Carbon Coated Magnetic Nanoparticles For Local Drug Delivery Using Magnetic Implants

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ABSTRACT

Bioferrofluids obtained from carbon coated iron nanoparticles are promising candidates for magnetic drug delivery. The carbon cages render the particles biocompatible, and provide a good support for drug adsorption. We propose a method in which gold plated permanent magnets are implanted directly in the affected organ, close to the tumour, by endoscopic techniques. The bioferrofluid charged with the chemotherapeutic agent is injected and the particles attracted to the magnet, then desorption of the drug takes place at the tumoral region. This method seems to be more promising, costless and effective than that based on the application of external magnetic fields. Preliminary results of drug adsorption and a preclinical experimental animal model are described.

Keywords: magnetic nanoparticles, magnetic implants, local therapy.

1 INTRODUCTION

Magnetic nanoparticles have been widely used for targeted delivery of chemotherapeutic agents for the treatment of solid tumours. The drugs are adsorbed or chemically bonded to the particles, and then the system is targeted to the site of choice with the aid of an external magnet, being the drugs eventually desorbed on the desired area over a long period of time [1,2,3]. This local treatment improves the efficacy of the chemotherapy, reducing dramatically systemic toxicity.

However, we consider that the use of external magnets presents serious limitations, the administration method is limited to an artery close to the tumour, and also well defined magnetic field geometries would be needed, depending on the tumor allocation, to perform completely targeteable magnetic drug delivery [4]. To avoid these inconveniences, we propose the implant of small permanent magnets directly into the affected zone, creating an internal local magnetic field, rather more effective than those produced by external magnets.

The carbon encapsulation of Iron nanoparticles or nanoparticles produced with other magnetic metals such as Nickel or Cobalt, renders the resulting particles biologically inert. Moreover, the carbon surface seems to be very effective for physical adsorption of drugs [5,6]. In addition, the carbon layers isolate the particles from each other, thus avoiding the problems caused by interactions between magnetic particles, as well as preventing bare metal nanoparticles from oxidation or degradation [7,8].

Size and magnetic properties are the key to obtain a stable, biocompatible ferrofluid, useful for the aim of our work. The size of the particles typically used in biomedical applications is lower than 1 micron, and usually below 500 nanometers. Smaller particles have a larger surface area and a low coercive force, something that prevents them from aggregation before the application of the magnetic field. On the contrary, they have lower magnetic susceptibility and saturation magnetization, and therefore they present a worse magnetic response. A balance is hence needed to meet the requirements for an effective control on the delivery of the drugs attached to the particles. By the discharge arc method we have obtained iron nanoparticles encapsulated in graphitic layers, suitable for the adsorption of chemiotherapeutic agents, in this case doxorubicin. With these particles a ferrofluid has been produced and its controlled delivery into the affected organ has been achieved using internal permanent magnets.

The biocompatibility test of this ferrofluid in blood is not as well specified as for solid surfaces in contact with blood and tissues. In our opinion circulating particles should be tested for rheology, activation of platelets and coagulation, and endothelial damage.

We will describe an experimental surgical model for magnet implants and test of the magnetic particles accumulation by histological analysis of different organs.

2 NANOPARTICLES SYNTHESIS AND BIOFERROFLUID PREPARATION

The production of carbon coated iron nanoparticles is accomplished by two methods, by the discharge arc method designed by Krätschmer-Huffman in 1990 [9], or by high energy ball mill grinding [6]. The Krätschmer method uses a cylindrical chamber, in which there are two graphite electrodes: a stationary anode containing 10 microns starting iron powders, and a moveable graphite cathode. 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. In the material collected from this deposit we found: carbon nanostructures, amorphous carbon and iron and iron oxides nanoparticles encapsulated in graphitic layers.

High energy grinding is performed in a ball mill. A suspension of iron micrometric powders and graphite powders in ethanol is grounded for several hours to obtain a viscous solution that is dried up to obtain the final product as fine iron carbon powders.

For both preparations, on-coated or partially coated magnetic particles, which are not biocompatible, were eliminated by chemical etching. For magnetic purification, stable suspensions of the particles are prepared in a surfactant solution: 2.5 g of SDS in 500 ml of distilled water. A field gradient produced by 3KOe permanent magnet was used for magnetic separation of this suspension. The purified material was washed with HCl 3M at 80ºC. The obtained magnetic material was dissolved and the coating carbon forms carboxylic groups, which, due to their hydrophobic nature, contribute to the stability of ferrofluid suspensions. The sample is finally heated at 350ºC in order to evaporate resulting amorphous carbon.

