

PROJECT FINAL REPORT



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Name of the scientific representative of the project's co-ordinator: Dr. Valentin DEDIU

Organisation: ISMN-CNR, VIA GOBETTI 101, 40129 BOLOGNA, ITALY

Coordinator e-mail: V.Dediu@bo.ismn.cnr.it

Coordinator phone: +39 051 6398507

Coordinator fax: +39 051 6398540

Project website address: <http://www.magister-project.eu/>

Final publishable summary report

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Executive Summary

MAGISTER project has successfully promoted a conceptually innovative idea introducing for the first time the notion of Magnetic Scaffolds (MagS) for tissue engineering and regeneration. The project has moved on both in vitro and in vivo approaches achieving important new knowledge applicable for foundational scientific activities, and valuable know-how for commercial usage and medical practices.

NEW CONCEPTS (Worldwide pioneering and leading role of MAGISTER)

- ❖ Before MAGISTER only magnetic nanoparticles (MNP) were investigated as magnetic materials related to the Biomedical field – MAGISTER introduced the concept of MAGNETIC SCAFFOLD and related supporting materials
- ❖ Magnetic Scaffolds have proven to promote the magnetic dragging of MNP-Bioagents aggregates (BIOAGs) both TOWARDS and INSIDE the scaffold (in vitro and in vivo demonstrations)
- ❖ Pioneering BIOAGs for the controlled delivery of both Vascular Endothelial Growth Factor (VEGF) and stem cells have been developed. The technology couples highly controlled MNP manufacturing with an innovative surface functionalisation method based on novel biocompetent hyperbranched peptides (dendrons)
- ❖ Magnetic Fixation of scaffolds for the first time proposed and its utility demonstrated
- ❖ Magneto-thermosensitive effects for the first time demonstrated in macroscopic objects

NEW MATERIALS (Worldwide pioneering and leading role of MAGISTER)

A NUMBER of biocompatible magnetic materials for scaffolds have been developed and fabricated:

IMPREGNATION – Hydroxyapatite, gelatine, coral, polymers have been impregnated with bio-shelled MNP either produced by consortium or commercially available – these materials constituted the first available batch of MagS for earlier in vitro and in vivo experiments. High magnetization values, low-intermediate biocompatibility was achieved.

SINTERING – mainly HA-magnetite combination. High magnetization values, intermediate-high biocompatibility.

CHEMICAL DOPING -> **OUTSTANDING RESULT** considering both SCIENTIFIC value and EXPLOITATION potential. HA:Fe first ever magnetic fully bioresorbable material. Medium magnetization values, full biocompatibility.

SURFACE FUNCTIONALISATION – Novel dendrons have been designed to functionalise the surface of MNP and to spatially control the exposure of functional groups capable of binding VEGF and significantly increase the magnetisation of stem cells and endothelial cells thus driving angiogenesis

BIOPLOTTING by using NEW materials – PCL-HA:Fe – innovative magnetic scaffolds for in vivo exp.

IN VITRO (Worldwide pioneering and leading role of MAGISTER)

- ❖ Magnetic guiding of BIOAGs inside MagS – demonstrated for both growth factors and stem cells bound to MNP.
- ❖ Demonstrated that even magnetically weak scaffolds increase the sticking probability of (magnetized) cells by about 15%.
- ❖ For the first time demonstrated a distinct side by side magnetically guided colonization of a scaffold fibre by two different types of cells
- ❖ Good biocompatibility detected on most selected materials

IN VIVO (Worldwide pioneering and leading role of MAGISTER)

- ❖ Demonstrated that Magnetic guiding of bio-agents inside MagS leads to an excellent tissue reconstruction with clear vascularization effects

- ❖ Revealed the orientation of scaffold material by magnetic lines during scaffold-bone reconstruction
- ❖ Demonstrated that magnetic fixation of scaffolds increases bone quality at the interfaces scaffold-old tissue by reducing micromotions
- ❖ Developed protocols for implantation of magnetic scaffolds and injection of magnetic bio-agents

Description of project context and objectives

The main driving idea of the project was the creation of a conceptually new type of bioactive scaffold able to be manipulated in situ by means of **magnetic forces**. This approach was supposed to generate scaffolds with characteristics such as **multiple use** and possibly **multipurpose delivery** in order to repair **large bone -defects** and **osteochondral lesions** of the skeletal system.

The Consortium elaborated, investigated and fabricated new types of bioactive scaffolds – magnetic scaffolds (MagS) - characterized by a strongly enhanced control and efficiently adjusted *tissue regeneration* and *angiogenesis*. The magnetic moment of the scaffolds enables them with the fascinating possibility of being *continuously controlled* and *reloaded* from an external supervising center with tissue growth factors. Such a magnetic scaffold can be imagined as a fixed “station” that offers a long-living assistance to implanted tissue engineering constructs, providing a unique possibility to adjust the scaffold activity to the personal needs of the patient.

The tissue engineering is a complex multistep therapeutic approach which requires a relatively long regeneration time to re-establish the full functionality in damaged tissues. Thus the temporal control of the various aspects of the tissue regeneration process is very important to allow optimal clinical outcomes. In bone, for instance, the restoration of the mechanical resistance to physiological stresses should be accompanied by angiogenesis, leading to a complete histomorphologically and biologically mature tissue. Such a temporal control is hardly achievable with the traditional scaffold approaches, where the growth factors are usually seeded before the implantation. Pre-loading limits the delivery of localised, controllable and long-term biochemical stimuli thus impairing the scaffold tissue regeneration potential.

The magnetic guiding process is already well known in nanomedicine, though before MAGISTER it has not been yet applied in the field of scaffolds. It has been elaborated mainly for drug delivery and tumor hyperthermal treatment and is guided in those cases by *external* magnetic fields. While this concept holds also for the guiding process towards a magnetic scaffold, the MagS presence modifies the magnetic flux distribution and leads to much higher concentration of magnetic “lines” near/inside the scaffold and, as result, to higher achieved magnetic field gradients, responsible for the magnetic attractive force. Indeed, making a link to drug delivery models for MNP, the motion of a magnetic nanoparticle inside the scaffold micro scale channel can be described similarly to the MNP in a blood vessel and is strongly governed by the field gradient.

The scaffold works like a magnetic focusing lens or local field amplifier: its relatively strong internal magnetization can be aligned in the same direction by relatively weak external field, so called coercive field.

It is very important to consider that the proposed scaffold materials can reach very high magnetization values in the applied external magnetic field, while after its removal their magnetization will drop by orders of magnitude (soft magnetic materials with low remanence). Thus the scaffold magnetization can be literally switched off after the tissue reparation.

The consortium adopted various parallel approaches for the construction of suitable magnetic scaffolds. MAGISTER combining the utilization of standard (high magnetization) magnetic materials with absolutely novel approaches with magnetic doping of most accepted biocompatible scaffold materials. The first method aimed at providing means for an early start of most of the research tasks, the second method aimed at generating a new class of magnetic biocompatible materials available for various present and future nano-bio-magnetic applications.

Among the standard magnetic materials the most important role was attributed to the magnetite, Fe₃O₄, material widely accepted in the field of nanomedicine (*drug delivery*,

hyperthermal treatment and other) and available for the consortium in the form of magnetic nanoparticles with bio-compatible coatings. Scaffolds produced from magnetite played a crucial role in the realization of the MAGISTER workplan and making available the demonstration of the proof of principle for the main objectives and in particular for the scaffold reloading challenges.

In parallel to this/these demonstrations the consortium had to introduce FOR THE FIRST TIME fully biocompatible magnetic scaffolds from such widely accepted materials like hydroxyapatite, bioactive glasses to scientific community (inserts to these materials from zirconium oxide and titanium oxide will be also investigated). Materials had to be magnetically doped and used in various ratio and geometries (bulk or coatings) for MagS fabrication. This last approach, delivered towards the end of the project, aimed at circumventing the important problem of leaving any non bio-resorbable magnetic inclusion (for example magnetite) inside the repaired tissue and will bring the MagS into clinical applications.

In parallel to these efforts for the MagS operation, the consortium planned to make further steps in the improving the general properties of the state of the art scaffolds and especially the angiogenesis efficiency by using new **bioactive nanostructured** phases and bio-hybrid composites, able to be integrated in the bone remodelling process and to faster induce the required stimuli *in vivo* and by designing **hierarchically organised** morphology, characterized by **interconnected structures** with preferential **unidirectional paths** for the free development of blood vessels.

Various bio-agents (BA) combinations such as stem cells and Vascular Endothelial Growth Factor (VEGF) had to be investigated for being seeded in MagS and investigated for achieving the most effective bone regeneration with a strong concurrent support to angiogenesis. Cells isolated from either adipose tissue (ATSC) or bone-marrow (BMSC) were used as the source of osteoprogenitors. Controlled gradients of VEGF (both recombinant protein and produced by progenitors, as parallel strategies) were the main choice of consortium as the angiogenic stimulus.

While in some experiments the scaffolds could be seeded by bio-agents following traditional methods, the most challenging objective of this project was to transport in vivo the BA towards the scaffold to provide scaffold reloading in real time. Such transport can be driven by chemically attaching the BAs to selected magnetic nanoparticles working as small locomotive in the magnetic field gradients created by external magnetic fields strongly modified in the vicinity or inside the magnetic scaffold. Considerable efforts were thus dedicated to elaboration and selection of various magnetic nanoparticles, pointing on both magnetic strength and biocompatibility, and, on the other hand, considerable efforts were dedicated to the functionalization of the MNPs in order to generate the bio-aggregates MNP-ligand-bioagent (BIOAGs).

For the MNP development and preparation, the project workplan adopted a strategy similar to that of MagS: a reasonable combination of highly magnetic MNPs and novel (elaborated by consortium) MNPs realized from magnetically doped biocompatible materials used in order to fulfil the objectives and to guarantee a proper time schedule for the project. These biomaterials were be used to functionalise the MNP surface and to act as nano-reservoirs for the controlled delivery of growth factors from them. The physico-chemical properties of these biomaterials was expected to be tuned to retain and delivery growth factors under the control of magnetic field and temperature changes. Likewise, their use for MNP surface functionalisation ensured the MNP interaction with progenitor cells thus magnetising them to allow their magnetic-driven delivery *in vivo*. While simple and available approaches were used for the proof of principle experiemnts, in the later stage of the project MNP from the same as scaffold material was planned to be utilized (doped HA, ZrO₂, TiO₂ and other) avoiding the necessity of their removal from the bio-environment. In this case the MNP will remain entrapped inside the scaffold. A fascinating option of a scaffold building in situ by magnetic guiding of building blocks (BIOAGs)

was also part of the investigation in a specially dedicated task where the general principles and requirements of this process had to be investigated and delivered.

