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Magnetic Particles in Biotechnology: From Drug Targeting to Tissue Engineering

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1. Introduction

Iron oxide nanoparticles are responsible to magnetic field allowing them to be manipulated, tracked, imaged and remotely heated. Such key features open up a wide field of applications in medicine which includes cell separation, magnetic force-based tissue engineering, MRI tracking of transplanted cells, magnetic drug targeting and hyperthermia.

In most applications reported in the literature, magnetic systems are typically composed of an inorganic core and an organic coating. Although cores have been made from different materials, iron oxide nanoparticles constituted of magnetite (Fe3O4) and maghemite (γ-Fe2O3) are used at a great extent. While the core provide nanocontainers with magnetic properties, the shell functions to (i) protect against core agglomeration, (ii) provide chemical handles for the conjugation of drug molecules, and (iii) limit opsonization. Additionally, shell coatings have been engineered to enhance pharmacokinetics and tailor in vivo fate. Organic shells main comprise phospholipid bilayered membranes or polymeric coating of dextran, for instance. Magnetic system design with such different materials can be achieved via a number of approaches, including in situ coating, post-synthesis adsorption and end-grafting. In fact, several methods have been proposed for their synthesis, coating, and stabilization, mainly comprising the precipitation route together with a surface functionalization step by means of polymers or surfactants. This point will be the focus of the next chapter section – "Producing magnetic particles."

Once produced, these magnetic carriers must meet certain criteria for use in the human body. For therapeutic purposes, magnetic carriers must be water-based, biocompatible, biodegradable, and nonimmunogenic. Besides, special care should be focused on the particle size, surface properties, magnetic properties, and administration route, as will be discussed in the third chapter section, entitled "Magnetic particles: concerns towards *in vivo* use."

The fourth chapter section comprises the applications of magnetic particles in the field of biotechnology. They can be divided into therapeutic and diagnostic ones. Chapter subsections will focus on both. Also discussed is a novel application of magnetic

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nanoparticles – the use of magnetic force for tissue engineering, termed "magnetic force-based Tissue Engineering (Mag-TE)." Since cells labeled with magnetic nanoparticles can be manipulated using magnets, this novel tissue engineering methodology using magnetic force and functionalized magnetic nanoparticles may hold great promise in reproducing *in vitro* patterned tissues for organ regeneration.

The fifth and last section of this chapter provides concluding remarks while addressing future perspectives in regard to magnetic particles in biotechnology.

2. Producing magnetic particles

2.1 Synthesis of magnetic carriers

In most applications reported in the literature, iron oxides, such as magnetite and maghemite, are the magnetic material of choice. The synthesis, coating, and stabilization of such particles will be discussed below. The most common synthetic route to produce magnetite (Fe_3O_4) is the coprecipitation of hydrated divalent and trivalent iron salts in an alkaline medium (A. K. Silva et al., 2008).

Nanoreactors can be employed for the precipitation reaction. They provide a constrained domain, which limits the growth of the particles. This method offers numerous advantages over the previous ones when higher homogeneity of size and shape are concerned. A discussion of these follows.

Microemulsions are colloidal nano-dispersions of water in oil (or oil in water) stabilized by a surfactant film. The synthesis of magnetic particles by this means is carried out when water droplets interact and exchange their contents. Experimental results have confirmed that the microemulsion method allows good control of the particles by preventing their growth and providing particles small enough to get stable magnetic fluids. On the other hand, magnetic particles prepared by coprecipitation may undergo aggregation. Microemulsions, which are thermodynamically stable dispersions, can be considered as truly nanoreactors that can be used to carry out chemical reactions and, in particular, to synthesize nanomaterials. The main idea behind this technique is that by appropriate control of the synthesis parameters, these nanoreactors can produce smaller and more uniform particles than the ones produced by other standard methods. Particle size was found to depend on the molar ratios of water and surfactant (Lopez-Quintela, 2003).

Liposomes are also used as nanoreactors for the precipitation as they provide a constrained domain, which limits the growth of the particles. Alternatively, encapsulation of magnetic particles into liposomes may be performed after synthesis (A. A. Kuznetsov et al., 2001). Magnetoliposomes have been found to be a promising approach that offers some unique advantages when the magnetic nanoparticles are applied in biological systems. Lipid systems present the advantage of low toxicity due to their composition, mainly physiological lipids, compared to the polymeric particles. In fact, encapsulation of the magnetic nanoparticles in liposomes increases their biocompatibility under physiological conditions, making them suitable for a large variety of biological applications. Furthermore, it is known that magnetic particles tend to agglomerate, and are chemically unstable with respect to oxidation in air. Encapsulation of the magnetic nanoparticles in liposomes protects them from aggregation and oxidation (Heurtault et al., 2003).

Concerning the production of polymer-based magnetic carriers, three different methods may be used. The emulsification/polymerization method has been successfully employed to produce magnetic microcapsules. In this process, particles are synthesized in the internal aqueous phase of an inverse emulsion/microemulsion. Afterwards, polymerization by a cross-linking agent takes place. In such microcapsules, the drug and the magnetic particles are in the inner compartment (Saravanan et al., 2004). Alternatively, polymer-covered magnetic particles can be produced by in situ precipitation of magnetic materials in the presence of a polymer that acts as a stabilizer.

Magnetic polymer nanoparticles have been produced in the presence of water-soluble dextran, poly (vinyl alcohol), sodium poly (oxyalkylene di phosphonates), carboxymethyl starch, and amylose starch, just to name a few. In all cases, magnetic particles are surrounded by a hydrophilic polymer shell. Such systems are functionalized by the introduction of chemical groups so that they are able to bind active molecules. For instance, dextran-coated magnetic particles, which are highly hydrophilic, uniform, and nontoxic magnetic carriers, may be activated by the periodate oxidation method. Thus, magnetic polyaldehyde dextran is formed and may be conjugated to different molecules (Hong et al., 2004).

