delivery vehicles.421 MagForce developed marketable products (NanoTherm®, NanoPlan® and NanoActivator™) for the local treatment of solid tumors (glioblastoma multiforme, prostate cancer and pancreatic cancer). The principle of the method is the direct introduction of MNPs into a tumor and their subsequent heating in an AMF. The water soluble MNPs are extremely small (approximately 15 nm in diameter), and contain an iron oxide core with an aminosilane coating. The MNPs are activated by an AMF, which changes its polarity 100 000 times per second. Thus heat is produced, raising the temperature of the cancer cells in the order of 5 °C. These MNPs have been already injected in patients with prostate cancer demonstrating stable intra-tumoral deposition of the MNP in the prostatic tissue for at least six weeks, which allows for a series of thermal therapy treatment without further injections.146,422 Magnetic hyperthermia for bone tumors reduction has also been studied showing a good clinical outcome.423

9. Remaining challenges

phóng xạ

To translate the preclinical settings into clinical applications for most of the magnetic biocomposites that have been mentioned along this manuscript a lot of questions should be cleared by intense basic scientific work. It will be only possible to answer most of the open questions by adding up the efforts of interdisciplinary research groups. In this section some of the general challenges for an extended biological application of MNPs will be mentioned and discussed.	làm rõ nhiều vấn đề rất nhiều Chúng ta chỉ có thể hiểu rõ được những vấn đề còn bỏ ngỏ chúng tôi sẽ trình bày và phân tích việc các
Firstly, the magnetic properties of the MNPs should be improved to enhance the magnetic resonance signal in MRI and to maximize the specific loss power increasing the efficiency of magnetic thermal induction. Probably it will be necessary to extend the use of ferrites such as CoFe2O4 and MnFe2O4 or the new fabrication of nanostructures like core-shell systems as it was recently demonstrated for improvements in hyperthermia applications.31 Toxicological studies of new magnetic biocomposites will have to be carried out. In the future, more general and robust bioconjugate chemistries for connecting biomolecules to particles will be also necessary. The scaling-up of the fabrication of most of the mentioned composites is still not possible. Another challenge in the development of coatings involving active biomolecules for MNPs is to limit the overall size of particles to below 100 nm, since MNPs larger than	tính chất từ độ tổn hao công suất đặc trưng các người ta đã chứng minh rằng nó bền vững và phổ dụng Việc mở rộng quy mô chế tạo đa số các composite được đề cập vẫn chưa được tiến hành việc các

100 nm are rapidly cleared by the liver and spleen.310 Applications such as drug delivery or hyperthermia will be favored with the development of new and improved magnetic biocomposites.	[redacted] các [redacted]
Regarding the application of magnetic nanoswitches, the current studies might lead to the development of implantable sensors offering long-term stability when placed in the body. However, the main drawback is the imaging enzyme activity in vivo which involves previous cytoplasmatic delivery of MNPs in the cells of interest (see Section 6.3).	Về vấn đề ứng dụng các [redacted] các cảm biến cấy ghép được có thời gian ổn định dài [redacted] liên quan đến sự dẫn truyền [redacted]
The fate of MNPs in magnetically labelled cells after their transplantation in an organism is also not fully understood and requires further study. It is well established that the MNP-induced signal hypointensity has a maximum (e.g. after 24 hours), and afterwards continuously declines but the reason is not completely clear. Different possible mechanisms like proliferation-dependent MNP dilution,	[redacted] trong cơ thể [redacted] lí do của hiện tượng này vẫn chưa rõ ràng

metabolic degradation, exocytosis and/or cell death followed by an uptake of free MNPs by invading macrophages, and transport to other organs/tissues in the body of the organism are currently being discussed. Possibly, not a single but an interplay of these mechanisms may cause the MR signal intensity decrease. In addition, this could be cell-type dependent and/or MNP- specific. Specifically the metabolic degradation could be influenced by a MNP-design with a biodegradable cover that allows a slow cleavage (retard formulation) in lysosomes and/or cytoplasmic localisation of the MNPs. The proliferation- dependent MNP dilution can be influenced by using nonproliferating cells or slow proliferating ones. Exocytosis of MNPs has been rarely investigated after magnetically cell labelling. This process could be cell-dependent as well as labelling-dependent. Cells that actively take up MNPs by endocytosis store the foreign material in lysosomes. In this subcellular compartment MNPs may either undergo metabolic degradation or may leave the cell via exocytosis. To omit the latter process cells can be magnetically labelled by physical methods such as electroporation or magnetofection. This guarantees cytoplasmic rather than lysosomal MNP localisation. On the other hand the bombardment of cells with MNPs leads to a much higher percentage of preparation-dependent cell death. This means that a greater number of cells is necessary to finally ensure of having	vào sự sinh sôi bởi các đại thực bào xâm lấn sinh vật hiện vẫn nghiên cứu qua lại giữa Sự pha loãng MNP phụ thuộc sự sinh sôi khi sử dụng các tế bào không sinh sôi hoặc sinh sôi chậm nghiên cứu sau khi đánh dấu tế bào bằng từ Các