To prepare the bioferrofluid 125 mg of the final product are suspended in 100 ml of gelafundine, a commercial succinylated gel commonly used as plasmatic substitute in blood. The solution is sonicated for several minutes.

3 CHARACTERIZATION

Dynamic Light Scattering experiments (DLS), Scan Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) have been used for morphologic characterization. High-resolution TEM HRTEM and Energy Filtered EFTEM images show that the coating of the iron particles is complete, thus rendering them biocompatible. Fe appears with a darker contrast whereas C, a lighter element, appears with a lighter contrast (fig. 1). Moreover HRTEM micrographs showed that the plane spacing observed in the nanoparticles correspond to the Fe2O3 Maghemite phase, whereas the plane observed at the encapsulation layer should be associated with the C graphite phase. From the EFTEM images iron (EELS peak at 708 eV, L3 peak) and carbon (EELS peak at 228, K peak) were selected separately. Once both images were obtained, an elementary map can be drawn, showing a coloured distribution of each of the elements: green for Fe and red for C (Fig. 1). Furthermore, sample composition could be also analysed by electron diffraction. Results obtained were in good agreement with those obtaines previously by HRTEM, as, we observe the presence of iron oxide in the core of the nanoparticle, crystallized in the cubic phase of the γ-Fe_2O_3 (Maghemite).

From the analysis of the HRTEM and EFTEM images a size distribution centred around 20 nm has been obtained for our particles. Dynamic Light Scattering was performed for all the samples, and the obtained average size was approximately 400 nm. The apparent contradiction between these results and the ones derived from HRTEM and EFTEM, is due to the fact that particles bigger than tens of nanometers are not visible by means of TEM techniques

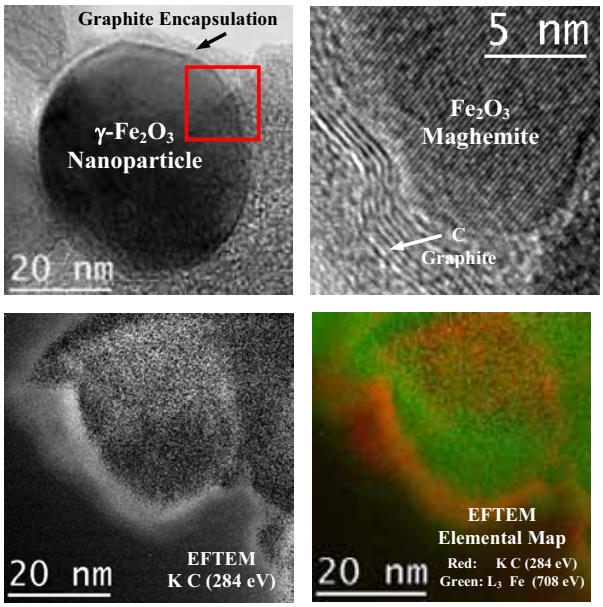


Fig. 1: HRTEM and EFTEM pictures of the iron-carbon naoparticles

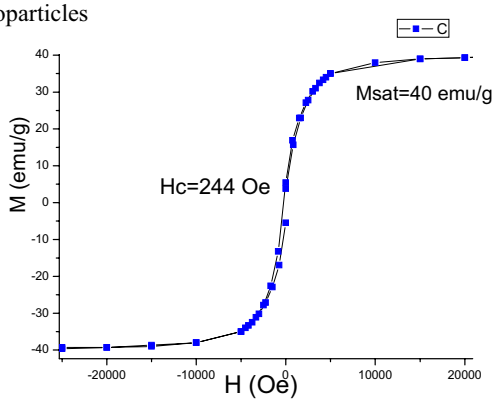


Fig. 2: Hysteresis loop at Room Temperatura for the iron-carbon particles.

because the electrons are only able to penetrate inside small enough particles.