Another method for producing magnetic polymer particles consists of separately synthesizing magnetic particles and polymer particles and then mixing them together to enable either physical or chemical adsorption of the polymer onto the magnetic material to be achieved (É. L. Silva et al., 2009).

3. Magnetic particles: Concerns towards *in vivo* use

For therapeutic purposes, magnetic carriers must be water-based, biocompatible, biodegradable, and nonimmunogenic (Häfeli, 2004; A. K. Silva et al., 2007a). Concerning the *in vivo* use, the following parameters are critical: (a) particle size, (b) surface characteristics of the particle, (c) concentration of the fluid, (d) volume of the fluid, (e) administration route, (f) duration/rate of the injection/infusion, (g) geometry and strength of the magnetic field, (h) duration of magnetic field application, (i) particle stability, and (j) magnetic properties. Physiological parameters of the patient organism are also important. They comprise: a) size, weight, and body surface, (b) blood volume, (c) cardiac output and systemic vascular resistance, d) circulation time, (e) tumor volume and location, (f) vascular content of target area, and (g) blood flow in that area (A. A. Kuznetsov et al., 1999; Lübbe et al., 1999).

Markedly, size is a crucial factor. Large microspheres can physically irritate the surrounding tissue or even embolize small blood vessels and capillaries. Besides, stable suspensions of dense particles larger than 2μm are rarely prepared, and it is difficult to inject suspensions of such particles through a catheter. On the other hand, very small particles (less than 0.1 μm in diameter) have a small magnetic moment. In such a case, magnetic forces may not be high enough to counteract the linear blood-flow rates in the tissue. As a consequence, the magnetic field may fail in successfully concentrating particles at the target organ, with also the possibility of a significant fraction of them accumulating in the liver (Häfeli, 2004; Lübbe et al., 2001).

Surface charge is known to play an important role in blood half-lives of particles. It is generally agreed that highly positively and negatively charged particles present a decreased circulation time. In such a case, particles undergo phagocytosis, resulting in distribution mainly in the liver or spleen. The clearance from circulation is mediated by interaction with cells, especially those of the reticulo-endothelial system. Functional groups on cell surfaces

alter the circulation time. A usual approach consists of grafting magnetic systems with PEG (polyethylene glycol), which may be achieved by the precipitation of the particles in the presence of this polymer. By such a technique, sterically stabilized carriers are produced due to the induced sterical hindrance, which avoids protein binding and macrophage recognition (Bulte et al., 1999).

Since the particles must be effectively controlled by the applied magnetic field, their magnetic properties, their dispersion, and their degree of agglomeration are important. It has been observed that an increase in stability of the particles leads to a decrease in toxicity. Low coercive force will prevent aggregation of the particles prior to superimposition of the field. As a result, superparamagnetic particles seem to be ideal. Besides superparamagnetism, high magnetic susceptibility and high saturation magnetization allow the particles to be effectively controlled by a relatively weak field (A. A. Kuznetsov et al., 1999).

The fate of magnetic particles also depends strongly on the administration route:

3.1 Intravenous administration

After intravenous administration, smaller particles are subject to rapid renal elimination or are removed by cells capable of endocytosis (i.e., by B and T lymphocytes), while larger ones undergo uptake by the liver, spleen, and bone marrow. The blood half-lives of many iron oxide nanoparticles administered in patients vary from 1 h to 24–36 h (Corot et al., 2006). Iron oxide particles present low toxicity and are well tolerated in the human body. Inside the cells, such systems are expected to be degraded relatively fast. In fact, degradation into iron (Fe) and oxygen is presumed to occur in intracellular lysosomes of macrophages under the influence of a variety of hydrolytic enzymes, low pH, and protein mobilization and utilization according to natural Fe pathways. The human body contains around 3–4 g of Fe, for example, in the proteins ferritin, hemosiderin, transferritin, and hemoglobin. As the magnetic nanoparticles start to break down, any soluble Fe becomes part of this normal Fe pool, which is then regulated by the body. A clinical dose would likely include just a few milligrams of Fe per kilogram of body weight, which is low compared to the total participating in Fe metabolism. Iron oxides have been shown to degrade *in vivo* and integrate Fe stores in the human body. Therefore, they are not expected to be toxic to the organism (Mornet et al., 2004). This assumption concerning the low toxicity was confirmed by the lethal dose LD50 of magnetic systems. For instance, the LD50 of dextran–iron oxide complex was found to be 2000–6000 mg of Fe kg–1 of body mass. Besides, the systemic safety of several iron oxide nanoparticles has been evaluated after injection in humans, indicating that these products have a satisfactory safety profile according to standard toxicological and pharmacological tests (Corot et al., 2006).

3.2 Subcutaneous and intratumoral administration

Small particles injected locally infiltrate into the interstitial space around the injection site and are gradually absorbed by the lymphatic capillaries into the lymphatic system. For this reason, subcutaneously or locally injected (intratumoral administration) nanoparticles can be used for lymphatic targeting, i.e., as a tool for chemotherapy against lymphatic tumors or metastases. In order to achieve a good uptake in regional lymph nodes following subcutaneous injection, colloidal carriers should be small (60 nm or smaller) and the surface of the particles should be neither too hydrophilic nor too hydrophobic. Concerning intratumoral administration, different studies indicate its feasibility (Hilger et al., 2002, 2005).