In addition to the reloading option, **magnetic scaffolds were investigated for a number of very important options** that could not be adequately addressed by other methods in use. One such aspect was the possibility to achieve **efficient scaffold fixation via magnetic forces** providing a very elegant and simple solution to the problems of fixation that many scaffolds meet. Indeed, because of their different physical characteristics, scaffold fixation represents a major clinical problem due to the difficulties in obtaining a stable interface between host bone and the scaffold. At present in the treatment of small osteochondral lesions, most surgeons do not use any fixation system, the stability of the scaffold being granted only by the fibrin clot and by the congruency between the prepared lesion site and the geometry of the scaffold. In the treatment of extensively damaged bone fixation is achieved by extremely complicated and invasive procedures (external fixations, intramedullary nails, plates and screws), requiring continuous control and often multiple surgical interventions with an obvious increase of health cost and complications.

A further attractive capability was the possibility to achieve a smooth tuning of the temperature of the scaffolds and separately of the MNPs by the absorption of an external variable magnetic field. This built-in feature would open the way to two applications: i) the use the MNP and the scaffolds as programmable delivery systems triggered by a temperature switch; ii) the use of the effect of a prolonged localized temperature increase on angiogenesis during the repair process.

Main objectives

- ⇒ **Elaboration and fabrication for the first time of *highly porous* magnetic scaffolds – MagS – by combining standard and innovative biocompatible magnetic nanomaterials with scaffold materials.**
- ⇒ **Functionalization of MNPs - biocompatible standard magnetic nanoparticles and new, non-conventional MNPs produced by the consortium (magnetically doped HA, ZrO₂, TiO₂)**
- ⇒ **Realization of a new process of vascularization by magnetically driven delivery of angiogenic growth factors, or of genetically engineered VEGF-expressing progenitors, towards the magnetic scaffolds (*in vivo* MagS reloading).**
- ⇒ **Translate the performance of individual components (scaffold, cells and BIOAGs) into a practicable tissue regeneration strategy by determining (a) the spatial distribution of the magnetic loading of cells and growth factors under the influence of external magnetic fields and (b) the optimal time intervals for sequential and/or simultaneous loading of the scaffolds with selected cells and growth factors.**
- ⇒ **Scaffold building in situ by magnetic guiding of BIOAGs for specific applications (small articular osteochondral lesions, treatment of vertebral body defect and other).**
- ⇒ In addition to the objectives related to the main driving idea of the project, *in vivo* reloading of MagS with **bio-agents**, the consortium aims to deliver few parallel developments with an important impact and eventually faster approval for clinical applications.
- ⇒ **New minimally invasive type of fixation of scaffolds** to be elaborated and experienced *in vitro* and *in vivo*.
- ⇒ Realization of **switch-on/switch-off command scheme for the delivery of the angiogenic factors and stem cells** by MagS through the precise **control of the temperature of the magnetic scaffolds** by the absorption of external AC magnetic fields.

- ⇒ **Advanced MagS simulators will be realized on bases of conceptually new 3D nanostructured geometry involving vessel simulation on micro- and nanoscales.**

The successful achievement of objectives and realization of the program was proposed to be measured against the completion of the project aims resulting in:

- ✓ in vitro and in vivo proof of principle of reloading of magnetic scaffolds with selected cells and growth factors
- ✓ in vitro and in vivo bone and osteochondral regeneration achieved by application of Magnetic Scaffolds for tissue engineering
- ✓ bringing the developed nano-materials to clinical experimentation and practice

Description of the main S&T results/foregrounds

During the whole duration of the project most of Partners had to face conceptually new scientific, technological and applicative challenges given the fully multidisciplinary character of the Project. The research activity and collection of results lead thus to clear added value accomplishments on both fundamental and application sides with respect to the previous consortium knowledge. The Consortium moved on this combining various approaches, including literature work, detailed discussions among partners and specially dedicated Workshops, bringing together Workpackages grouped by tasks and objectives and clearly open for the whole Consortium.

Important for the project proceeding and scientifically sound and innovative results have been achieved already in the initial part of the project as result of significant efforts. This relates mainly to the fully innovative fabrication of magnetic scaffolds, significant modification and improvement of magnetic nanoparticles quality, development of BIOAGs (MNP functionalized to either VEGF or selected stem cells), development of general concepts on in vitro magnetic guiding inside the scaffolds, modeling, first in vivo experiments. The biocompatibility and nanotoxicity check of the new or modified materials was continuously in the “on” state during the period.

A very significant success in the initial period undoubtedly was represented by the development and fabrication of innovative materials suitable for the fabrication of magnetic scaffolds (MagS), conceptually new bio-tool proposed for the first time in this project. This work was characterized by results and achievements well exceeding the planned in the DoW work. At least six complementary approaches have been invented and realized by the Consortium for the fabrication of magnetic scaffolds. All these approaches are fully innovative and have never been reported previously.

This enabled the Consortium with means for starting important in vitro and even in vivo investigations in the early stage of the project (already at Months 12-18) which was crucially vital for the fulfillment of such an intense and innovative working program as that of MAGISTER. Among the scaffold magnetization approaches, three main methods have been selected as most representative and efficient for the Objectives realization: 1) impregnation of commercial bio-hybrid hydroxyapatite-collagen scaffolds with ferrofluids 2) MagS via biologically inspired mineralization of Collagen fibers and contemporary incorporation of Magnetite and 3) realization of polycaprolactone (PCL) based magnetic scaffolds incorporating magnetite nanoparticles, while at the later stage (see below) this third solution was enormously enriched

by substituting magnetite by the first ever bioresorbable magnetic material – Iron doped Hydroxyapatite. It is important to mention that all these developments were strongly collaborative involving a number of partners.

All three developed approaches resulted sufficient for the realization of magnetic guiding, as shown both experimentally in the period 18-48 months and by modeling at the early stage, the magnetization inside the scaffold achieving values in the 1-30 emu/g interval.

In this first stage the magnetization had a homogeneous distribution over the scaffold. In addition to homogeneous magnetic scaffolds, the consortium developed few approaches able to provide MagS with internal magnetic gradients, ranging from 0.1-0.2 T/m to hundreds T/m – the whole range being suitable for the magnetic guiding of available MNPs. Some of the developed magnetic scaffolds have been already checked for in vitro and in vivo biocompatibility and good results were achieved (see below). Investigation of other MagS with respect to this is in progress in accordance with project workplan.

Magnetic Nanoparticles (MNPs) of outstanding quality were developed, combining superparamagnetic behavior with very high magnetization value. Thus 10 nm magnetite MNPs covered with PAA (polyacrylic acid) showed magnetization values up to 60 emu/g, quality well above the standard commercial offer. Moreover, it was demonstrated that the MNPs are characterized by single crystalline structure. It has been decided that these nanoparticles satisfy most of the consortium needs and their utilization will back the fulfilment of most related deliverables.

An important decision taking by Consortium was to fully rely on superparamagnetic nanoparticles for magnetic guiding issues, abandoning the efforts on (ferromagnetic) multi-domain MNP (MD-MNP) as not yet sufficiently developed. The latter offer in principle a manipulation ability similar to that of superparamagnetic MNP (forming no aggregations) but the state of the art of MD-MNP does not yet allow to avoid aggregate formations, preventing so far their application in bio-field. These considerations matured through long discussions within the Consortium but also through discussions with worldwide experts not involved directly in MAGISTER (for example a significant input to this was given by discussions with magnetic experts at Trinity College, Dublin).

The material science oriented work on MNP development and fabrication pursued in a straight and fruitful collaboration with that of scaffold magnetization especially as far as magnetic doping of biocompatible materials was concerned – saving thus considerable efforts and funds.

In parallel to the scaffold line MAGISTER promoted considerable efforts on the second most important actor of magnetic guiding – bio-agents bound to magnetic nanoparticles and constituting the so called BIOAGs (magnetic shuttles). Here the consortium moved on two lines: MNP-VEGF and MNP-SC, the latter utilizing a number of different stem cells.

The development and fabrication of MNP-VEGF BIOAGs has achieved the optimisation of “at least” (as stated by DoW) two types of functionalised bare MNPs and two types of functionalized coated MNPs as a final step to achieve bio-competent magnetic carriers (BIOAGs). Moreover the Consortium has optimised an excess of BIOAGs types (overall 6 types rather than 4).

A novel platform for recombinant expression of VEGF in order to provide the consortium with a standardized source of growth factors was developed.

An advanced research performed on selected mesenchymal cells expressing controlled levels of VEGF put the basis for a further functionalization allowing the development of the second class of BIOAGs: selected cells-MNP. A significant work in this direction involved also the magnetization of stem cells by inserting MNPs inside the cell.

A new (not planned in DoW) BIOAG strategy – polymeric sponges functionalized with MNPs, suitable for magnetic guiding and controlled release was promoted too. The work on BIOAGs proceeds thus as planned in DoW with few additional solutions, not foreseen before.

The Consortium work in the middle period of MAGISTER (19-36 Months) has been strongly dominated by *in vitro* tasks – not more pure development, but *in vitro* tests and applications of developed materials together with the initial part of the *in vivo* experiments have clearly assumed the leading role in the project. Nevertheless, the development and fabrication of novel magnetic biocompatible materials has proceeded its successful paths, moving to more sophisticated approaches and more complex materials.

The research stile in the intermediate parts of the project were even more strongly multidisciplinary: by this time biologic and medical partners have become familiar with magnetic issues, magnetists and material scientists learned bio-problems, chemical approaches not only bridged and linked nanoparticles to bio-agents but also bridged different disciplines, while industrial partners showed more and more interest to exploitable achievements. The interaction and collaboration between partners has further increased, reaching the level of a very friendly but responsible and fruitful partnership.

In this *in vitro* dominated part of the project, the major achievement was unambiguously the successful demonstration of the midterm Proof of Principle (PoP). This demonstration encompassed the loading of *magnetic scaffolds* by two types of angiogenically active bio-aggregates (BIOAGs), containing different amounts of magnetic nanoparticles (MNP) for magnetic guiding. Namely, the BIOAGs were represented by MNP-growth factor aggregates and by cells loaded by magnetic nanoparticles. It was shown that the magnetic control of such BIOAGs allows their efficient orientation towards and into the scaffold, while the magnetism of the scaffold play an active role in distributing the BIOAGs inside the scaffold following internal magnetic gradients.

Such processes, successfully foreseen in the MAGISTER working plan, represented the first time realization of the magnetic loading of desired bio-agents not only via an external magnetic field, but also using internal magnetic gradients of these new bio compatible porous systems. The latter guarantees clear advantages for the loading of scaffolds with angiogenic factors (and more generally with various required bio-agents) given the ability to drag bio-materials attached to MNP not only inside the scaffold but also generate special distributions characterized by sub-millimetric or even 10-100 micron scales (corresponding to established magnetic gradients).