enough labelled cells that are viable. Instead of physical labelling it is also possible to modify MNPs by transmembrane localisation peptides (e.g. HIV tat peptide). Although this method is associated with a small percentage of preparation-related cell death, all additional materials used in the synthesis of MNPs must be FDA-approved. The same is true for transfection agents used to enhance MNP cellular incorporation.	tỷ lệ chết tế bào phụ thuộc quá trình điều chế cao hơn màng với tỷ lệ phần trăm chết tế bào phụ thuộc điều chế nhỏ tăng cường
Robust protocols are necessary to effectively label cells with MNPs. This includes that neither the Fe-concentration used nor the total in vitro labelling procedure/preparation steps should markedly influence cellular functions like viability, proliferation, differentiation, migration, and chemotaxis for example. Moreover, this also implicates that neither free MNPs nor labelled cells might induce harmful reactions in the organism. The latter point is in preclinical settings largely neglected.	Điều này có nghĩa là nồng độ Fe được sử dụng cũng như tổng số quy trình đánh dấu trong ống nghiệm/các bước điều chế không được ảnh hưởng nhiều đến chức năng tế bào điều này cũng nói lên rằng các MNP tự do cũng như các tế bào đánh dấu không được

Besides data concerning the biodistribution it is important to know what happened with the particles in long-term observations. Especially when non-degradable materials such as mesoporous silica are used it is necessary to investigate their fate and their influence on organs/tissues after different time points. A lot of work is necessary until all of these questions are fully answered that is a pre-requisite to implement the MNP-technology into clinical applications.

chúng ta cũng cần biết điều gì xảy ra với các hạt

10. Outlook

Colloidal MNPs possess a broad spectrum of interesting properties that make them useful for biological applications. MNPs based on superparamagnetic iron oxide offer the privileged status of being accepted for clinical purposes. Until now, they have been used in humans for MRI diagnosis but in a near future they are expected to be also used for therapeutic issues and thus becoming theranostic agents. MNPs can be easily synthesized, they can be made colloidally stable, they are inexpensive, and they can be conjugated with biological molecules in a straightforward way. The lack of interference from complex diamagnetic biological matrix, the use of non-radiative and non-toxic detection techniques, and the possibility of analyte magnetic separation and collection are among the peculiar advantages of the use

có những ưu điểm vượt trội đã được chấp nhận sử dụng cho các mục đích lâm sàng

trong điều trị

điều trị-chẩn đoán

chúng có thể được tạo ra ở dạng keo ổn định

Không cần can thiệp từ nền sinh học nghịch từ phức tạp, sử dụng các kỹ thuật phát hiện

of MNPs for diagnosis. Due to their magnetic properties, they are especially interesting in drug delivery because apart of the possibility of tagging their surface, that almost all kind of NPs have, they can be driven inside the organism by the application of an external magnetic field gradient to the target area of the body where the therapy has specifically to act.	không độc hại và không phóng xạ, và khả năng phân tích tách từ và tập hợp chất truyền dẫn gradient từ trường bên ngoài cần tác động
Gene therapy, anticancer treatments and tissue regeneration are between the most challenging clinical applications of MNPs. Regardless of the good tolerance that some MNPs have shown, the long-term outcome of the MNPs in the body will still need to be determined if their use in medicine wants to be extended. In depth analysis of the potential risks associated with the intensive use of inorganic MNPs cannot be further delayed, including the factors related with epigenetic phenomena and long-term cardiovascular effects. Thanks to their broad utilization for research purposes and to their potential in clinical practice, MNPs represent an ideal model to attempt to set up a comprehensive and acceptable nanotechnology platform for the accurate classification of such risks, for the identification of general protocols for the evaluation of nanomaterial safety toward human health and environmental protection, and for the certification of nanoparticle-based drugs and contrast agents for extensive medical application.	

Nowadays, it is universally recognized that a disciplinary point of view is largely insufficient to face a similar challenge. However, with the joint efforts of chemists, physicists, biologists, pharmacologists, radiologists and clinical doctors, soon the mirage of exploiting molecular nanoclinics to assist conventional diagnosis and therapy will become reality.

Abbrevations

Ab antibody
AMF alternating magnetic field
CLIO cross-linked iron oxide magnetic nanoparticles
DC dendritic cells
DOX doxorubicin
EMA European Medicines Agency
EPR enhanced permeation and retention
FA folic acid
FDA US Food and Drug Administration
mAb monochlonal antibody
MNP magnetic nanoparticle
NP nanoparticle

PTX paclitaxel
PEG polyethylene glycol
MR magnetic resonance
MRI MR imaging
SPION superparamagnetic iron oxide NP

[redacted]