3.3 Oral administration

Magnetic particles exhibit strong potential as externally modulated oral systems for both *in vivo* imaging and targeted drug delivery. In this approach, imaging agents or drugs can be localized to specific sites through the application of an external magnetic field. Similarly to the other routes of administration, the fate of the particles in the gastrointestinal tract is closely related to the particle size. Particles under 5 μm can be removed via lymphatic drainage, particles up to 500 nm can cross the membrane of epithelial cells through endocytosis, and particles less than 50 nm can achieve the paracellular passage between intestinal epithelial cells. The use of the magnetic force to delay the transit of orally administered drugs may become an attractive strategy for enhancing the efficacy of orally delivered systems. Despite the promising properties, magnetic particles may dissolve in acid media (Silva-Freitas et al., 2011). Such possible particle loss could reduce the efficiency of the magnetic system used as a drug carrier. In order to avoid it, magnetic particles may be coated using polymers to protect them from the gastric environment. Our recent studies have shown that xylan and Eudragit®S100 coatings are able to protect magnetite from the gastric pH, successfully preventing particle degradation (A. K. Silva et al., 2007b; É. L. Silva et al., 2009).

4. Applications of magnetic particles in the field of biotechnology

4.1 The therapeutic applications

4.1.1 Magnetic drug targeting

One of the major problems related to drug administration is the difficulty in targeting a tissue or an area of the body. After administration, drugs tend to distribute to various organs in a process that occurs depending on the physicochemical properties of the molecule. In order to reach an acceptable therapeutic level at the desired site, large amounts of the drug must be administered. However, only a part of the dose will actually reach the intended tissue or disease site, while the other fraction can cause toxic side effects at non-target organs (Häfeli, 2004; A. K. Silva et al., 2010).

Magnetic drug targeting (MDT) presents a solution to this problem by using magnetic particles as controllable carriers of therapeutic agents which are either encapsulated or attached to the surface of these particles (Mangual et al., 2011). Due to its non-invasiveness, high efficiency, quick-impact, and reduced toxicity in the non-target regions, many researchers are engaging in this area (Cao et al., 2011).

MDT typically uses an external magnetic field source to capture and retain magnetic drug carrier particles at a specific site after being administered in the body. The accumulation of the magnetic particles in the desired area depends on the interplay of the magnetic forces, fluid resistance, and diffusive motions (Cao et al., 2011).

There are some significant limitations of MDT. One limitation is the gradient problem, once the retention of the magnetic particles is quite low due to the relatively weak nature of the magnetic force, which must overcome the hydrodynamic force (A. K. Silva et al., 2006). It can be difficult to use external magnets to target areas deep within the body by the fact that the strength of the magnetic field generated from a permanent magnet decreases sharply with distance (Mangual et al., 2011).

An approach to overcome this limitation uses implant assisted-MDT that takes advantage of a magnetic implant placed inside the body that can become magnetized in the presence of

an external magnet creating a localized magnetic field around the implant and increasing the magnetic force on nearby magnetic particles (Mangual et al., 2011).

In addition, magnetic properties and the internalization of particles depend strongly on the size of the magnetic particles. Some hydrodynamic parameters, such as blood flow rate, particle concentration and infusion route play significant roles. Also, there are several forces acting on magnetic particles in a viscous environment and magnetic field, such as magnetic force due to all field sources, viscous drag force, inertia, gravity, thermal kinetics, particle fluid interactions and inter-particle effects such as magnetic dipole interactions, electric double layer interactions, and van der Waals force (Babincova & Babinec, 2009).

The possibilities of MDT applications have drastically increased in recent years. In the clinical area of human medicine, these particles are being used as delivery systems for several drugs, mainly chemotherapy drugs. Such approach may represent a new method to treat cancer. Also, MDT has been used in radiotherapy and immunotherapy. Fig. 1 demonstrates a schematic MDT model for cancer treatment (Alexiou et al., 2011).

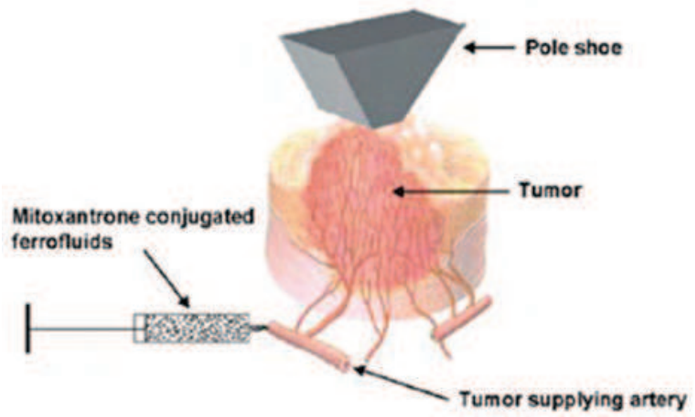

Fig. 1. Schematic drawing of MDT for tumor treatment. Reprinted from the *Journal of Magnetism and Magnetic Materials*, Vol 323, C Alexiou, R Tietze, E Schreiber, R Jurgons, H Richter, L Trahms, H Rahn, S Odenbach, S Lyer, Cancer therapy with drug loaded magnetic nanoparticles--magnetic drug targeting, 1404-1407, Copyright (2011), with permission from Elsevier. License number 2690150177916.

Our research group has developed a new technology for oral delivery of drugs for the treatment of *Helicobacter pylori* infections. The focus of this work was the development of a stomach-specific formulation of amoxicillin based on drug-containing Eudragit®S100 microparticles with a magnetite core. This could be the first attempt to prepare a magnetic system for local delivery of antibiotics, in particular for the treatment of *H. pylori* infections (É. L. Silva et al., 2009).