In addition to this (guiding/loading) achievement, an important result not foreseen by the MAGISTER was registered by the consortium. It has been found that the magnetization of scaffolds increases the efficiency of cell sticking to the scaffold walls, providing additional advantages to the utilization of magnetic scaffolds for tissue regeneration.

In parallel to the bio-tests of the previously developed materials, the development of novel biocompatible magnetic materials proceeded in a more intensive (than extensive) way. As clearly realized by the consortium and strongly recommended by the project officer and project technical assistant, a strong selection of materials was performed restricting the research to three most appealing and efficient materials (all based on hydroxyapatite matrix), while withdrawing four other candidates. In addition to these, efforts were still dedicated to the late (on the project scale) candidate material, out of the list, namely to bio-active glasses based magnetic materials.

The list of THREE Materials for magnetic scaffolds to be fabricated and applied in the second half of the project was:

A) HA:Fe scaffolds – most innovative

A.1 Pure(ISTEC):

Main application: injectable scaffolds (D1.5)

A.2 With polymeric support (IMCB + ISTEC) PCL/HA:Fe – this material combination features the inclusion of the fully bioresorbable magnetic hydroxyapatite material (chemically doped with Fe – outstanding MAGISTER achievement) in polymeric matrix.

Main application: in vitro and in vivo with possibility to have both homogeneous magnetisation and gradient one.

B) Hybrid impregnated HA/Collagen (ISMN, ISTEC) – highest magnetisation achieved.

Main application – in vitro for magnetic guiding experiments, no gradient achievable.

C) HA-magnetite (ceramic) – high magnetization and gradients achievable

Main application: most appropriate at the moment for in vivo experiments

Withdrawn materials : Pure Fe₃O₄; PCL:Fe₃O₄; Silk; Gelatine

Noteworthy, the research along the line of selected materials brought the consortium to outstanding results. The most remarkable result featured the achievement of pure and stoichiometric magnetic phase of Fe doped hydroxyapatite. The results were patented jointly by three partners (Patent MI2010A001420) and published in a number of high level journals. This achievement opened extremely promising general perspectives for the very application of magnetic materials in medicine: **the iron doped hydroxyapatite material constitutes the first ever developed fully bioresorbable magnetic material** characterized by reasonably high superparamagnetic strength (sufficient for many applications). This material could thus seriously challenge the magnetite, recently dominating the market of magnetic materials for medical field (mainly in the form of magnetic micro and nanoparticles). The magnetite, while showing higher magnetic values, is not biocompatible by itself and is made biocompatible by special shells, featuring no bioresorbability indeed.

Hence, this outstanding achievement represents clearly one of the most exploitable results of MAGISTER.

The consortium moved beyond the simple powder fabrication from the described above material, preparing Poly(caprolactone) based scaffolds with iron doped hydroxyapatite as well as first batches of fully bioresorbable MNPs.

The scaffolds based on physical mixtures of hydroxyapatite and magnetite, made biocompatible but not bioresorbable, have also evolved in materials fully compatible with *in vitro* and *in vivo* experiments of MAGISTER.

On the other hand, considering the last objective in the area of magnetic scaffolds research, successful preliminary studies have indicated good perspectives for the development of injectable scaffolds on the basis of materials from the selected list.

Important successes have been achieved on the route of BIOAGs (bio-aggregates) development and fabrication. The development of protocols pursued two lines involving BIOAGs based on linking MNPs to growth factors (1) or to progenitor cells (2).

As an important previous step functionalisation of various types of MNP, in particular Fe₃O₄@PAA, has been actively and successfully pursued. The most suitable formulations were used for the production of BIOAGs that were achieved either by the optimization of VEGF uploading on the MNP or by the use of the functionalized nanobeads for cell magnetization. The work led to the selection of MNP with novel biofunctionalities adaptable to both the VEGF and cell magnetic carriers. VEGF stability when bound to the BIOAGs as well as release kinetics were

also studied under various conditions, considering the project objective related to the remote control.

Finally, the most important achievement of partners involved in BIOAG fabrication is the ability to deliver to the consortium BIOAGs in amount necessary for all the planned *in vitro* and *in vivo* experiments.

Thermo-sensitive magnetic materials have been successfully developed pursuing the line of magnetically activated remote control of the drug or bio-active release. Two parallel approaches were developed by the consortium involving dendrimer and hydrogel solutions. Both approaches provided encouraging results as clear evidence of molecular collapsing were obtained around the target temperature value (32-52°C). In addition hyperthermic properties of some selected magnetic scaffolds upon high frequency magnetic field was also providing an added value to this activity as it showed the potential of remote release not only by MNP-drug aggregates (the only approach of the pre-MAGISTER research) but also within the scaffolds, promoting thus conceptually different solutions involving the magnetic scaffolds as multiple drug store with selective and on command expulsion.

The work on this topic culminated with the proof of principle that VEGF can be released from BIOAGGs by the generation of a controlled thermal stimulus that is derived from the energy loss of magnetic nanoparticles under specific magnetic field conditions. The generation of heat at the surface of the nanoparticles was exploited for VEGF release by the functionalisation of the magnetic nanoparticles with thermo-responsive generation 3 poly(epsilon-lysine) semi-dendrimers with an ascertained binding activity for VEGF. The thermo-responsive character was conferred to the poly(epsilon lysine) semi-dendrimers by the presence of two elastin-like sequences embedded in the first branching generation of the macromolecule, while the VEGF-binding affinity was provided by the tethering of the uppermost branching generation of the semi-dendrimer with molecules of carboxybetaines. The final dendrimers (C G3K ELP CB16) were uploaded with VEGF according to protocols previously optimised during the project to achieve the final BIOAGGs. The BIOAGGS manufactured at University of Brighton in scaled-up and reproducible conditions were generally tested in Santiago de Compostela for experiments of VEGF under magnetic field and then returned to UoB for studies of VEGF release. It found out that in less than five minutes of magnetic stimulation, the temperature increase was enough to attain 42 (°C) in agreement with undertaken commitments.

In vivo part of the project has been basically concentrated in the second part of the project, and became especially forceful in the last 18 Months. *Starting from the Months 30 an intense activity was generated by the consortium for the preparation and realization of main in vivo experiments regarding in vivo magnetic loading as well as in vivo test of other objectives of MAGISTER. Guided by medical partners the consortium developed a detailed plan for the utilization of the knowledge established under in vitro part of the investigation as well as of all the available in vivo knowledge available at consortium and external sources. The number of animals for any given experiment, the exact quantity of required materials, the roles of different partners including the exact assignment of the places where the experiments will be performed were assembled into a detailed table available to the consortium.*

Among this the task dealing with “in vivo bone regeneration and neoangiogenesis through MAGNETIC SCAFFOLDS, MNPs-VEGF (BIOAGG) delivery and MAGNETIC GUIDING” undoubtedly represented the most challenging deliverable result of MAGISTER, being effectively an in vivo

Proof of Principle of the main ideas and concepts behind the project . It represented a massive and widely multidisciplinary knowledge transfer of the materials and methods developed during the whole project duration in preclinically validated animal models.

The analysis of results collected on a number of animal models clearly indicated the achievement of NEOANGIOGENESIS (i.e neo-vessels formation) following the implantation of magnetic scaffolds (MagS) and magnetic guided delivery of MNP-VEGF. BONE regeneration occurred after magnetic scaffold implantation into the bone defect: it occurred both at the surface and inside the scaffold.

The acquired batch of data represents by no doubts a heritage of the consortium and its complete statistical evaluation and full understanding will proceed well beyond the end of the project. Nevertheless the data induce unmistakably optimistic conclusions regarding neoangiogenesis and bone regeneration via magnetic guiding. Moreover, the very imaging of neoangiogenesis and tissue regeneration was successfully fulfilled. Hystological sections of the regenerated bone tissue after Mags implantation and subsequent delivery of VEGF-MNPs and magnetic guiding clearly demonstrated new vessels formation elucidating both the VEGF role in promoting tissue regeneration and differentiation and its distribution via magnetic guiding.

This finding is crucial and opens the possibility to apply MAGISTER materials and methods in clinical practice for the treatment of large bone defects, where scaffold colonization and new-vessel formation represent the major limitations to obtain better and faster recovery of limb anatomy and function.

Moreover, the evaluation of Local and Systemic toxicity on in vivo animal model has revealed no adverse effects and hence promoted the investigated materials for various possible in vivo applications, significantly rising up the exploitable potential of MAGISTER outputs.

Considering further clinical issues, sets of clinical definitions were developed and continuously updated after 18, 36 and 48 Months.

In addition to material science and in-vivo/in-vitro magnetic guiding issues, magnetic fixation, a line moving in the project in parallel to the magnetic guiding, has successfully concentrated on calculations of magnetic forces for different possible configurations and their experimental tests. It has been shown that for osteochondral defects the magnetic fixation can successfully overcome the weight of the scaffold by at least one order of magnitude. Magnetic scaffolds can be efficiently fixed to the osteochondral region via at least three different scaffold-magnet configurations: external permanent magnet ring (EM) placed close to the bone and acting on the scaffold with both magnetizing effect and magnetic attraction; small permanent magnet pins (PM) implanted into the bone under the scaffold; utilization of pins from magnetic stainless steel instead of those from permanent magnets.

For long bone (diaphysial) defects it has been found that magnetic forces can only be used in addition to the standard mechanical fixation of scaffolds, and thus cannot substitute it completely. The fixation nevertheless can be significantly improved through the attractive magnetic forces.

Before moving to in vivo experiments the results of simulations have been compared with experimental data achieved by Magneto-Mechanical Laboratory System, a specially designed innovative system at partner University of Bologna. A good agreement between experimental measure and simulation results has been shown.

Remarkably the evaluation of in vivo fixation of the selected scaffolds by magnetic attractive forces, has been successfully concluded and pointed-out extremely interesting advantages

related to the fixation of magnetic scaffold through magnetic forces. In the context of “interface tissue engineering” magnetic forces resulted in highly integrated interfaces between the host bone and the regenerating tissue: magnetic lines seem to fasten and to participate to scaffold integration and tissue maturation.

Important to underline that very good results have been achieved considering the biocompatibility of the magnetic pins. Available bio-coating methods have been adjusted to the needs of in vivo experiments performed in the project.

The modeling part related to fixation was already mentioned. In addition to such studies MAGISTER dedicated considerable efforts to the development of innovative software and innovative models related to cell proliferation conditions and some aspects of the magnetic guiding.

