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Magnetic nanoparticles for local drug delivery using magnetic implants

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Abstract

Magnetic nanoparticles are good candidates used for the targeted delivery of anti-tumor agents. They can be concentrated on a desired region, reducing collateral effects and improving the efficiency of the chemotherapy. We propose a method in which permanent magnets are implanted by laparoscopic technique directly in the affected organ. This method proposes the use of Fe@C nanoparticles, which are loaded with doxorubicin and injected intravenously. The particles, once attracted to the magnet, release the drug at the tumor region. This method seems to be more promising and effective than that based on the application of external magnetic fields.

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Keywords: Magnetic nanoparticle; Magnetic implant; Biocompatibility; Local therapy; Drug delivery

1. Introduction

The targeted delivery of anti-tumor agents adsorbed on the surface of magnetic nanoparticles is a promising alternative to conventional chemotherapy. The particles, loaded with the drug, are concentrated at the target site with the aid of an external magnet. The drugs are then released on the desired area over a long period of time [1–6]. This local therapy could improve the efficiency of the treatment, reducing systemic toxicity.

However, we consider that the use of external magnets has serious limitations. The administration method is limited to an artery close to the tumor, it can only be applied to the treatment of superficial organs and, moreover, well defined magnetic field geometries are needed to perform an efficient magnetic drug delivery [7–9]. To avoid these drawbacks, we propose the implant of small permanent magnets directly into the affected zone, creating an internal local magnetic field gradient, which would be rather more effective than those produced by external magnets. Our method allows a selective treatment of tumors localized in deep organs were a large field gradient of the order of 5 kOe/cm is achieved at the vicinity of the magnet. A recent theoretical approach modeled the behavior of magnetic fluids under the influence of a permanent magnet stent [10–13]. Preliminary in-vitro results of blood compatibility and drug uptake and release as well as a preclinical in-vivo experimental animal model are described, showing the reliability of the proposed method.

2. Materials and methods

2.1. Preparation of the magnetic biofluids

The magnetic particles used for this research are Fe@C 200 nm core-shell nanoparticles, synthesized by means of the arc-discharge method [14]. The Krätschmer-Huffmann

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method [15] uses a cylindrical chamber with two graphite electrodes: a stationary anode containing iron powder (ALFA-AESAR) with a beginning size of 10 μm, and a moveable graphite cathode (ALDRICH). An arc is produced between the graphite electrodes in a helium atmosphere. The graphite electrode is sublimed and builds up a deposit on the inner surface of the chamber. The material collected from this deposit consisted of carbon nanostructures, amorphous carbon, and iron and iron oxide nanoparticles encapsulated in graphitic layers. Non-coated or partially coated magnetic particles, which are not biocompatible, were eliminated by chemical etching. For magnetic purification, stable suspensions of the particles were prepared in a surfactant solution made from 2.5 g of SDS in 500 ml of distilled water. A field gradient produced by a 3 kOe permanent magnet was used for magnetic separation of this suspension. The purified material was washed with 3 M HCl at 80 °C to dissolve the exposed magnetic material and to form carboxylic groups on the carbon coating. This hydrophobic coating contributes to the stability of the magnetic suspensions. The resulting powder was then filtered.

To prepare the biocompatible magnetic suspensions 100 mg of particles were suspended in 100 ml of Gelafundine®, a commercial succinylated gel commonly used as plasma substitute of blood, and sonicated for 15 min. Human blood from 5 healthy volunteer donors was tested in vitro, before and after being mixed with the particle suspension in different dilutions (1 ml of blood and 0.06; 0.12; 0.24 and 0.5 ml of particles). The same procedure was followed for the ex-vivo tests in 10 noninjected control New Zealand rabbits and 10 test animals after intravenous injection of 1 ml of the particle suspension in Gelafundine®. Blood samples were taken 10′, 30′ and 24 h after the injection of particles.