4.1.2 Magnetofection

Several systems, including viral and non-viral carriers, have been developed to transfer foreign genetic material into cells with the aim of enhancing gene transfer *in vitro* and *in vivo*. Viral vectors can provide a high transfection rate and a rapid transcription of the genetic material inserted in the viral genome. However, viral carriers present potential problems for patients, such as the immunogenicity of the viral proteins, lack of desired

tissue selectivity, potential for oncogenesis due to chromosomal integration, and generation of infectious viruses due to recombination.

Non-viral vectors are less immunogenic, are easy to produce in large scale and capable of delivering large genetic material, exhibit enhanced biosafety, and can be associated with tissue targeting. However, unlike viral analogues that have evolved to overcome cellular barriers and immune defense mechanisms, non-viral gene carriers exhibit significantly lower transfection efficiency compared with the viral ones. Among the non-viral vectors, the most used compounds are cationic and biodegradable polymers, lipids, liposomes, and niosomes (He et al., 2010).

In order to overcome those disadvantages, a new transfection method called magnetically guided gene transfection or magnetofection has been developed. Magnetofection employs magnetic nanoparticles combined with transfection agents to form magnetic gene vectors so that the vectors can be rapidly concentrated on the surface of target cells under the attraction of a magnetic field (Yunfeng, 2010).

Since the first reports on magnetically enhanced nucleic acid delivery, it has become a well-established method and has been predominantly used to potentiate viral and non-viral gene delivery. The nucleic acids can be directly associated with magnetic nanoparticles in naked form or can be incorporated into a complex composed of magnetic particles and other components, e.g., cationic lipids and polymers. Fig. 2 shows the principle of magnetofection *in vitro* (Schillinger et al., 2005).

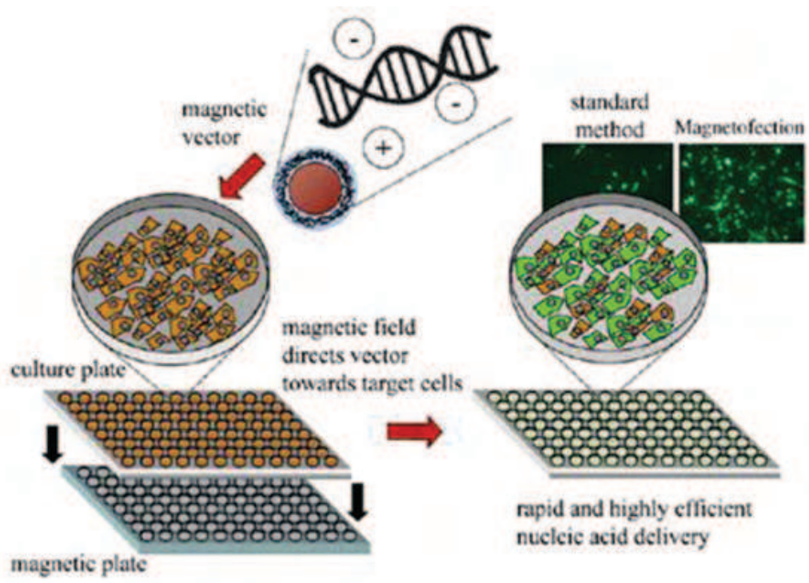

Fig. 2. Illustration of magnetofection in cell culture. The magnetic force pulls magnetic nanoparticles with surface-bound gene vectors toward the cells and results in rapid kinetics and high efficiency nucleic acid delivery. Reprinted from the *Journal of Magnetism and Magnetic Materials*, Vol 293, U Schillinger, T Brill, C Rudolph, S Huth, S Gersting, F Krötz, J Hirschberger, C Bergemann, C Plank, Advances in magnetofection--magnetically guided nucleic acid delivery, 501-508, Copyright (2005), with permission from Elsevier. License number 2690150501010.

The magnetofection process has substantial advantages over traditional transfection methods. The process time is substantially reduced; high transfection rates can be obtained with lower vector doses; an increase in the gene transfer efficiency can be reached; and gene delivery can be achieved with non-permissive cells. The association of gene vectors with superparamagnetic nanoparticles under a magnetic field can boost the efficiency of many vector types up to several hundred-fold and this technique is available for most gene vectors. The accumulation of the vectors in the target area and the dose–response relationship with a smaller amount of DNA required for sufficient gene expression can be enhanced by magnetofection methods (Holzbach et al., 2010).

4.1.3 Magnetic embolization

The conventional cancer treatment is based on chemotherapy and radiotherapy, which are effective treatment, but these systemic approaches typically have an effect on both healthy and disease tissues (Yang et al., 2011). Therefore, it is necessary to study novel strategies to eradicate cancer cells. An alternative approach to overcome these disadvantages is magnetic embolization, which is a new technique in cancer therapy presenting less toxicity than chemotherapy and less invasiveness than surgery.

Magnetic embolization consists of injecting (in a blood vessel) a magnetorheological (MR) fluid, which is a suspension of micron-sized magnetizable particles such as Fe or iron oxide particles (e.g., magnetite). The microscopic structures of these fluids change in the presence of a magnetic field, which leads to a phase transition from a liquid to a solid. MR fluids solidify only under a magnetic field. A seal is formed, which mechanically blocks the tumor blood vessels, causing its death. Once the field is removed, thermal energy makes the fluid return to its original liquid state (J. Liu et al., 2001).

Flores and Liu showed that blocking the fluid flow is possible within a single-tube system, simulating one blood vessel. They found that the seal remains stable even at pressures exceeding those found inside human arterioles and capillaries (Flores et al., 1999; J. Liu et al., 2001). Despite some promising results, it is difficult to obtain selective embolization of small blood vessels when these are positioned at a large distance from the magnetic field source (e.g., approximately >3 cm inside the body of the patient). In this case, a suitable magnetic field intensity and gradient in the vicinity of the vessel is required (A. K. Silva et al., 2010).