A successful development of an innovative mathematical model describing the loading and transfer of BIOAGS in magnetic scaffold has accompanied the more experimental-oriented work described above. The model has been basically developed by using parameters of magnetic fields and objects experimentally defined and calculated by the Consortium. An additional approach (magneto-diffusion model) has been proposed for the description of MNPs penetration into a generic material – scaffold material or tissue. Thus a full mathematical description of magnetic parameters of magnetized scaffolds and magnetic nanoparticles has been realized.

A full mathematical description for in vitro oxygen and nutrients transfer from vascular compartment of developing tissue to osteogenic cells was built, enabling also the possibility of time dependent description of osteogenic cell development. The most distinct feature of the presented model is to account for vessels (capillaries) situated in inner space of the scaffold pore and actively contributing to the metabolism. A good agreement between the results of numerical modelling of cells density with known experimental data was found.

Oxygen transfer and bone maturation in magnetic scaffolds for in vivo conditions was also developed, involving the interconnection with blood vessel density and hence nutrient /oxygen concentration. It accounts for the formation of capillaries from the preloaded vascular cells and the ingrowth of the capillaries from the surrounding tissue.

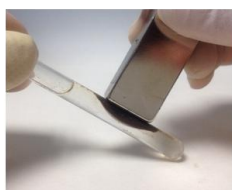
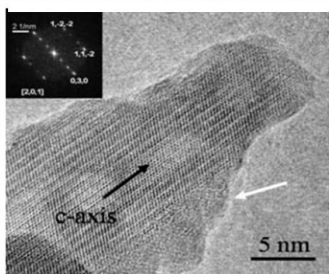
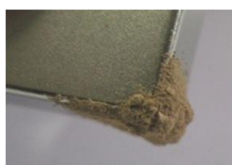
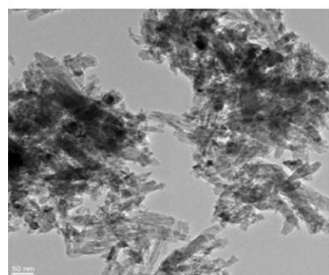
Finally, extensive calculations and simulations of the fully innovative nano-shuttle objects have resulted in the real fabrication of the object. Samples of large surface-to-volume magnetic shuttles have been produced by a combined fabrication process consisting in three steps: anodic porous alumina fabrication (to replicate the column nanostructure in the surface of the shuttle), UV-photolithography (to pattern the base of the shuttle) and finally the electrodeposition of the shuttle with Fe as magnetic material. All steps in the different stages of the fabrication process has been optimised (up to a reasonable limit) to obtain the shape of the shuttle initially envisaged in the project. General dimensions of the shuttle can be tailored on purpose by adequate selection of parameters, down to shuttle sizes of about 1.7 microns side and nanocolumns in the nanometre range from 60 to 160 nm.

Continuous biocompatibility and biotoxicity tests were performed for all the developed materials, objects and aggregates. This provided a validation or vice versa an exclusion of given material or given morphology. Noteworthy partners involved in this kind of research, and especially EMPA partner, had to develop conceptually innovative protocols and approaches able to deal with such a novelty as magnetic materials. By no doubts this knowledge has an important impact towards further research in the field and towards important future selections for clinical or industrial applications.

Main results achieved

The following part represents the collection of MOST IMPORTANT and MOST INTERESTING results achieved by MAGISTER and features also an explicit mentioning of the SCIENTIFIC and APPLICATION IMPACT of these results.

1) Bio-active and bio-resorbable magnetic nanoparticles Fe(II)Fe(III)-doped HA (Fe-HA MNPs)



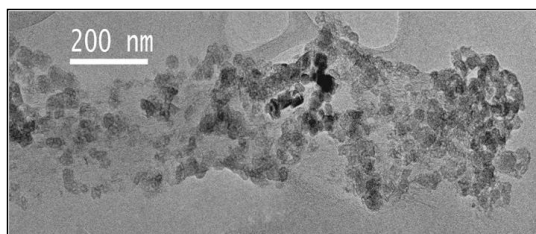
Description: Nano- and macro-morphologies of the developed Fe-HA nanoparticles characterized by an intrinsic magnetization.

Scientific Impact: The Fe-HA present modified cell parameters due to the substitution of calcium with Fe(II) and Fe(III) ions into the lattice. This ions occupying specific coordination positions confer to the particles an intrinsic magnetization at room temperature and a remarkable hyperthermia under the effect of applied electromagnetic field.

Application Impact: This innovative biologically active ceramic phase with superior level of biocompatibility and bioresorbability than magnetite is suitable to obtain magnetic nanoparticles for theranostic aims or to

transport and selectively release on site specific bioactive molecules. The Fe-HA was also useful to load 3D polymeric scaffolds to fabricate bioresorbable and biomimetic magnetic materials as granulate and Injectable magnetic paste.

2) Biologically inspired hybrid magnetic scaffolds for osteo-chondral and/or bone tissue regeneration (hybrid-MagS)



Description: Collagen/Fe-doped-hydroxyapatite bio-hybrid magnetic scaffolds for bone tissue and osteo-chondral tissue regeneration.

Scientific Impact: A biologically inspired nucleation of superparamagnetic iron doped hydroxyapatite (Fe-HA) on assembling collagen fibers was performed. During the nucleation of Fe-HA crystals on collagen fibers the two iron species (Fe(II) and Fe(III)) have been introduced into the apatitic lattice in different Ca crystallographic positions with a specific coordination generating in

situ a mineral phase endowed with intrinsic superparamagnetism.

Application Impact: The developed bio-hybrid magnetic scaffold endowed with improved biocompatibility due to the nucleation of a bioresorbable magnetic phase on collagen fibers have been obtained with different magnetization and mineralization gradients in order to regenerate bone and osteochondral tissues and to guide functionalized MNPs directly to the implant sites.

3) Bio-hybrid Collagen/Hydroxyapatite + Fe₂O₃ 30/70wt% composites for bone tissue regeneration (hybrid-MagS)



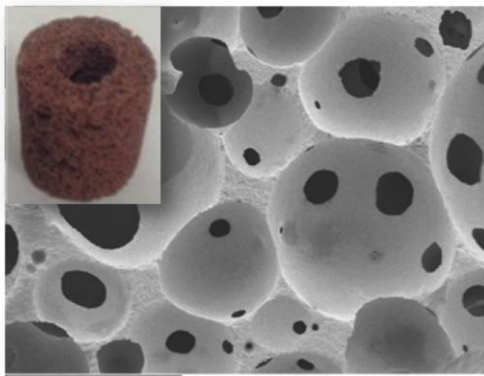
Description: bio-hybrid Collagen/Hydroxyapatite + Fe₂O₃ magnetic scaffold for bone regeneration.



Scientific Impact: In this approach, magnetic bio-hybrid composites have been obtained by impregnation of hydroxyapatite/collagen hybrid scaffold with magnetite based nanoparticles (Fe₂O₃). This technique permits to reach so far highest magnetization values and to develop scaffold with a magnetization gradient.

Application Impact: The hybrid-MagS with different gradient of magnetization and mineralization are suitable for bone tissue regeneration, for the in vitro and in vivo magnetic loading by an external magnetic field and the magnetic fixation.

4) Porous ceramic magnetic scaffolds based on HA and Fe₂O₃ for bone tissue regeneration (MagS)



Description: Porous ceramic magnetic scaffolds (MagS) made of Hydroxyapatite/Magnetite (90/10 wt%).

Scientific Impact: Porous ceramic magnetic scaffolds (MagS) made of ceramic/ceramic composite Hydroxyapatite/Magnetite (90/10 wt%) were obtained by a controlled ceramic process.

Application Impact: these MagSs are suitable for bone replacement and magnetic fixation. The attractive magnetic forces occurring between Mags

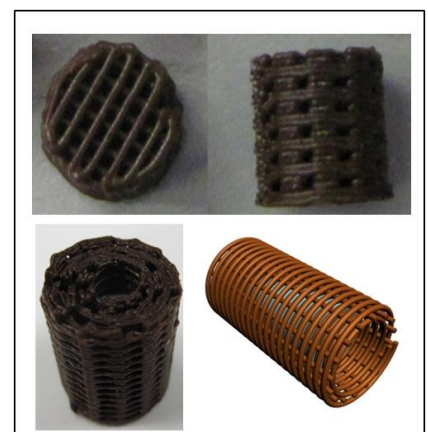
and the implanted biocompatible magnet are able to avoid the micro-movements at the scaffold-bone interface and promote a faster integration of the scaffold.

5) Polymeric magnetic scaffolds (PCL/Fe-doped-hydroxyapatite)

Description: 3D polymeric Magnetic porous scaffolds based on PCL/Fe-HA 80/20wt% nanocomposite

Scientific Impact: The PCL/Fe-HA composite scaffolds have been designed, modelled and manufactured by 3D Bioplotting technique that is suitable for realizing scaffolds with complex morphology and magnetization gradients.

Application Impact: The PCL-Fe-HA composite scaffolds are suitable to guide the bone tissue regeneration in vivo. This scaffold may allow to develop customized solutions for difficult surgical reconstruction particularly for significant bone loss in which early load bearing is needed. Magnetic fixation was performed in-vivo, to this aim a cylindrical scaffold incorporating a permanent cylindric magnet was designed to match the geometry of the diaphysis of the selected animal model.



6) Injectable bio-active and bio-resorbable magnetic scaffolds (Fe-HA based)



Description: injectable bioresorbable magnetic paste

Scientific Impact: Starting from the magnetic Fe-doped-hydroxyapatite (Fe-HA) nano-powder was developed a ready-to-use,

injectable and bioresorbable magnetic scaffold.

Application Impact: The injectable-MagS are useful as bone void filler for orthopedic and maxillofacial surgery. The bone graft material is able to speed up the bone regeneration and improve bone quality respect to non magnetic paste. Magnetic pastes requires a less invasive surgery and can be used to fill small bone defects cavitory bone defects such as cyst or tumor removal, filling cages for Posterior Lumbar Interbody Fusion (PLIF).

7) Analytic balance-based magnetometer

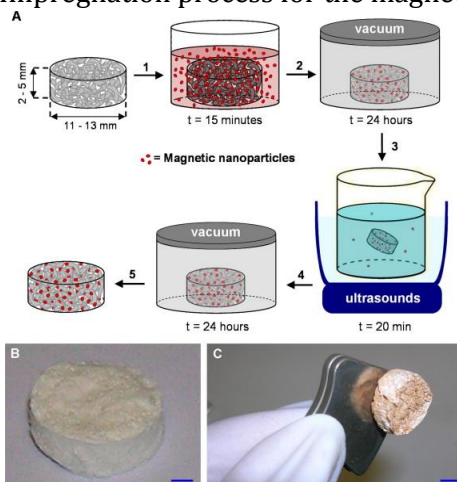


Description: Picture of the prototype of the analytical balance-based magnetometer

Scientific Impact: This magnetometer makes it possible to carry out simple magnetic characterizations (e.g. hysteresis loops) without the need to access a specialized laboratory.