2.2. In-vitro experiments

To test the biocompatibility of the particles in contact with circulating blood, we performed a series of hematological and rheological tests. These hemorheological parameters are usually recommended for biocompatibility [16], conventional radiological contrast media [17] and paramagnetic nanoparticle magnetic resonance imaging [18] testing. We have followed these protocols and adapted them to nanoparticles in the blood stream. Nanoparticles are similar in shape and length to large circulating plasma proteins such as fibrinogen and immunoglobulins. As these proteins have a decisive influence on plasma and blood viscosity, we have some concern on the possible effect of the nanoparticles on these two parameters. Moreover, another important parameter includes the possible increase of blood erythrocyte aggregation, which could have a dramatic effect on the micro-vessel circulation.

The blood viscosity was measured with a Brookfield® DV-3 cone-plate viscometer at different shear stresses (230, 23 and 5.7 s^{-1}). Plasma viscosity was also measured with a capillary viscometer (Fresenius). We have determined the erythrocyte aggregation using a Mirenne aggregometer after 5 and 10 s of stasis and after 5 and 10 s of very low shear stress (3 s^{-1}). The applied shear stress simulates the circulating blood in veins, capillaries and arteries. We have also done a complete blood cytometry, including differential leukocyte counting, using a hematology cell counter (Coulter LH-750®). Coagulation tests performed include the prothrombin time (PT), thromboplastin time, fibrinogen (ACL-9000® coagulation analyzer and Hemosil IL® kit), and D-dimer immuno-turbidimetry (turbiquant D-dimer Dade-Behring®).

All coagulation tests before and after mixing of human blood with different concentrations of nanoparticles were within the normal range. No D-dimer levels over 200 μg/liter were detected. Higher levels would be expected in case of activation of the coagulation system. There are neither statistical, nor clinical significant differences between control blood and blood with different concentrations of particles. The results of human blood rheology are summarized in Table 1. The ex-vivo tests with rabbit blood samples showed no significant differences between blood samples taken up at different times after the injection of particles. PT and activated partial thromboplastin time (APTT) were slightly longer in test rabbits than in controls but the difference was not statistically significant (Table 2). From a clinical point of view, some test rabbits were in a very mild hypo-coagulation state that did not exceed the recommended value for prevention of thrombosis during

Table 1
Mean values of blood viscosity (centipoises) in five samples

	Human Basal	Human 0.06 cm^3	Human 0.12 cm^3	Human 0.24 cm^3	Human 0.5 cm^3
BV 230 s^{-1}	4.44	4.36	4.45	4.33	4.1
BV 23 s^{-1}	6.7	5.0	7	5.4	6.2
BV 5.7 s^{-1}	7.2	7.6	8	6.8	6.4
EA 5M	3.4	3.4	3	2.9	2.9
EA 5M 1	10	8.9	8.4	7	6.9
EA 10M	8.3	7.6	7.6	7.1	8.8
EA 10 M 1	22.5	20.6	18	18.6	20.1

BV = blood viscosity at different shear rates (s^{-1}); EA = erythrocyte aggregation index; EA 5/10 M = integration time at 5 and 10 s of stasis, conventional units; EA 5/10 M 1 = integration time at 5 and 10 s at very low shear rate.

Table 2
Coagulation tests in rabbits

	Controls $x \pm SD$	Experimental $x \pm SD$	P (Mann–Witney)
PT	7.067 ± 0.071	9.6 ± 2.5	0.08
APTT	57.025 ± 26.4	65.920 ± 30	0.253
FIBRINOGEN	248 ± 149	381.8 ± 85.5	0.0388
D-DIMER	< 200	< 200	

PT = protrombin time (s); APTT = activated partial tromboplastin time (s); D-DIMER (μg/l).

surgery. PT and APTT experimental/control ratios were 1.35 and 1.15, respectively. The recommended ratios in humans for prophylaxis of thrombosis range from 2.0 to 3.0. The dispersant may contribute in part to this mild hypo-coagulation state in test rabbits, which is still within the tolerable limits. Fibrinogen was moderately elevated in test rabbits, as expected, because fibrinogen is an acute-phase reactant. Nevertheless the slight rise in fibrinogen is in the upper-normal range; it is within the tolerable limits and can be attributed to the stress produced for invasive procedures. Hemoglobin, erythrocytes, leukocytes (including differential count) and platelets were in the normal range both before and after the intravenous administration of particles. There were neither statistical, nor clinical significant differences.