A possible solution to this problem may be obtained by a new approach referred to as magnetic resonance navigation (MRN). This has been proposed to steer and track in real time endovascular magnetic carriers in deep tissues to target areas of interest (Pouponneau et al., 2010), and restrain the systemic carrier distribution. MRN is achieved with a clinical magnetic resonance imaging (MRI) scanner upgraded with an insert of steering coils. Therefore, MRN can overcome the problem of a weaker magnetic field in deep tissues observed with an external magnet. By controlling chemoembolic material distribution, MRN could improve embolization and drug concentration in the tumor area while limiting chemoembolization of healthy blood vessels and the hepatic complications (Kennedy et al., 2010).

MRN significantly controlled the distribution of these therapeutic particles as compared to the control. More importantly, a decrease in the TMMC (Therapeutic Magnetic Microcarrier) levels in the untargeted lobe was obtained. Steering efficiency was higher with the left steering compared to the right steering. Therefore, MRN was more efficient in preserving the right liver lobe from the chemoembolization than left lobes. Only one-third of the TMMC dose reached the right lobe without steering because of the natural difference in

blood supply between the lobes (the right lobes have less weight than left lobes) (Pouponneau et al., 2011) (Fig. 3). Thus, further work is necessary to confirm the feasibility of MRN applied to magnetic embolization.

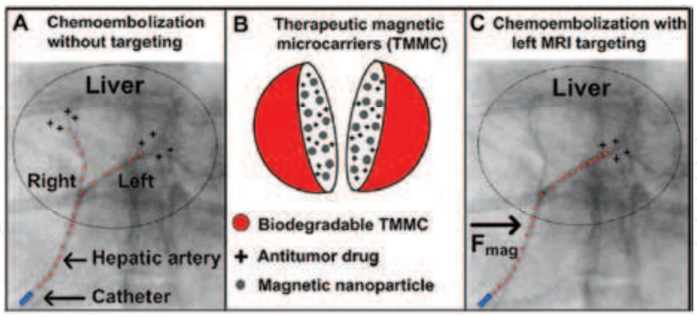

Fig. 3. Representation of MRI targeting with PLGA microparticles loaded with the doxorubicin and magnetic FeCo nanoparticles for liver chemoembolization. Images A and C are fluoroscopy images of the rabbit hepatic artery with superposed images of the microparticle distribution without (A) and with (C) the MRI targeting. On image A, the microparticles are released from the catheter in the artery and distributed to both lobes. Image B illustrates a schematic representation of a cut of the microparticles loaded with an antitumor drug and magnetic nanoparticles embedded into a biodegradable matrix. Image C displays the MRI targeting of the left bifurcation using the magnetic force (Fmag) to preserve the right lobe from the chemoembolization. Reprinted from Biomaterials, Vol 32, P Pouponneau, J-C Leroux, G Soulez, L Gaboury, S Martel, Co-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation, 3481-3486, Copyright (2011), with permission from Elsevier. License number 2690150825494.

4.1.4 Tissue engineering

Advances in cell therapy research gave rise to a fast-growing multidisciplinary field that integrates knowledge of engineering, biology and medicine. Tissue engineering (TE) is a promising technology for overcoming the organ transplantation limitations related to organ donor shortage. It consists of appropriately using cells, materials, and physics/biochemical processes to restore, maintain, or improve tissue function (Fig. 4).

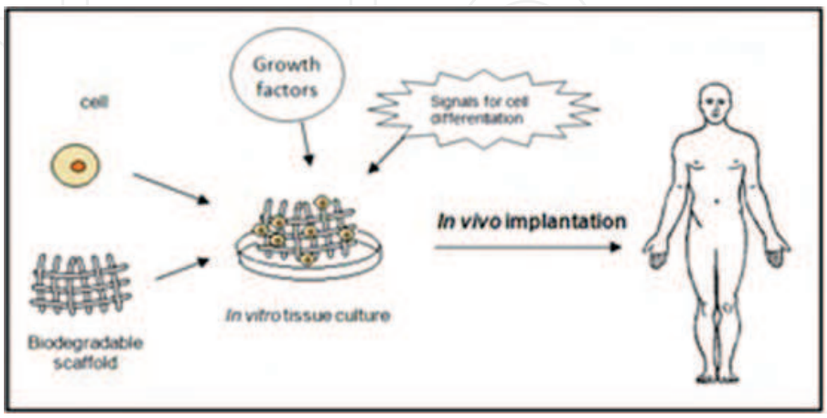

Fig. 4. Schematic picture of tissue engineering. The combination of a scaffold, cells and soluble factors facilitate the formation of structural and functional tissue units.

Despite successful efforts and results, tissue-engineered constructs lack structural complexity. Well-defined spatial cell organization is required in the attempt to reproduce living tissue complexity and succeed in creating functional tissue constructs. In this regard, several cell patterning methods such as microcontact printing and lithography have been developed. However, these methods demand specialized surfaces to be used as substrates and fabrication is time consuming. In order to bypass these shortcomings, innovative active patterning approaches have been based on the use of an external force. This constraint may be generated by an electric field, as in the dielectrophoresis technique, or by an optical trap. Alternatively, Ino et al. (Ino et al., 2007, 2008) suggested the use of magnetic force to induce two-dimensional patterning of magnetically-labeled cells on submillimetric scales.