Magnetization measurements were performed with a SQUID magnetometer (MPMS-5S, Quantum Design). The magnetization isotherms (fig 2) can be understood as the superposition of two different magnetic behaviours, consistent with the existence of two different particle size ranges in our samples. On one hand, a high saturation magnetization and a high coercivity is observed, even at room temperature. This suggests the existence of large ferromagnetic particles (several hundreds of nanometers, in good agreement with the DLS results) in our sample, with a blocking temperature above room temperature. This fact is confirmed by field cooling (FC) and zero-field cooling (ZFC) magnetization measurements (data not shown). On the other hand, the high field susceptibility displayed by the hysteresis loop is due to the existence of small particles (tens of nanometers, according to the TEM results), showing superparamagnetic behaviour.

The presence in our samples of large particles showing ferromagnetic behaviour could be a drawback for a drug delivery method in which an external magnetic field was applied. For those methods superparamagnetic particles are required: once the magnetic field was removed, the particles would stay with zero remanence, avoiding aggregation due to the dipolar interaction between their respective magnetizations. This aggregation would not allow the biological absorption and eventual excretion of the particles by the body. On the contrary, in our method, based on the implantation of permanent magnets in the affected area in the body, the particles are attracted to a stronger magnetic field and stay attached to the tumour, releasing the drug for a long period of time. Once the drug has been released, the particles remain aggregated and attached to the magnet. Since the narrowest capillary in the body has several microns of diameter, and considering the high magnetization found for these particles, the obtained particle sizes seem adequate to achieve a good magnetic response of the ferrofluid to the internal magnet and, at the same time, they are small enough to avoid thrombosis due to aggregation of the particles.

The specific surface area of our nanoparticles is a key parameter in order to test their capability of adsorbing and desorbing a drug. It was measured by the BET method [10]. The obtained adsorption and desorption areas were 35 m^2/g and 14 m^2/g respectively (fig 3). Lower values of the specific surface area have proved to be effective for a faster desorption [11]. Therefore our particles are suitable for being used as drug delivery vehicles. With the aim of increasing the specific surface area, activated carbon particles are also being synthesized, thus improving the capability of our system to adsorb chemiotherapy agents.

4 ADSORPTION PROPERTIES

Kinetics of adsorption was measured following the procedure described by Kuznetsov in reference [5]: A 100 µg/ml solution of doxorubicin hydrochloride was mixed with 1 mg/ml of distilled water. The suspension was

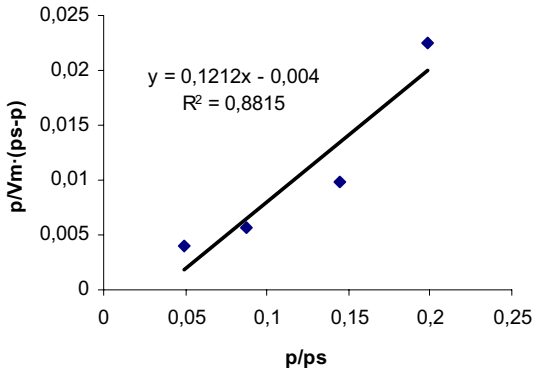
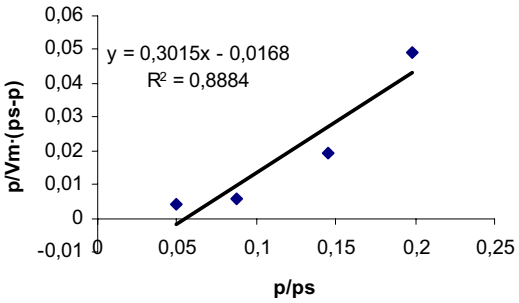


Figure 3: BET adsorption (uppper image) and desorption (lower figure) isotherms for calculation of the specific area of our iron-carbon nanoparticles.



incubated on a shaker at room temperature, and samples were taken after 5, 15, 30, 60, 90, 120 and 180 minutes. The adsorbent particles were sedimented 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 4). Adsorption seems to reach saturation after 3 hours of incubation. In agreement with the low specific area found for the particles, the first results show that the amount of drug adsorbed is relatively low, a fact that will have to be improved for future applications in *in vivo* experiments. Smaller particles or a more porous surface (as the activated carbon) encapsulating the particles could be the solution for this issue.