Application Impact: The set up responds to the increasing need for magnetic characterizations in biological laboratories, which are commonly equipped with analytical balances. For a fraction of the cost of research-level magnetometers, it is possible to add the kit to the balance, without interfering with its usual task of weighing. The discussion on the possible commercialization of the kit is in progress.

8) Impregnation process for the magnetization of scaffolds for bone regeneration



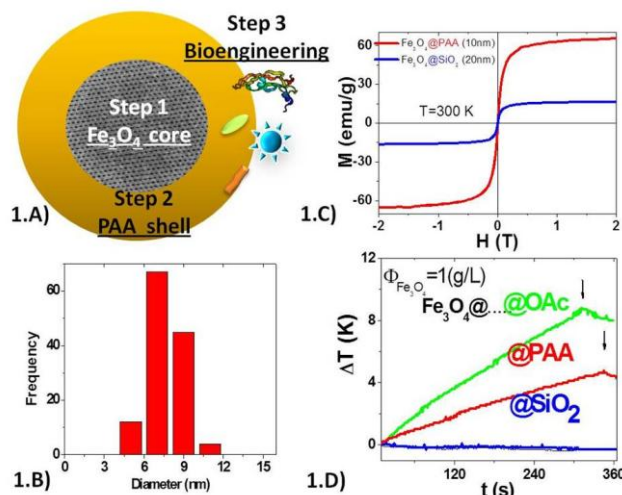
Description: Impregnation process of a scaffold with a ferrofluid. The scaffold is sufficiently magnetized to stick to a magnet.

Scientific Impact: This technique can be used for several materials, such as collagen and hydroxyapatite/collagen composites. It makes it possible to investigate magnetic scaffold systems in an affordable and repeatable way.

Application Impact: The flexibility of the method means that we can magnetize many different systems, of almost arbitrary shape and made of several different materials. The simplicity of the process

means that it can potentially be used in a clinical setting for real time magnetization of tailored scaffolds.

9) Multi-engineered Biocompatible Super-Paramagnetic (SPM) Fe₃O₄ nanoparticle (NP) coated with Poly-acrylic-acid (PAA) NPs for multipurpose magnetic delivery and safety mild thermal therapies.



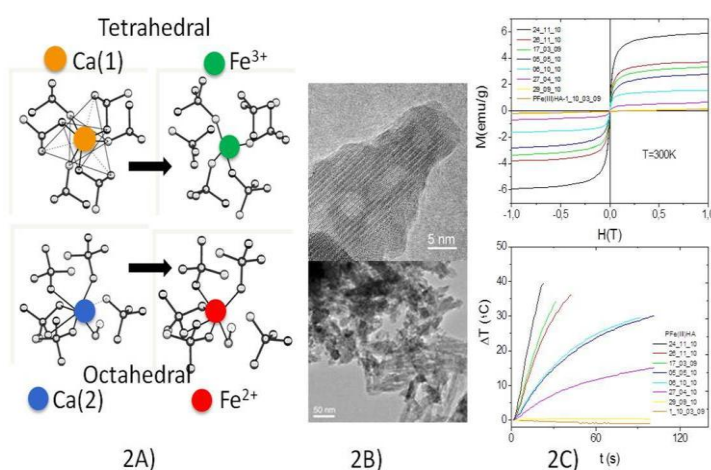
Description: The proof of concept of the multi-engineering strategy has been successfully done by integrating a SPM magnetite core coated by a biocompatible PAA shell obtaining NPs with 1.A) high core cristallinity and 1.B) precise monodispersity, and good C)&D) magneto-thermal performances, physicochemical stability for long periods; functionalizable surface.

Scientific Impact: These very small SPM Fe₃O₄ cores (8nm) coated with biocompatible Poly-acrylic-acid(PAA), demonstrates the feasibility of the multi-

engineering strategy to obtain NPs with magnetic functionality in the core and bioactivity in the coating shell.

Application Impact: These NPs open the door to in-situ magnetic hyperthermia drug and cell growth factors delivery applicable to oncological therapies with enhanced tissue regeneration offering simultaneous in situ MR imaging possibilities.

10) Bioresorbable Intrinsically Magnetic Hydroxyapatite (HA) Scaffold mimicking Fe₃O₄ structure



Description: Successful proving of concept of optimized chemically doping 2A) Iron ions into the Calcium positions in HA scaffold with 2B) avoidance of magnetite secondary phases formation. The new intrinsically magnetic phase of HA shows 2C) magneto-thermal performances suitable for biomedical applications.

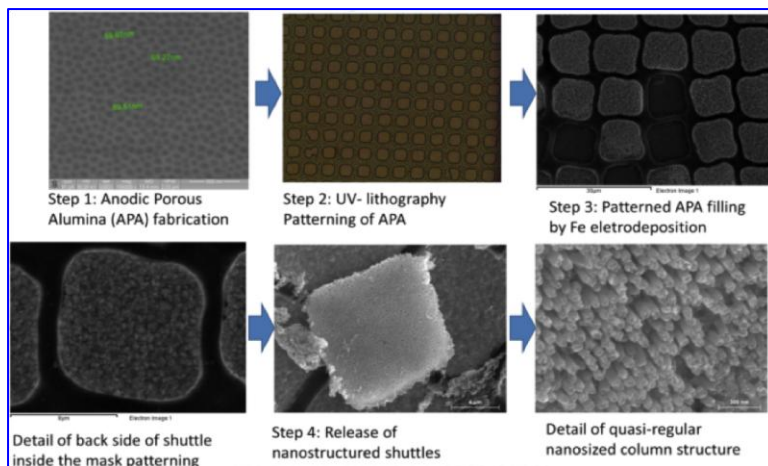
Scientific Impact:

A new bioresorbable and intrinsically magnetic HA scaffold has been engineered and its

magnetic functionalities can be effectively controlled by the amount of magnetic doping.

Application Impact: These MNPs open the door of traditional applications of multipurpose magnetic hyperthermia (in-situ drug and biomolecules delivery, cancer therapy) or MR imaging possibilities by a bioresorbable nano-drug carrier.

11) Nanostructured magnetic shuttles with high surface-to-volume ratio.



Description: Samples of large surface-to-volume magnetic shuttles have been successfully fabricated by a combined process in three steps: anodic porous alumina (for nanostructuring), UV-photo-lithography (to pattern the base of the shuttle) and finally the electrodeposition of the shuttle with Fe as magnetic material. General dimensions of the shuttle can be tailored on purpose by adequate selection of parameters, down to

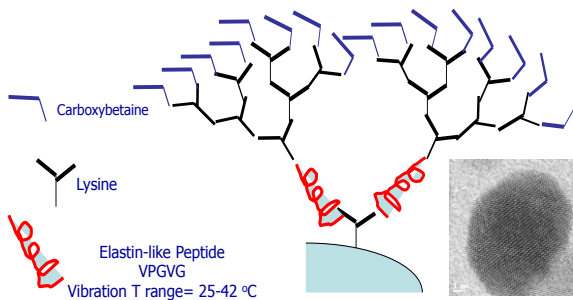
shuttle sizes of about 1.7 microns side and nanocolumns in the nanometre range from 60 to 160 nm.

Scientific impact: Fabrication of a regular nanostructure of columns of controlled diameter, height and period.

Application Impact: The high surface-to-volume ratio enables a higher loading of active factors to be delivered in an individual and controlled way to the area to be healed.

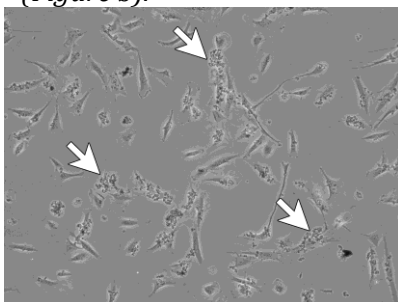
Applications in other fields have been also foreseen (i.e. non-volatile memories, tagging)

12) Magnetic Nanoparticles Functionalization with Biocompetent Semi-Dendrimers as Shuttles for Growth Factor and Stem Cell Controlled Manipulation and Delivery



Description: Magnetic shuttles for VEGF and stem cells in the form of magnetic nanoparticles (MNP) were developed. The affinity to both the growth factor and the stem cells was obtained through the exposure of monomers of carboxybetaine (CB) at the uppermost branching generation of poly(epsilon-lysine) biocompetent semi-dendrimers (BSD). BSD were produced with and without an elastin core making the whole macromolecule thermoresponsive (Figure a).

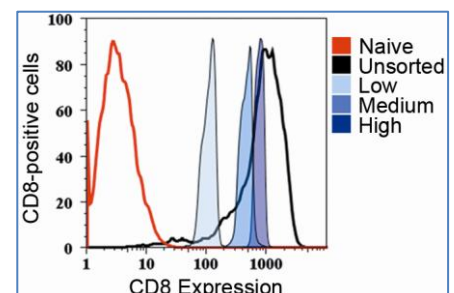
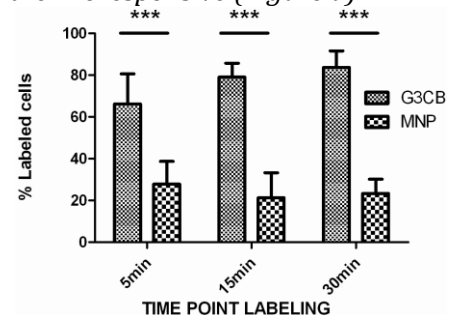
The BSD covalent grafting was obtained through the development of 18 -nm diameter MNP presenting a poly(acrylic acid) coating (Figure a, insert) and through a mild surface chemistry. Shuttles with a hydrodynamic radius of 36 nm, a VEGF binding capacity of 3.64 VEGF $\mu\text{g}/\text{mg}$ of nanoparticles and an efficiency of stem cell magnetization higher than 80% after only 15 min of incubation were obtained (Figure b).



Uniquely, the cells were magnetized through glyocalyx decoration rather than internalization (Figure c, arrows).

Further, genetically modified mesenchymal progenitor cells could be rapidly purified by flow cytometry to yield populations that homogeneously expressed a

specific safe and therapeutic VEGF level (Figure d).



Scientific Impact: For the first time, insights were provided unveiling the ability of synthesizing BSD exposing CB that were able to interact with: (i) VEGF improving its bioavailability to the endothelial cell VEGF receptors thus stimulating angiogenesis, (ii) the stem cell glycocalyx. Integrated with the magnetic shuttle these BSD allowed the magnetization of the stem cells without the need for internalization or for antibody-based recognition. Decoration of purified VEGF-expressing cell populations provides an advanced bioderivative with combined potentials for: 1) efficient magnetic delivery; 2) tissue regeneration (mesenchymal progenitors); and 3) vascularization (controlled VEGF expression).