This group developed a novel methodology for cell patterning using magnetic force and magnetite cationic liposomes (MCLs), which contain 10 nm magnetite nanoparticles. MCLs were used as carriers to introduce magnetite nanoparticles into target cells since the positively charged particle surface interacts with the negatively charged cell surface. Given that cells labeled with magnetic nanoparticles can be manipulated by using a magnet, they were able to seed labeled cells onto a low-adhesive culture surface through the use of magnetic force to form a tissue construct (Ino et al., 2007). This technique was designated magnetic force-based tissue engineering (Mag-TE). By using it, complex cell patterns (curved, parallel, or crossing motifs) were successfully fabricated from several cell types (Ino et al., 2007). This group also showed that magnetically labeled keratinocytes were accumulated using a magnet, and stratification was promoted by a magnetic force to form a sheet-like 3D construct. Transmission electron microscopy revealed the presence of intercellular adhesion proteins, desmosomes, within keratinocyte sheets constructed by Mag-TE. This result indicated that the proposed method enabled intercellular contact and preserved adhesion proteins. For this reason, patterns were found to resist after magnetic force removal and manipulation was also feasible.

Following a related approach, Frasca et al. applied magnetic forces to create a 3D cell assembly with tuneable size and controlled geometry. Cells were magnetically labeled using anionic citrate-coated iron oxide nanoparticles. Focalized magnetic force ensured an efficient entrapment of the cells at the magnet vicinity. This technology could be applied with no restriction regarding the physicochemical nature of the substrate, the cell type, or the geometry of the imposed magnetic constraint (Frasca et al., 2009). The same group demonstrated that magnetic force-assisted cell seeding provided effective cell seeding into 3-D porous scaffolds. Moreover, precise spatial cellular organization inside the scaffold could be achieved by means of magnetic microtips that develop high magnetic forces.

4.1.5 Magnetic hyperthermia

Hyperthermia is a technique that increases the temperature of the local environment of a tumor, resulting in changing the physiology of diseased cells and finally leading to apoptosis. Depending on the degree of temperature rise, hyperthermia treatment can be classified into different types. In thermo ablation, a tumor is submitted to high temperatures of heat >46 °C (up to 56 °C), causing cells to undergo direct tissue necrosis, coagulation, or carbonization. Moderate hyperthermia (41 °C <T< 46 °C) has various effects both at the cellular and tissue levels. Diathermia uses lower temperatures (T<41 °C) for the treatment of rheumatic diseases in physiotherapy.

During moderate hyperthermia, which is traditionally termed hyperthermia treatment, cells undergo heat stress resulting in activation and/or initiation of many intra- and extracellular degradation mechanisms, including induction and regulation of apoptosis, signal transduction, multidrug resistance, and heat shock protein expression. The tissue-level effects include pH changes, perfusion, and oxygenation of the tumor microenvironment (Santos-Marques et al., 2006).

Nevertheless, traditional hyperthermia treatment presents several challenges: 1) unavoidable heating of healthy tissue resulting in burns, blisters, and discomfort; 2) limited penetration of heat into body tissues; and 3) thermal under-dosage in the target region, a nearly unsolved problem in the case of bone of the pelvis or scull, which shields deep tissues, often yielding recurrent tumor growth (Tanaka et al., 2005).

Improvements in this technique yielded the magnetic hyperthermia treatment, which has a number of advantages compared to conventional hyperthermia treatment: 1) cancer cells absorb magnetic nanoparticles (MNPs), therefore increasing the effectiveness of hyperthermia by delivering therapeutic heat directly to them; 2) MNPs can be targeted through cancer-specific binding agents, making the treatment much more selective and effective; 3) the frequencies of oscillating magnetic fields generally utilized pass harmlessly through the body and generate heat only in tissues containing MNPs; 4) MNPs can also cross the blood-brain barrier, and thus can be used for treating brain tumors; 5) effective and externally stimulated heating can be delivered at cellular levels through alternating magnetic fields; 6) stable MNPs can be administered through a number of drug delivery routes; 7) MNPs used for hyperthermia are only a few tens of nanometer in size and therefore allow easy passage into several tumors whose pore sizes are in the 380–780 nm range; 8) compared to macroscopic implants, MNP-based heat generation is much more efficient and homogeneous; 9) MNP-based hyperthermia treatment may induce antitumoral immunity (Ito et al., 2005); and 10) the last but most important aspect is that MNP-based hyperthermia can also be utilized for controlled delivery of drugs (Lu et al., 2005). This additional feature opens up possibilities for the development of multifunctional and multi-therapeutic approaches for treating a number of diseases.

For MNP-based hyperthermia, a general procedure involves the distribution of particles throughout the targeted tumor site, followed by generation of heat to the tumor using an external AMF. The absorption efficiency of any material to generate heat due to AMF is measured in terms of a specific absorption rate or specific loss of power. These terms are generally used to define the transformation of magnetic energy into heat. For a majority of applications, it is desirable to have higher temperature enhancement rates.

Heat generation can be attributed to two different phenomena: relaxation and hysteresis loss. The relaxation is of two types: Brownian and Néel. Brownian relaxation is due to the physical rotation of particles within the medium in which they are placed (external dynamics) and is hindered by the viscosity that tends to counter the movement of particles in the medium. Heat generation through Néel relaxation is due to rapidly occurring changes in the direction of magnetic moments relative to crystal lattice (internal dynamics). This is hindered by the energy of anisotropy that tends to orient the magnetic domain in a given direction relative to crystal lattice. For intracellular magnetic fluid hyperthermia, Néel relaxation is the major contributor for heat release (Hergt & Dutz, 2007).