The above data measured in normal human laboratory tests showed good biological compatibility. Mild adverse effects, however, could be detected with more sophisticated coagulation tests that will be performed in a second step. These effects include aggregation of platelets, prothrombin fragment $1+2$ and thrombin–antithrombin complex. Platelets aggregates were detected neither by the cell counter nor by microscopic examination. However, we have not explored a possible inhibition of platelet aggregation yet. This can be done with different inductors: thrombin, epinephrine or collagen. Prothrombin fragment $1+2$ and thrombin–antithrombin complexes detect minimal activation of coagulation, but are not routine tests and usually reserved for research purposes in human pathology [19]. We have in mind to adapt these techniques to rabbit blood and explore them in a second step.

The capability of the particles to spontaneously adsorb and release a drug has been studied using magnetic separation and UV spectroscopy. The kinetics of adsorption was measured following the procedure described by Kuznetsov: a 100 μg/ml solution of doxorubicin hydrochloride was mixed with 1 mg/ml of the particles in distilled water [20]. The suspension was incubated on a shaker at room temperature, and samples were taken after 5, 15, 30, 60, 90, 120 and 180 min. The adsorbent magnetic phase was magnetically separated in a gradient field produced with a 3 kOe permanent magnet, and the optical density of the supernatant measured with a UV spectrophotometer at 498 nm. The concentration of the adsorbed drug was then calculated (Fig. 1). We observed that the adsorption was completed after 3 h of incubation. The adsorption and desorption areas of the particles, obtained by the BET method, were 35 and 14 m^2/g, respectively.

The doxorubicin desorption kinetics was studied in trying to simulate in-vivo conditions. Five milligrams of the particles were incubated in a solution of doxorubicin, separated magnetically, and resuspended in 5 ml of human plasma. The suspension was incubated at 36 °C under mild stirring. The magnetic phase was magnetically separated, and the optical density of the supernatant measured with a UV spectrophotometer at 296 nm in 2 h intervals during the first day and every 8 h thereafter. The particles slowly released the drug, and after 3 d desorption was complete (Fig. 2). Although it needs to be proven in in-vivo experiments, this result confirms that doxorubicin is released from the surface of our particles at a slow enough rate to reach the affected organs and efficiently treat a tumor.

