

Publishable summary

Introduction

The EU-funded project MAGNIFYCO (Magnetic Nanocontainers for Combined Hyperthermia and Controlled Drug Release) has the aim to design, assemble and fabricate a new generation of multifunctional nanostructures for performing combined hyperthermia and controlled drug release, specifically targeted to cancer cells. These multiple tasks are made possible by combining three components within the nanostructures: a) magnetic nanoparticles that allow detection by MRI, provide magnetic targeting towards tumors, treat cancer by hyperthermia, and thermally induce drug release; b) nanocontainers, such as *Tobacco mosaic virus*, zeolites, biological vesicles, polymeric nanogels, solid lipid nanoparticles, or self-assembled peptides, that protect the drug from enzymatic degradation and are sensitive to external stimuli, e.g. heat and pH change, which control the release of chemotherapeutic drugs; c) antibody fragments (Fabs) attached to the surface of the containers or of the magnetic particles, that allow selective delivery of the structure to ovarian cancer cells, which overexpress the receptor FR α . The individual building blocks and their assemblies are characterized with respect to physical, chemical, and biological features, followed by dissemination of the newly acquired knowledge. Additional *in vitro* experiments are ongoing, and will allow understanding the performance of the novel nanotools. Given the long-term objective directed towards application in patients, in the second part of the project *in vivo* animal studies will be carried out with the most successful magnetic nanocontainers.

The Consortium

The Consortium of partners was built for achieving the multiple aims of the Magnifyco project, hence an interdisciplinary collaboration among chemists, physicists, and biologists with expertise in basic aspects and in translational medicine was required. Proposed was a European network collaboration between academic partners, who develop new solutions for nanofabrication and characterization, and industrial partners, whose key expertise is in fields close to the Magnifyco research activities. The responsible of Magnifyco is the Italian National Research Council (CNR, Italy; Teresa Pellegrino), represented by the National Nanotechnology Laboratory Institute (NNL). CNR is in charge of functionalizing inorganic magnetic nanoparticles, and of developing stimuli-responsive polymeric nanovectors, in strong synergy with the Italian Institute of Technology (IIT, Italy, Albert Figuerola). IIT is additionally developing and studying novel types of colloidal iron-based nanoparticles. Four of the other academic partners are developing various types of nanocontainers: *Tobacco mosaic virus* nanotubes produced by CIC nanoGUNE (nanoGUNE, Spain, Alexander Bittner); zeolites as hard nanocontainers produced by the University of Twente (uni-Twente, Netherlands, Luisa de Cola); self-assembled peptides produced by the Universidade de Santiago de Compostela (USC, Spain, Juan R. Granja), and the mammalian vesicles produced by the Laboratoire Matières et Systèmes Complexes (CNRS, France, Claire Wilhelm). The Universidad Complutense de Madrid (UCM, Spain, Miguel A. Garcia) and the Instituto Nazionale dei Tumori (INT, Italy, Silvana Canevari) are both involved in the characterization of the materials. While UCM contributes to the structural and magnetic characterization of the nanostructures, INT offers support for the *in vitro* characterization, and will be involved in the *in vivo* characterization of the best performing nanocontainers with ovarian tumor models. Three companies are members of the consortium: MagForce

Nanotechnologies (Magforce, Monika Fischler, Germany), with a strong expertise in *in vivo* hyperthermia characterization based on iron oxide nanoparticles; Nanovector (Nanov, Paolo Gasco, Italy), experts in solid lipid nanoparticles for drug delivery; Dompè Pharma (Dompe, Italy, Candida Cesta), who develop and produce antibody fragments on a large scale.

The overall project plan

The aims of the Magnifyco project are: Synthesis and evaluation of new inorganic nanomaterials for hyperthermia treatment; preparation and investigation of various types of soft and hard materials for drug encapsulation; assembly of the magnetic nanoparticles with the nanocontainers; *in vivo* applications of the best performing magnetic containers. The project is divided into 8 specifically defined and interconnected work packages (WPs). The WPs 1 and 2 provide the building blocks, magnetic inorganic nanoparticles and soft and hard nanocontainers, which are then assembled in the WP3. The WP4 deals with the functionalization of the magnetic nanoparticles and/or the nanocontainers with the antibody fragments. The nanomaterials obtained by these WPs are characterized magnetically and structurally in the WP5, and they are tested on tumors cells in *in vitro* studies in the WP6. The prototype device that exhibits the best performing features for drug delivery and hyperthermia treatment will then start the WP7 for *in vivo* applications. In the WP8, started at month 18, MagForce will function as “end user company” and will help the consortium to identify possible products for further industrial development.