A number of types of magnetic nanomaterials have been investigated for magnetic hyperthermia. Some of the well-known hyperthermic agents based on iron oxide are magnetite and maghemite nanoparticles. Recently, a high heating performance of 1300–1600W/g was reported based on FeCo metallic nanoparticles (Nojima et al., 2010). However, iron oxide-based MNPs continue to attract attention due to their lack of toxicity, excellent biocompatibility, and metabolization, which is carried out by heme oxygenase-1 to form blood hemoglobin and hence maintain Fe cell homeostasis in cells. In addition, magnetite was found to be superior to cobalt nanoparticles with respect to its high Curie temperature, saturation magnetization (90–98 emu/g), and lower toxicity in preclinical tests (Martina et al., 2008).

In vivo studies using magnetic hyperthermia have been conducted by Matsuoka et al. (Matsuoka, 2004). They have developed magnetic cationic liposomes based on superparamagnetic iron oxide nanoparticles and investigated their *in vivo* efficacy for hyperthermia treatment of hamster osteosarcoma. Magnetoliposomes were injected directly into the osteosarcoma and then subjected to an alternating magnetic field. The tumor was heated above 42°C, and complete regression was observed in 100% of the treated hamsters. Therefore, these results demonstrate the feasibility of magnetic hyperthermia.

4.2 The diagnostic uses

4.2.1 MRI contrast agent

MRI is a noninvasive and sensitive technique to obtain images by non-ionizing radiation. As it associates rapid *in vivo* acquisition of images, long effective imaging window, fine signal intensity contrast, high temporal and spatial resolution, and simultaneous information about physiology and anatomy of the desired area, MRI quickly became one of the major tomographic imaging modalities (G. Liu et al., 2011).

The images formed are the result of several parameters such as proton density, relaxation times (T1, T2, T2*), water diffusion, nuclear alignment, radio frequency excitation, spatial encoding, etc., providing a digital representation of tissue characteristics. The relative difference between the signal intensity of two adjacent regions is called contrast, and this difference is translated using a color scale (normally the grey scale for MRI). The spin–lattice (1/T1 = R1) and spin–spin (1/T2 = R2) relaxation rates of the water protons in tissues, which are the most important intrinsic factors for contrast, are dependent upon the local environment of the protons, so that different tissues will relax at different rates (Yurt & Kazanci, 2008). However, for specific studies of evaluation at the molecular and cellular level, the MRI sensibility is lower, being necessary the use of a contrast agent or even a selective binding attached to a contrast agent, in order to differentiate the target area (Lalatonne, 2010).

Contrast agents are normally defined based on their relaxation properties, their magnetic properties, and their biodistribution. When defining a contrast agent based on relaxation properties, the efficiency is described by the longitudinal and transverse relaxivity R1 and R2, respectively. The relaxivity reflects the change in the relaxation rate as a function of the contrast agent concentration. The relaxivities are affected by the size and the composition of these particles (Yurt & Kazanci, 2008).

To design a contrast agent, the choice of core and monolayer material is a critical step because this composition determines the primary physical and chemical properties besides reactivity, solubility, and interfacial interactions. Most common core among the MRI contrast agents are paramagnetic lanthanide metals (gadolinium, manganese and dysprosium ion complexes) and superparamagnetic magnetite particles (iron oxides) (Yurt & Kazanci, 2008). Iron oxide particles are widely investigated in MRI applications as they alter the relaxation times of tissues in which they are present and due to the low toxicity when compared to gadolinium chelates (Lalatonne, 2010). In this context, the superparamagnetic particles, which can be superparamagnetic iron oxide (SPIO) particles, ultrasmall superparamagnetic iron oxide (USPIO) and oral magnetic particles (OMPs), appear as preferred materials because (a) they have magnetic characteristics, (b) they are composed of biodegradable Fe, (c) their coating can be functionalized with various ligands, (d) they provide the greatest signal changes per unit of metal, and (e) they are easily detectable by light and electron microscopy (Bulte & Kraitchman, 2004).

Superparamagnetic iron oxides have substantially larger T2 relaxivity compared with gadolinium chelates in current clinical use, typically by an order of magnitude or more. This increase is confirmed by a superior magnetization. The T1-relaxivity can also be much higher for iron oxides than for gadolinium chelates. In addition, iron oxide nanoparticles may offer several advantages over existing agents due to their accumulation in macrophages combined with an intravascular distribution and higher relaxivity values (Bulte & Kraitchman, 2004).

Another field of research in development aims to use superparamagnetic contrast agents in drug delivery applications for real-time monitoring of drug distribution to the target tissue, as well as to follow the effect of therapeutics on the progression of disease.

4.2.2 Magnetic cell tracking

There is a great need to develop improved means of monitoring transplanted cells *in vivo*. A recent methodology involves the use of magnetic particles for intracellular magnetic labeling of cells. This technique, called magnetic cell tracking, allows *in vivo* tracking of implanted cells via MRI. Magnetic cell tracking can be used as a non-invasive tool to provide unique information on the dynamics of cell movements within and away from tissues *in vivo*. Alternatively, magnetic cell tracking could be applied in the future to monitor cell therapy in patients. Both approaches require magnetic labeling of cells as well as methods for analysis and evaluation of cell labeling (Vuu et al., 2005).

The magnetic cell tracking technique may overcome the limitations of individual *in vivo* imaging methods including low sensitivity, low resolution, or low soft tissue contrast. MRI provides excellent soft tissue contrast and due to its high resolution, MRI can be used for the visualization of single cells against a homogeneous background (Himmelreich & Dresselaers, 2009).

Several methods have been developed to incorporate sufficient quantities of iron oxide nanoparticles into cells. These methods mainly concern the prolonged incubation of the cells with the particles resulting in their passive internalization. Another possibility is the introduction of functional ligands chemically linked to the particles, in order to increase the uptake by cells. Besides, the transient increase in the membrane permeability using a

magnetic field (magneto-electroporation) may result in a quick cytoplasmic accumulation of the magnetic particles (Dousset et al., 2008).