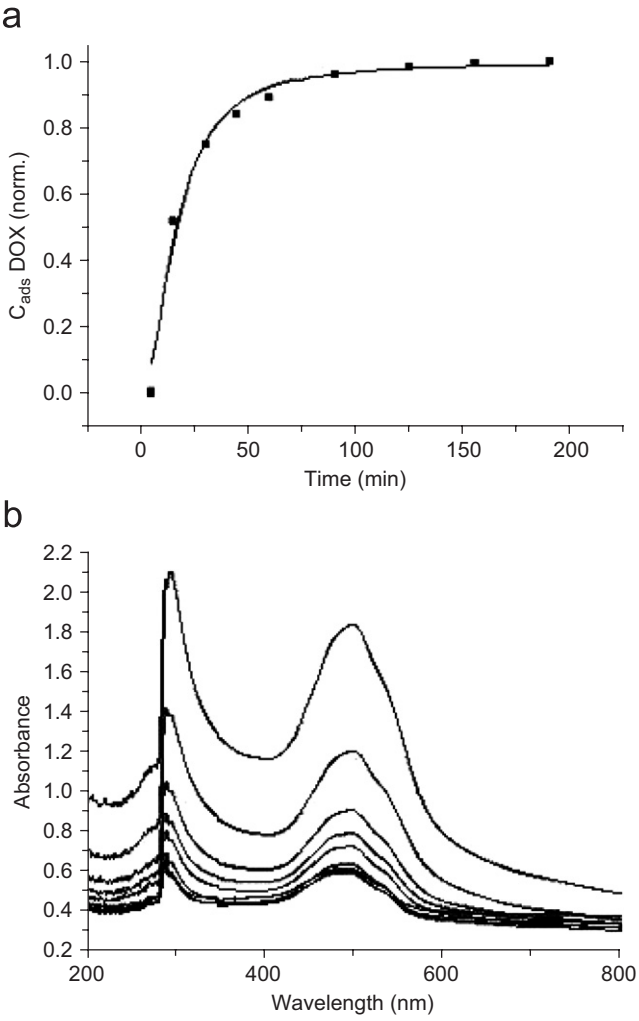

Fig. 1. (a) Kinetics of doxorubicin adsorption by the iron–carbon particles. (b) The concentration of doxorubicin, as a full spectrum, adsorbed on the particles' surface is plotted versus time of incubation.

2.3. In-vivo experiments

Stable suspensions of the carbon coated iron nanoparticles were tested in an in-vivo preclinical animal model. Magnetic implants were inserted into the main organs of 36 New Zealand rabbits and the capability of the magnet to attract the magnetic particles was tested. The experimental procedure was previously evaluated by the Ethics Committee for Animal Research of the University of Zaragoza, code PI 19/04. During surgery, the rabbits were under general anesthesia with endotracheal intubation. An intravenous needle was inserted in the marginal ear vein, fixed in it, and a perfusion of a physiologic saline solution

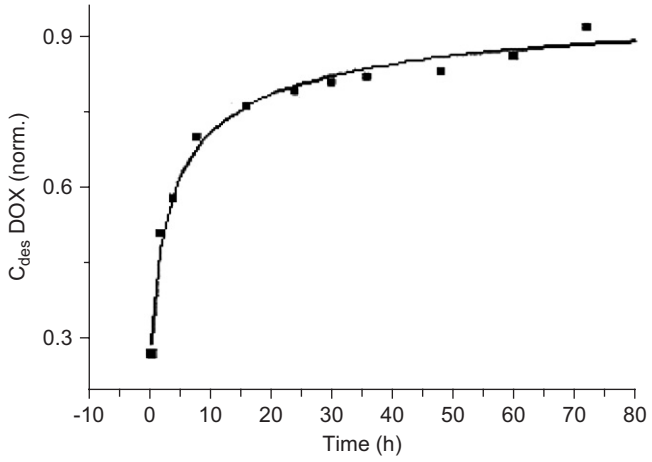

Fig. 2. Kinetics of doxorubicin desorption from the iron–carbon particles.