Results obtained at mid-term

The EU project Magnifyco deals with an innovative and multivalent approach of cancer treatment by exploiting new nanometric magnetic containers. In the first 18 months of the Magnifyco project, efforts have been dedicated to fabricate various types of nanocontainers and to merge them with magnetic nanoparticles. This required to include research objectives such as control of the nanostructures' size and shape, understanding of the hydrophilicity/hydrophobicity properties of the nanocontainers, and their colloidal stability. It was possible to associate magnetic nanoparticles to zeolites, to polymeric nanogels, both pH-responsive (Langmuir, 26, 10315, 2010) and thermo-responsive (Nanoscale, 3, 619 2011), and to decorate *Tobacco mosaic virus* with magnetic nanoparticles (ACS Nano, 8, 4531, 2010). In addition, magnetic nanoparticles were linked to biogenic vesicles from endothelial cells or macrophages in two different geometries, first decorating the vesicles' membrane (Nanomedicine 5, 727, 2010), and second encapsulating them within the vesicles (Biomaterials 27, 7061, 2010). Self-assembled peptides use, envisaged to be used as nanocontainers, have shown severe problems of solubility in physiological media. However, dimer-forming models have been studied (Chem. Asian J. 6, 110, 2011; Amino Acids DOI10.1007/s00726-011-0886-2), and a new strategy for the application of this type of nanocontainers has been designed, namely attaching magnetic nanoparticles to peptide dimers, which include a cytostatic drug in a sandwich like-configuration between the peptide moieties.

Depending on the type of nanocontainers, the most suitable drugs, already in use in ovarian cancer treatment, have been identified. Drug encapsulation and release capacities have been tested after applying the proper stimuli (acidic pH, temperature and alternating magnetic field) directly to the magnetic nanocontainers. Within the different nanocontainers so far developed and tested, it was found that for zeolites coated by the polymeric PNIPAM it was not possible to achieve a temperature-controlled release since the polymer network blocks the zeolite pores.

However, preliminary *in vitro* studies on tumor cells have been performed with magnetic nanocontainers that have already passed those tests (Advanced Materials, 23, 787, 2011, Nanoscale 3, 619 2011).

In parallel, a broad variety of inorganic nanoparticles, synthesized by thermal decomposition methods, have been produced and magnetically characterized. The continuous feedback between the groups involved in the synthesis, and those involved in the magnetic characterization, has allowed excluding those particles that have poor magnetic performance for hyperthermia. On the basis of this screening, iron-platinum alloy particles of 9 nm in diameter, particles of the ferrite Fe_2MnO_4 of 9 nm in diameter, and iron oxide nanocrystals prepared by seeded growth (diameters between 4 and 18 nm) have been excluded (manuscript submitted), as well as extremely small Fe_3O_4 crystals on virus particles (manuscript in preparation). Of the other magnetic nanoparticles, those that have shown the most promising magnetic features will be further exploited by the whole consortium in the second part of the project. The functionalization with antibody fragments (Fabs) has already been achieved on some of the magnetic nanoparticles, and preliminary binding studies have shown their binding specificity and improved targeting to the antigen of interest, the $\text{FR}\alpha$ receptor. This finding, which goes behind the planned activities, could become a key result. In fact, in the case that it will be impossible to directly functionalize the containers with the Fabs, functionalization of the containers with magnetic nanoparticles that bear the antibody fragment could become a valid alternative.

In conclusion, important results have already been obtained after 18 months of this 3-year project, and therefore the experimental data collected so far allow us to forecast a successful outcome.