Some other examples of magnetic cell tracking applications include labeling mesenchymal stem cells, haematopoietic progenitor cells, Schwann cell transplants, neural stem cells, and NK cells.

4.2.3 Monitoring the gastrointestinal motility

The evaluation of the large intestine motility is usually made by intraluminal manometry, radiology, or scintigraphy. Most of the current knowledge about motility of the large intestine was generated by intraluminal manometry. Despite its providing quantitative assessment, intraluminal manometry is obviously invasive and uncomfortable for patients. Radiology offers qualitative or, at best, semi-quantitative information, and carries the risk of significant radiation exposure. Gamma-scintigraphy also imposes radiation exposure and depends on the availability of expensive equipment (Ferreira et al., 2004).

Among other methods, the investigation of intestinal movements by Magnetic Marker monitoring is considered to be a useful diagnostic tool. The colon exhibits complex motor patterns with variations of frequency and amplitude yielding compaction and movement of its contents along its extension. The arrival of a meal into the stomach is consistently associated with the unleashment of contractions of the large intestine, which causes movements of the colonic content, called gastrocolic reflex, and can be observed by an increase in the motor activity of the colon (Ferreira et al., 2004).

The oral route is still by far the most common way used for the administration of pharmacologically active substances. This is mainly due to the ease of administration and the general acceptance by the patients. Knowledge about the performance of dosage forms in the gastrointestinal tract is essential for the choice of the optimal formulation technology (Weitschies et al., 2010). In order to overcome restrictions that are associated with the use of radioisotopes, an alternative method for the investigation of the behavior of solid dosage forms in the gastrointestinal tract was developed. It is based on the labeling of the dosage as a magnetic dipole by means of incorporation of trace amounts of ferromagnetic particles, recording of the magnetic dipole field using biomagnetic measurement equipment, and data evaluation applying techniques established in magnetic source imaging (MSI). This method is known as Magnetic Marker Monitoring (MMM) or Magnetic Moment Imaging (MMI). (Goodman et al., 2010; Weitschies et al., 1994).

MMM is a new technique for the investigation of the gastrointestinal transit of magnetically marked solid drug dosage forms (Weitschies et al., 1999). The magnetic labeling of the dosage forms is achieved by the incorporation of small amounts of remanent ferromagnetic particles and their subsequent magnetization tracking. After ingestion of one magnetically marked dosage form, its magnetic dipole field is recorded during its gastrointestinal transit. Multichannel superconducting quantum interference devices (SQUID), developed for the detection of extremely weak biomagnetic fields, are employed for the measurement of the magnetic field (Drung, 1995). Finally, the parameters describing the magnetic dipole, i.e., its location r =(x, y, z) and its magnetic moment m=(m_x, m_y, m_z), are estimated from the recorded data by means of fitting procedures. After ingestion, their magnetic dipole field is recorded, and by means of fitting procedures, the location of the marked dosage form is

estimated from the recorded data. The disintegration behavior is also assessed by this technique. The induction generated by the magnetic dipole moment of the oral dosage form during disintegration is used for the investigation of its mechanism and quantitative determination of the process (Weitschies et al., 2001a, 2001b).

Additionally, MMM has been applied for the determination of the performance of disintegrating and non-disintegrating solid dosage forms such as tablets, capsules, and pellets in the gastrointestinal tract, as well as for the determination of the *in vivo* drug release from modified release products such as enteric-coated tablets and enhanced release tablets (Weitschies et al., 2005a).

The combination of MMM with the pharmacokinetic measurements (pharmacomagnetography) enables the determination of *in vitro–in vivo* correlations and the delineation of absorption sites in the gastrointestinal tract (Weitschies et al., 2005b). The results obtained with MMM can also serve as a data base for the development of improved pharmacokinetic models.

5. Conclusion and perspectives

The use of magnetic particles in the medical field opens new prospect of selective treatment of local tissues where efficiency is increased through local concentrations while, at the same time, general side effects can be avoided. However, the use of magnetic carriers in the human body imposes several requirements on the magnetic carriers. Magnetic carriers must be water-based, biocompatible, biodegradable, and nonimmunogenic. Besides, special care should be focused on the particle size, surface properties, magnetic properties, and administration route, for example. In most of the reports in the literature, iron oxides are the material of choice for the development of magnetic systems for therapeutic purposes.

Several methods have been proposed for their synthesis, coating, and stabilization. Magnetic systems produced by different methods have found many applications in biotechnology. The safety aspect, the non-invasiveness, and the high targeting efficiency are promising advantages for the use of magnetic particles in therapeutics. The current challenge still consists of totally controlling the biocompatibility, stability, biokinetics, and properties of the particles. By incorporating advances in surface engineering, molecular imaging, and biotechnology, magnetic systems have great potential to enable physicians to diagnose and treat diseases with greater effectiveness than ever before.

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Biotechnology is the scientific field of studying and applying the most efficient methods and techniques to get useful end-products for the human society by using viable micro-organisms, cells, and tissues of plants or animals, or even certain functional components of their organisms, that are grown in fully controlled conditions to maximize their specific metabolism inside fully automatic bioreactors. It is very important to make the specific difference between biotechnology as a distinct science of getting valuable products from molecules, cells or tissues of viable organisms, and any other applications of bioprocesses that are based on using the whole living plants or animals in different fields of human activities such as bioremediation, environmental protection, organic agriculture, or industrial exploitation of natural resources. The volume Advances in Applied Biotechnology is a scientific book containing recent advances of selected research works that are ongoing in certain biotechnological applications. Fourteen chapters divided in four sections related to the newest biotechnological achievements in environmental protection, medicine and health care, biopharmaceutical producing, molecular genetics, and tissue engineering are presented.

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