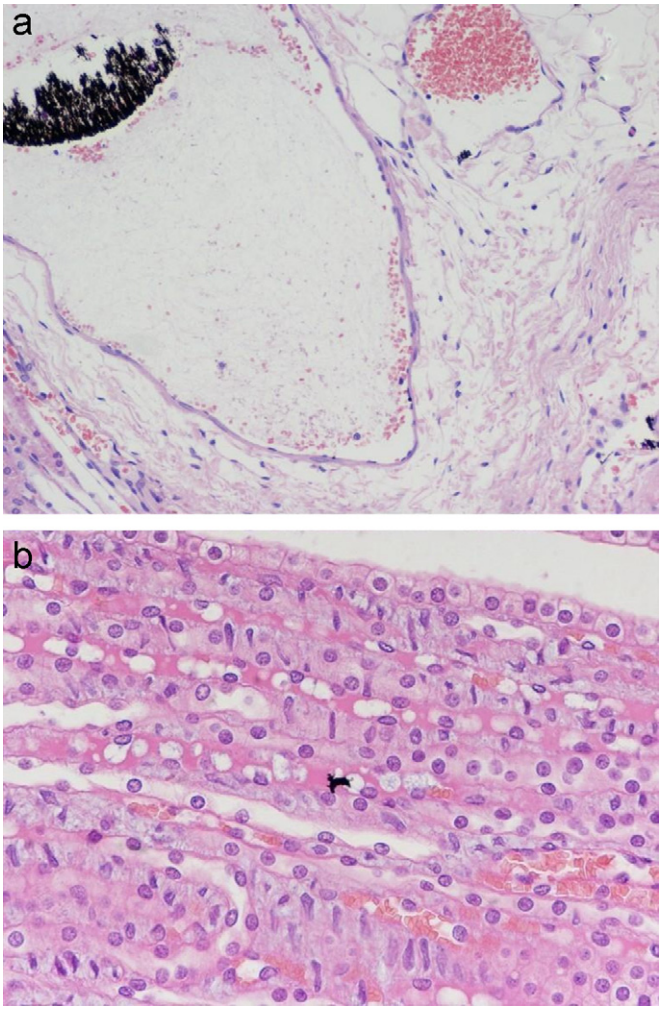

Fig. 3. Histopathology analysis. (a) Image of left kidney in which a magnet has been inserted (hematoxylin and eosin stain, 200 × magnification). The nanoparticles are clearly visible and oriented in the direction of the magnetic field gradient. (b) Right control kidney (400 × magnification). Practically no particles are observed.

was initiated. Next, the peritoneal cavity was insufflated with CO_2 through a Veress needle and a minilaparoscopic optic was introduced using a 5 mm trocar. Then, through an 8 French plastic trocar, and under endoscopic control, the lower pole of the left kidney was punctured, inserting a 4×2 mm cylindrical gold coated permanent magnet of neodymium–iron–boron. The right kidney was also punctured but no magnet was inserted. The purpose is to reproduce the same tissue damage as in the other kidney, and to have a control organ to compare the concentration of the particles attracted to the targeted organ. After controlling the absence of bleeding, the trocars were removed and the cutaneous insertion orifices were sutured.

Two milliliters of the particle suspension were injected intravenously in the marginal ear vein of the rabbit. Different groups of animals were sacrificed by terminal anesthesia after half an hour, 2, 7, 14, 21 and 28 d, and the main organs (lungs, liver, spleen, kidneys and spine) were extracted for histological study.

All the results obtained in histological analysis performed in samples obtained from different organs, showed the lack of Perls' stain reaction, as was also observed in haematological test. These results confirmed the good encapsulation of the magnetic particle core, as confirmed by the morphological characterization of the particles [15]. From histological studies, we observed a larger particle concentration in the implanted kidney in comparison to the nonimplanted one. Although it is difficult to quantify the results, a significant quantity of the particles concentrates on the kidney in which a magnet has been inserted. This is an indication of the capability of the magnet to attract particles specifically to the target organ (Fig. 3). Unfortunately, a relatively large amount of the particles was also concentrated in liver and spleen, whereas a very small amount of particles was found in the lungs of some animals. Despite the inert surface of our material, the size of the particles was big enough to be recognized by the macrophages of these organs, which capture, retain and eventually excrete the particles after several days. This fact could be a drawback for the purpose of delivering chemicals to specific targets in the body but, on the other hand, it could also be helpful for other applications, such as treatment of liver or spleen tumors, or for the treatment of leishmaniasis [21]. The obtained results open a new research direction that is under development to confirm these assumptions.

In order to avoid the distribution of particles to the reticuloendothelial system, the biofluid was directly injected into the aorta. This procedure significantly increased the amount of nanoparticles concentrated at the target organ. Two alternatives for capture of particles by the macrophages are currently under investigation. One alternative consists of pegylating the particles, another on the previous treatment with clodronate liposomes which suppresses the action of the macrophages [22].

3. Conclusions

In this work we propose a novel method of drug delivery based on implanting a permanent magnet in the affected organ. In-vitro experiments showed the blood biocompatibility of the obtained magnetic fluids. We confirmed the high efficiency of our Fe@C nanoparticles in the uptake and release of doxorubicin. In-vivo experiments prove the adequacy of the method to concentrate nanoparticles at the region where the magnet was implanted.

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