The expected final results and their potential impact and use

The Magnifyco target is motivated by the need to find more efficient and less invasive cancer therapies. The ovarian carcinoma, chosen as a tumor model for the study, represents the principal cause of death among gynecological cancers. Since initial symptoms are indistinguishable and non-specific, this form of cancer is often diagnosed in an advanced stage of the disease. So far, surgery is the preferred treatment of the disease, associated with chemotherapy, which is used after surgery to treat residual disease. In this project an alternative approach is proposed, based on hyperthermia combined with drug delivery, upon injection of drug-loaded magnetic nanocontainers into the specific tumor site. This system should have the potential to combine hyperthermia with a drug-therapeutic approach, and thus to be more effective than either method used on its own. To establish the response of the magnetic nanocontainers in tumor therapy, a locoregional treatment was opted for, targeted to intraperitoneally growing solid tumor masses, and to tumoral ascites, both of which are reasonable models for achieving a high tumor drug uptake, due to their confined localization.

Heat therapy, also called hyperthermia, has made huge advances, thanks to the current developments of nanometric heat-generating “foci”, that can be activated by externally applied magnetic fields. Iron oxide nanoparticles as heat sources have decisive advantages over macroscopic implants and more invasive surgery treatment. Being available in the form of colloidal suspensions, they can, like contrast agents, be injected through a variety of non-invasive routes. Their subsequent distribution within the body can be controlled by various targeting strategies, thereby optimizing their concentration in therapeutic target zones, or even specifically within tumor cells. The nanoparticles are sufficiently small to cross biological barriers, and therefore heat can be generated very close to and even inside the targeted cells.

Finally, the heat generated in this way is more homogeneous than that obtained with macroscopic implants. It is also proportional to the local concentration of nanofoci, and it is limited solely to the site of nanoparticle accumulation. To date, magnetic nanoparticles based on Fe_2O_3 and Fe_3O_4 have been proven to be useful materials for hyperthermia.

In Magnifyco, the heat generation from new types of nanoparticles, prepared by colloidal methods, is under investigation. The aim is to find new iron-based inorganic nanoparticles that provide at the same time higher colloidal stability and more efficient heat generation, thereby reducing *in vivo* dosing and toxic side effects.

Additionally, the materials being implemented as nanocontainers represent a new generation of drug carriers. Most of the nanocontainers (all of them when functionalized with magnetic particles) have never been explored so far, therefore new know-how is generated by Magnifyco. Finally, a decisive point is the active targeting by means of surface functionalization of the magnetic nanocontainers with specific antibody fragments (Fabs), in order to obtain high selectivity in tumor targeting.

Given the multifunctionality of the nanodevices, the investigated tools hold huge promises in cancer treatment, which should be extended also to other forms of cancer.

Final scenario forecast

The results obtained in the first 18 months are offering to the Magnifyco Consortium the possibility to identify promising products for performing hyperthermia and drug release at the same time, within the planned 36 months period. An already satisfying scenario would be the identification of magnetic nanocontainer prototypes, which will work not only *in vitro* but also *in vivo*. However, a successful scenario would be the identification of magnetic nanocontainers that can allow systemic administration, besides having better hyperthermia performance (at lower doses of iron oxide nanoparticles) than available so far. The ideal magnetic nanocontainers would have also additional key features: They would be easily sterilized, they would offer versatile procedures for scaling up their fabrication, and they would not show immunogenicity.

On the medium to long time scale, one can envision that the Magnifyco results will contribute at the social level to: i) A direct significant increase in life expectancy of ovarian cancer patients, due to the efficacy of the treatment, and due to the reduced toxicity of the treatment as a consequence of its targeting; ii) an indirect improvement of the quality of life of the relatives of the patients; iii) a future wider social impact if the approaches proposed by Magnifyco are successful, due to new perspectives in the therapeutic intervention against other solid and also haematologic tumors.

The benefits of the application of a nanotechnological approach, as compared to conventional pharmacological treatments, are expected to be accompanied by a direct health service cost reduction due to: i) Less drug needed for an equivalent efficacy, as a consequence of its encapsulation and targeting; ii) reduced hospitalization time as a consequence of reduced toxicity; iii) indirect reduced social cost in term of reduced “disease absence from work” (it should be taken into account that ovary cancer incidence is at a median age of 55-60 years, i.e. at an active working age).

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