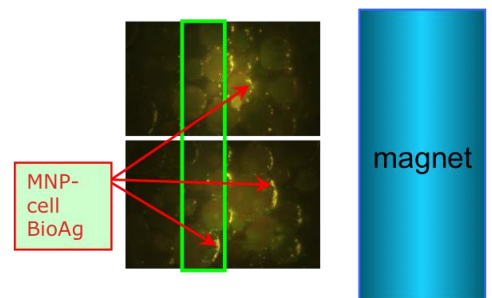
Application Impact: A breakthrough technology was achieved through the manufacture of biospecific magnetic shuttles in GMP conditions which are highly efficient tools for the manipulation and delivery of growth factors and stem cells in: (i) clinical applications (controlled delivery); (ii) pre-clinical standardized testing (e.g. VEGF ELISA kits, VEGF purification) and manipulation methods (e.g. stem cell isolation procedures).

13) Magnetically guided loading of MagS with BioAg

Description: MNP-loaded cells contain fluorescent marker. Magnetically-guided cell localisation can be focused on specific scaffold regions e.g. green box outlined

Scientific Impact: Novel demonstration of magnetic control over 3D scaffold loading efficiency. Mid-Term Proof of Principle.

Application Impact: Novel method for 3D cell assembly, with use in other WPs

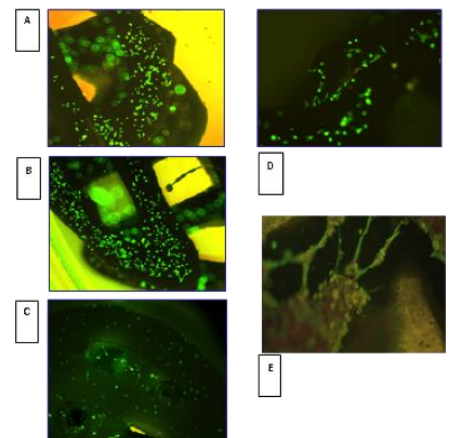


14) Angiogenesis in Magnetically guided loading of MagS with BioAg

Description: A, B, C: 3.5h after scaffold loading. D, E: 7h after scaffold loading. A, B: cell layer formation on scaffold surfaces, horizontal plane. C: Scaffold, longitudinal section. D, E: Tubule formation, scaffold horizontal plane.

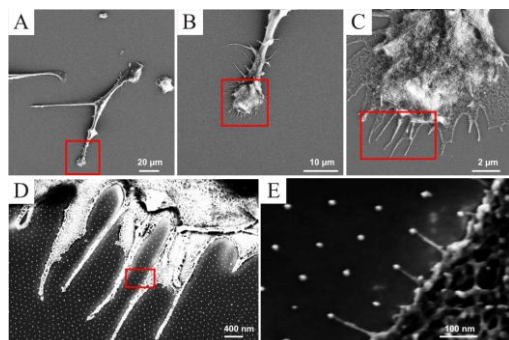
Scientific Impact: Novel system capable of producing 3D vascular structures through controlled assembly of angiogenic cells by magnetic forces

Application Impact: Critical for tissue regeneration – control over implant angiogenesis. In vitro assembly of 3D vascular structures for commercially- focused cell-based analysis.

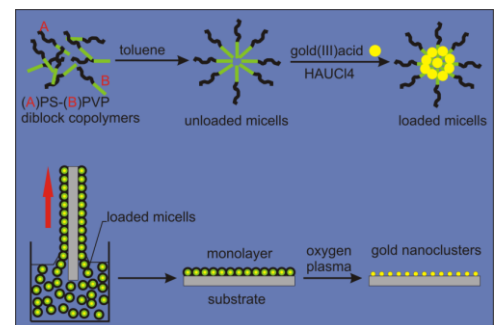


15) Surface-nanostructured scaffolds

Description: Block co-polymer micelle nanolithography; variable distance (PS length dependent);



transferable to polymers, 3D polymer surfaces (micro-channels); bio-functionalisation via thiol linker, cell anchorage demonstrated by cryo-SEM.



Scientific Impact: Novel Technology for bio-functional modification of polymer surfaces to enable nano-positioning of anchored cells and enhanced surface interaction

Application Impact: Improved bio-compatibility of medical devices – wide clinical application. Standardised cell assembly in vitro – improved reproducibility of commercial cell-based analysis

16) Magnetic BioReactor

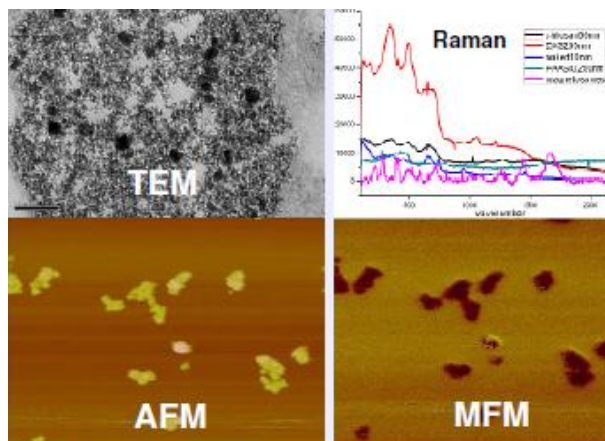
Description: Magnetic bioreactor designed and built at ISMN Bologna; designed as a glove-box: UV sterilization, thermo-statically-controlled sample holder; allows easy scaffold insertion and BioAg introduction, with microcirculation for scaffold effective perfusion

Scientific Impact: Enables detailed, reproducible loading of MagS and controlled application of magnetic fields essential to characterize and optimize magnetically-guided loading of BioAgS into 3D structures

Application Impact: Standardised MagS loading essential for future clinical tissue regeneration applications



17) Magnetic guiding using biologically-generated magnetosomes



Description: Magnetosome production in mesenchymal stem cells after Mms6 transfection; magnetosome growth / stability characterized; MSC functional differentiation capacity retained

Scientific Impact: Creation of cell-BioAg without MNP loading ; sustained Mms6 expression maintains cell magnetic responsiveness.

Application Impact: Innovative tool to create magnetically-guided cell populations for in

vivo/in vitro uses.

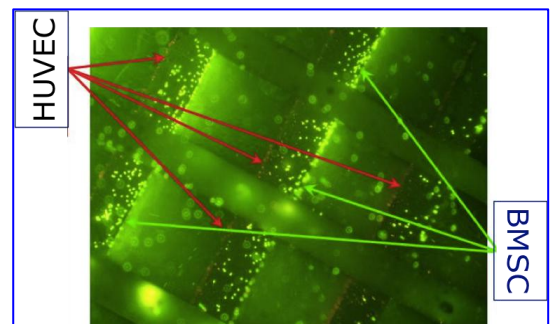
18) 3D Co-Colonisation of MagS with Angiogenic and Osteogenic Cell Populations

Description: Osteogenic bone marrow stem cells (green) and human vascular endothelial cells (red) localized in multiple layers in extruded Fe-hydroxyapatite scaffold; localized growth of both cell populations

Scientific Impact: Innovative technique for focused guiding and discrete localisation of dual cell populations within a single 3D scaffold space.

Application Impact: Innovative tissue regeneration strategies based on implants with dual functionalities. Novel in vitro models with commercial application in cell-based screening.

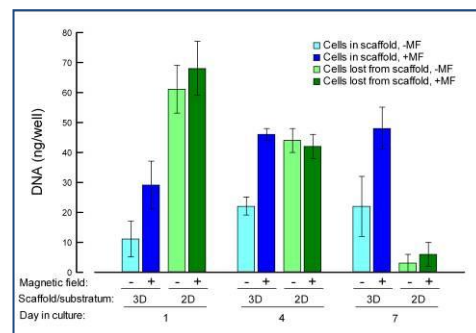
19) 3D Cell-Based Analytical Platform



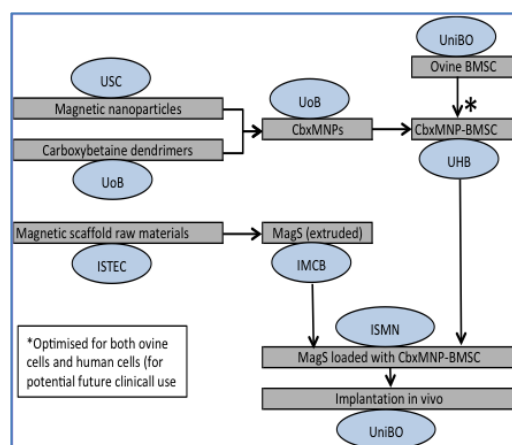
Description: MNP-loaded BMSC seeded into PCL/Fe-hydroxyapatite scaffolds. Assay of scaffold-associated cells showed more consistent performance after magnetically-guided loading

Scientific Impact: 3D cell-based assay platform with consistent cell loading, better assay reproducibility.

Application Impact: Potential commercial uptake in leading-edge commercial 3D cell-based analysis



20) Prototype Strategy for MagS Use in Vivo



Description: Co-ordinated protocol for synthesis and assembly of methods and materials capable of delivering loaded MagS as tissue implants using magnetic forces.

Scientific Impact: In vitro strategy allowing ethically-approved in vivo study under WP5.

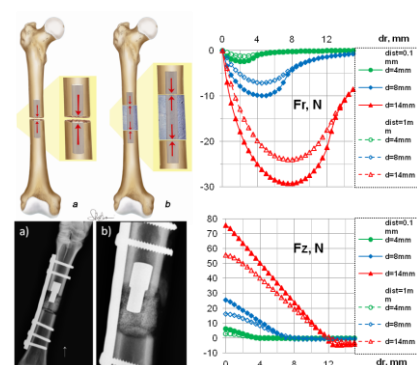
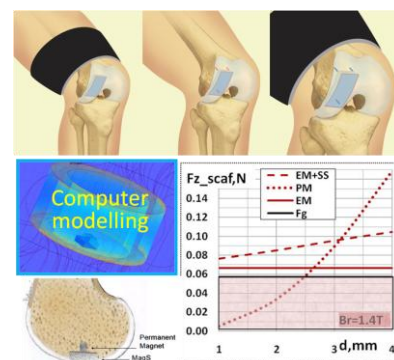
Application Impact: Potential new strategy for osteochondral tissue regeneration using magnetically-constructed and guided implants.

21) Analysis and simulations for magnetic fixation of osteochondral MagS

Description: There were defined three magnetic configurations generating sufficient forces to fix osteochondral MagS.

Scientific Impact: Novel method of scaffold fixation using MagS and internal PM or external EM permanent magnets or stainless steel pins with EM.