As a next step, *in vitro* desorption kinetics in human blood

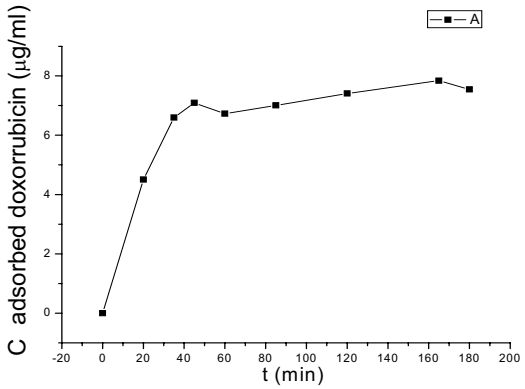


Fig 4: Kinetics of doxorubicin adsorption by the iron-carbon particles

is soon to be tested.

5 HEMATOLOGIC TESTS OF THE FERROFLUID

We have measured plasma viscosity with a Brookfield capillary viscometer, and plasma viscosity at different shear stress (230 s^{-1}, 23 s^{-1} y 5.7 s^{-1}) with a Brookfield plate-cone viscometer. The test was performed in New Zealand rabbits with circulating nanoparticles and *in vitro* human blood. We have determined as well erythrocyte aggregability after 5 and 10 seconds of stasis and after 5 and 10 seconds of very low shear stress (3 s^{-1}). The shear stress simulates those of circulating blood in different areas of veins and arteries.

We have not enough data at present for an statistics analysis, but the preliminary data suggest that 1 ml of nano-particles at a concentration of 12.5 mg/ml of Gelafundine injected in rabbits (n=3) do not modify blood and plasma viscosity and that erythrocyte aggregability remains within normal limits, with very small variations without clinical significance. The blood samples were taken before, and 10 minutes after the venous injection of the particles.

We have tested as well human blood at different concentration of particles (5 ml of blood and 0, 0.06, 0.12, 0.24 and 0.5 ml of ferrofluid). We obtained normal values in all tests, differing less than 10% in relation to our own series of normal controls. We could not determine plasma viscosity for technical difficulties due probably to interference of the particles with optical or magnetic inner controls of the viscometer.

The particles can be seen in blood smeared as black point in May-Gruendwald-Giemsa and Perls stain. The green-blue die characteristic of iron in Perls stain is absent, which is an indication of the good encapsulation of the iron by graphitic layers. All the particles are extra-cellular, both in rabbits (circulating particles "in-vivo") and human (mixture of the ferrofluid and blood "in-vitro"). We did not find magnetic particles neither in circulating monocytes nor granulocytes. This means that iron is well coated and that the particles have not been withdrawn from circulation for phagocytes.

6 "IN-VIVO" EXPERIMENTAL TESTS

The rabbits were submitted to general anaesthesia with endotracheal intubation. An intravenous needle was inserted in the marginal ear vein, fixed in it and a perfusion of a physiologic saline solution was initiated. Then, through an 8 French plastic trocar, a 4x2 mm cylindrical permanent magnet (neodymium-iron-boron covered with gold) was inserted percutaneously in the muscle mass of the forelimb of the animal. Next, the peritoneal cavity was insufflated with CO_2 through of a Veress needle and a laparoscopic optic was introduced by using a 5 mm trocar. Under endoscopic control, the lower pole of the left kidney was punctured, inserting in it the other magnet. Similar procedure was followed to implant a magnet in the right lobe of the liver at the right hypocondrium level. After controlling the absence of bleeding, the trocars were retired and the cutaneous orifices of insertion were sutured.

To prepare the bioferrofluid, 125 mg of our nanoparticles were suspended in 100 ml of gelafundine, a commercial succinylated gel commonly used as plasmatic substitute of serum blood. The solution was sonicated for several minutes and then 1 ml of the fluid was injected intravenously in the marginal ear vein of the rabbit. After half an hour, the animal was sacrificed by terminal anaesthesia and the main organs extracted for histological study.

Preliminary results obtained in histologic analysis performed in samples obtained from differents organs, showed the lack of Perls stain reaction, as was also observed in hematologic test. These results confirmed once more the good insulation of the magnetic particles core. From histologic studies, we observed a larger particles concentration in the implanted kidney repect to the non-implanted one. This is an indication of the capability of the magnet to atract particles. More work is being developed in this direction to quantify and confirm the formed assertions.

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