Application Impact: Innovative technique of scaffold fixation with additive possibility to reduce interface micromotions, to induce magneto-mechanical stimulation and to assist targeted drug or cell delivery.



22) Analysis and calculations for long bone magnetic fixation

Description: Long bone magnetic assisted fixation using permanent magnet rods set rigidly into the medullary canals and in the scaffold core for cases of fracture and scaffold treatments. Lateral and axial magnetic forces

Scientific Impact: Novel magnetic assistance to traditional bone fixation capable improve bone regeneration.

Application Impact: Innovative technique permitting to

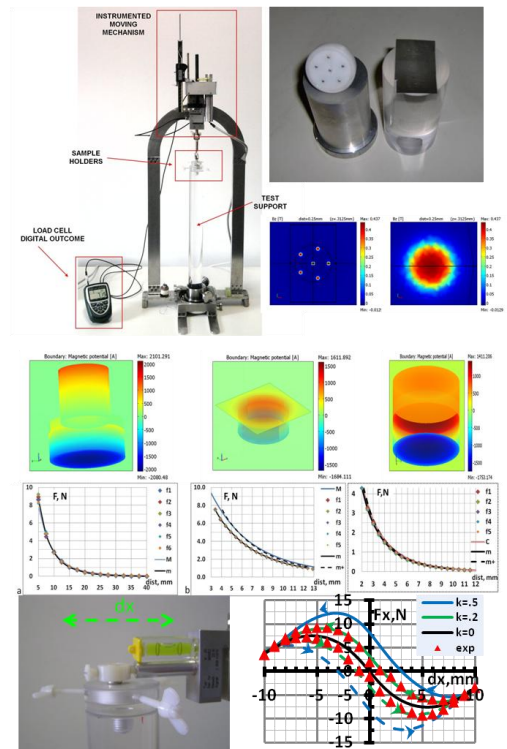
reduce interface micromotions, to induce magneto-mechanical stimulation and to assist targeted drug or cell delivery.

23) Magneto-Mechanical Laboratory System

Description: Have developed a Magneto-Mechanical Laboratory System (MMLS) as a suitable test of modelling and calculation results as far as fixation issues are concerned. A very good agreement between experimental and calculated data for different magnetic materials and configurations was found. Both axial and lateral forces were validated.

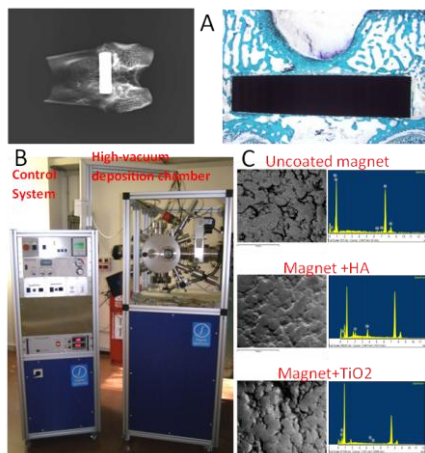
Scientific Impact: the MMLS is a conceptually new simulator instrument for the definition of the methodology for MagS fixation in selected bone or osteochondral defects.

Application Impact: the MMLS validated the results obtained from calculations and simulations by finite element method. It can be useful to simulate fixation and other orthopedic applications.



24) Permanent magnet biocompatible coating

Description: A- TiO₂ encapsulated magnet to set in the rabbit femoral condyle. B- Pulsed Plasma Deposition (PPD) System. C- SEM images and EDX characterizations of the uncoated magnet and after deposition of TiO₂ and HA.



Scientific Impact: Innovative PPD technique for permanent magnet coating. Confirmation of biocompatibility of TiO₂ encapsulated magnets and Parylene coated magnets. Good tissue regeneration and no inflammation near encapsulated and coated magnet in in vitro and in vivo experiments.

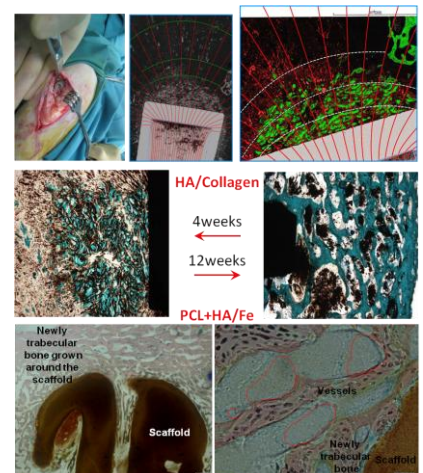
Application Impact: Such high quality bio-compatible coatings advance at least three application proposals: the first deals with the application of the innovative PPD method for bio-coating, a work performed by MAGISTER for the first time; the second deals with material selection, that is TiO₂ coating for the bio-compatibilisation of various alloy, metal and oxide objects to be used in vivo and in vitro; the third one deals with the possibility to utilize permanent magnets in vivo for any needed application (well beyond fixation).

25) In vivo bone MagS fixation

Description: Ha/Collagen or PCL+HA/Fe scaffolds implanted in lateral femoral condyles of rabbits or sheep near NdFeB permanent magnets.

Scientific Impact: Fairly good fixation achieved. A good bone regeneration using different MagS materials. Additional (unexpected) result: tissue and MNPs orientation follows magnetic field lines.

Application Impact: Fixation method which has good chances to achieve clinical applications in a short-medium time scale. The orientation of regenerating magnetized material along the magnetic lines put the basis for future control of the bone morphology via the magnetic field control.



26) In vivo long bone scaffold fixation



Description: Figure: HA long bone scaffold fixed by metal plate with addition magnetic fixation using NdFeB permanent magnet rods.

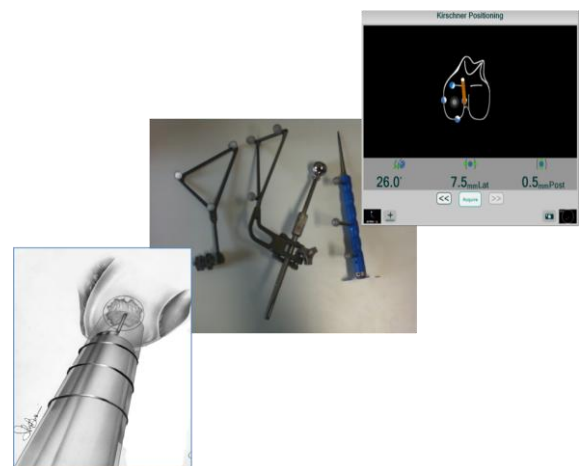
Scientific Impact: New magneto-mechanical concepts proposed and developed.

Application Impact: Fully innovative complementary fixation technique (additional to the traditional mechanical fixation) able to enhance considerably the interface stability for the osteochondral scaffold treatment and hence able to guarantee higher quality tissue regeneration.

27) Navigated surgical instrumentation (Computer Assisted Surgery –CAS) for Mags implantation

Description: Navigated surgical instrumentation for MAGs implantation allows more accurate permanent magnets and magnetic scaffolds positioning and more repeatable bone-osteochondral defects creation for pre-clinical animal studies.

Application Impact: Allows clinical manipulation of magnetic scaffolds and more in general of any magnetic object to be surgically implanted. Instrumentation conceptually ready for clinical validation.

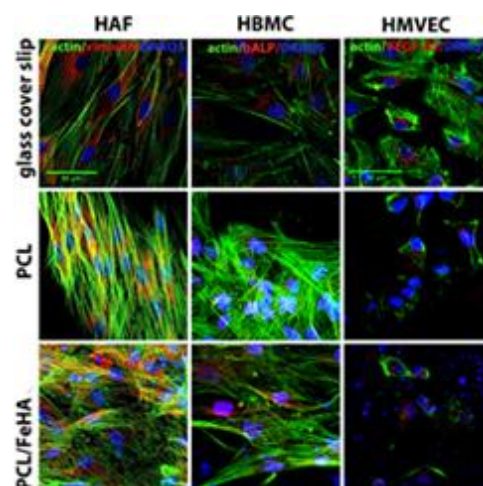


28) Proof of biocompatibility using a sequence of tests

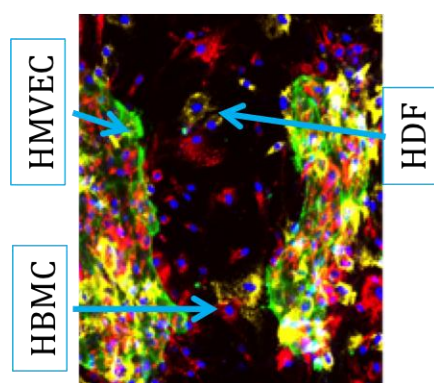
Description: Different cell type 2-D cultures to evaluate cell adhesion. Representative PCL and PCL/FeHA materials.

Scientific Impact: By using a test sequence starting with ISO10993-5 tests and subsequently evaluating cell adhesion, proliferation and differentiation a clear prognosis regarding the biocompatibility of the material can be made.

Application Impact: The proposed test sequence may help to speed up evaluation of biomaterials by omitting at each phase problematic candidates of the selected set of test materials.



29) Development of a three cell types co-culture set-up for biomaterial evaluation



Description: Osteogenic human mesenchymal stromal cells (HBMC, red), human microvascular endothelial cells (HMVEC, green) and human dermal fibroblasts (HDF, orange) co-culture.

Scientific Impact: The result represents a pioneering work as far as co-cultivation of 3 cell types and separate analysis of their populations using flow cytometry analysis (FACS).

Application Impact: This achievement opens new ways to screen in vitro for bioactive surfaces in respect of which cell types and consequently what type of tissue may expected to

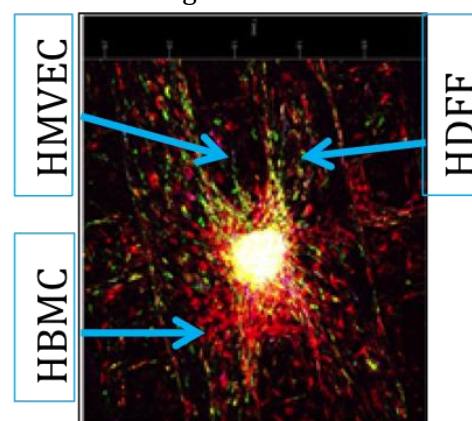
be formed at the implant surface.

30) Outgrowth of three cell types out of an organoid culture on a PCL based Magnetic Scaffold

Description: Osteogenic human mesenchymal stromal cells (HBMC, red), human microvascular endothelial cells (HMVEC, blue) and human dermal fibroblasts (HDF, green) growing out of a multicellular organoid colonizing in multiple layers a magnetic PCL composite fibre mesh.

Scientific Impact: The result represents a pioneering work implementing 3D organoid assembled of 3 cell types to determine the biomaterial colonization.

Application Impact: This achievement opens new ways for in vitro screening for bioactive surfaces.



31) Biocompatibility of Novel Magnetic scaffolds

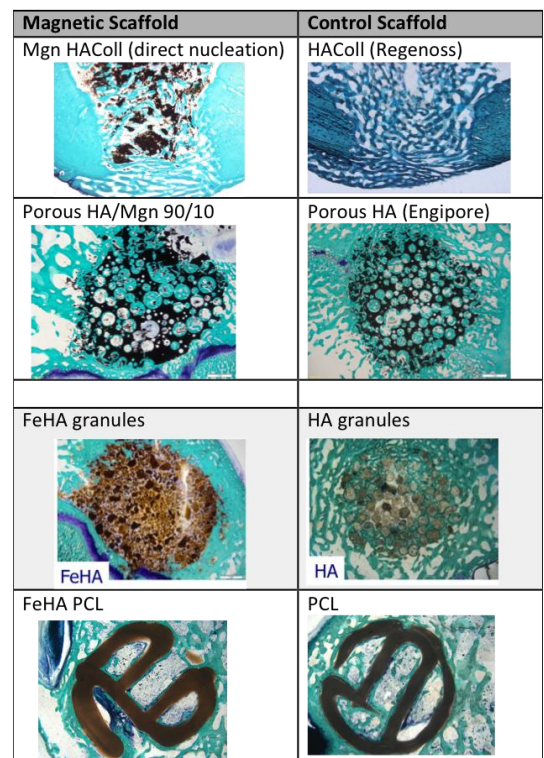
Description: All the magnetic scaffolds produced by WP1 were biocompatible. Biocompatibility was investigated on rabbit model and results include comparison with no magnetized control biomaterials. Histological results on the samples show no significant acute inflammatory or

chronic inflammatory/immunological response. It is also clear that there are no toxic effects on the organs.

The figure shows the biocompatibility and bone regeneration of the new magnetic scaffolds (left) and their NON magnetic control.

Scientific Impact: The new magnetic biomaterials developed represent a new platform to discover new insights and information regarding cell-cell, cell-biomaterial, tissue-biomaterial interactions and their relationship with the induced magnetic field.

Application Impact: The biocompatibility of the magnetic biomaterials represents the first step for their application into pre-clinical and clinical application in the treatment of bone and osteochondral critical defects

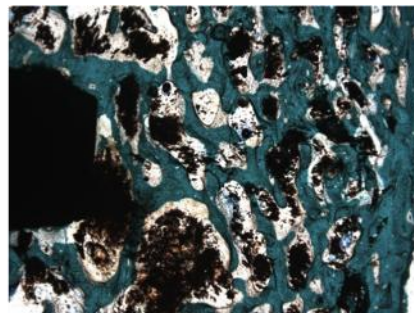
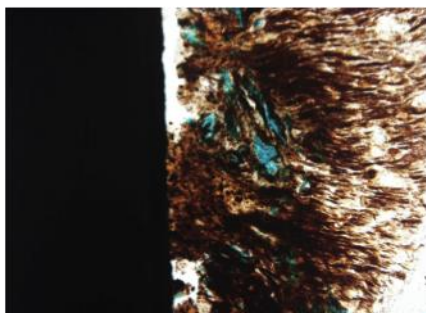


32) Hierarchical structured regeneration guided by magnetic forces

Description: The magnetic scaffold responded to the magnet, and it was oriented according to magnetic field lines and moreover cells proliferation and matrix deposition followed these lines, it was obtained a hierarchical structured regeneration guided by magnetic force.

Scaffold orientation "in vivo" according to the magnetic lines after 4 weeks (left image); bone tissue regeneration after 12 weeks (right image)

Scientific Impact: Collagen is the most essential structural extracellular matrix protein in the human body that determines material properties of dense connective tissues as well as connective tissue stromal elements of parenchyme organs. The possibility to change the magnetic biomaterial architecture after implantation, therefore offers the possibility to shape



the regenerating tissue according with specific characteristics of the tissue. This concept is translatable to all the field of tissue engineering.

Application Impact: In bone and osteochondral

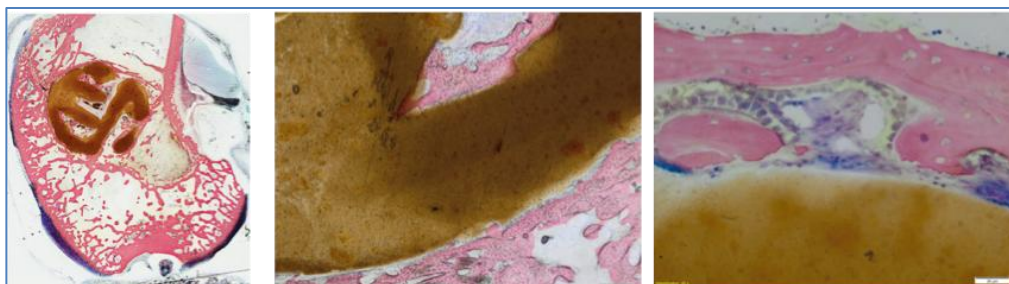
tissue engineering the orientation and organization of the regenerating tissue provide biomechanical characteristics specific of the anatomical region to be regenerated, for example: the sponge bone of the metaphysis or the cortical bone of the diaphysis. The described finding

opens conceptually new possibilities for medicine, and namely promoting bone regeneration with an a priori decided morphology.

33) Enhanced angiogenesis and osteogenesis in vivo

Description:

33.1) Small animal model (with relatively short follow-up)

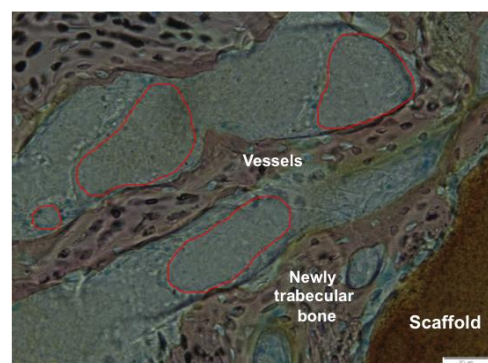


a) In vivo implantation of MagS pre-loaded in vitro: FeHA PCL scaffold implanted in a critical bone defect of the lateral

aspect of the femur of the rabbit, pre-seeded in vitro by magnetized Bone Marrow Stem Cells (BMSCs) expressing high VEGF levels.

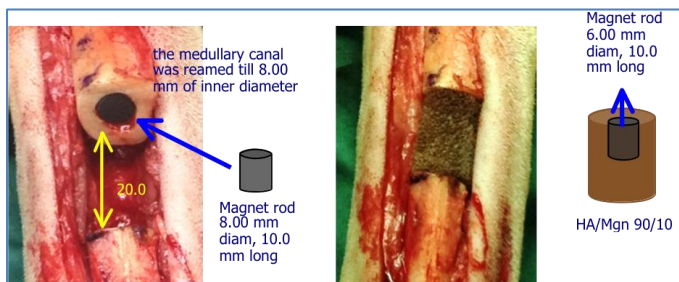
b) In vivo implantation of MagS and injection MNPs-VEGF

FeHA PCL magnetic scaffold implanted in a critical bone defect of the lateral aspect of the femur of the rabbit, close to a 3x3 mm parylene coated permanent magnet, and subsequent MNPs-VEGF delivery

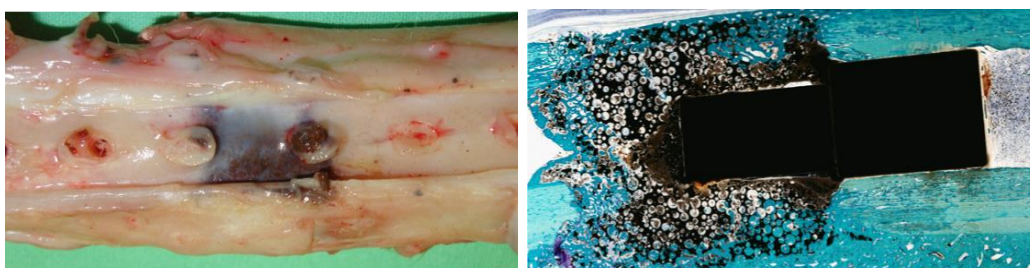


33.2) Big animal model

A critical bone defect of the sheep metatarsus implanted with magnetic porous HA and reloaded with BIOAGS with enhanced VEGF carrier properties which were obtained through the surface functionalisation of PAA-coated MNP with biocompetent dendrons able to bind VEGF. This challenging experiment aimed at translating materials and methods achievements developed during the MAGISTER project



as close as possible to practicable clinical practice. Magnetic Porous HA scaffold (90/10) implanted in a 2 cm critical bone defect of the sheep metatarsus: magnetic fixation was performed only at proximal interface (one magnet inside the medullar channel, one magnet inserted into the scaffold)



in order to allow comparison between magnetic and non magnetic fixation

Sheep metatarsus

explanted after 4 months (left), histological image of the sample (right)



MicroCT 3-D images of bone regeneration inside the magnetic scaffold after reloading with VEGF- MNPs and fixated at proximal interface with permanent magnets: more bone regeneration and more differentiated cortical bone regenerated at the magnetic interface.

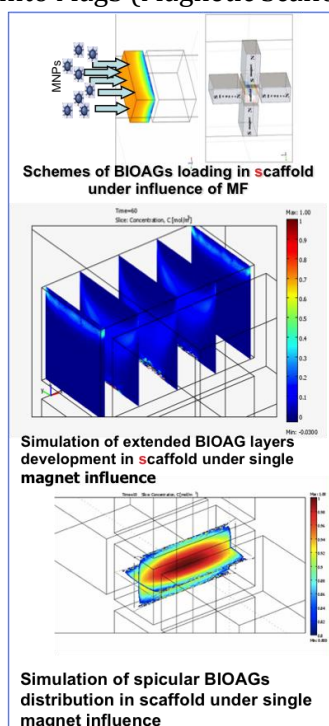
Scientific Impact: The small and big animal model experiments assessed an innovative method of delivery to the biomaterial-regenerating tissue specific growth-factors, VEGF in this case. The results demonstrated the efficacy of the method and have set the basis to translate the approach to other fields of tissue engineering and in general of medicine.

Application Impact: We foresee a potential huge clinical impact of the new method of drug delivery through magnetic guiding based on magnetized biomaterial and

external/internal permanent magnets. The VEGF delivery demonstrated to promote new vessels formation and bone regeneration. This strategy provide a new method to treat large tissue defect where poor vessels formation and insufficient cells biomaterial colonization represent the causes of implant failure.

The fixation effect produced by the permanent magnets offers an valuable instruments for the fixation of the biomaterial in the site of implantation and therefore its application will pave the way to new approaches in the field of interfaces tissue engineering, moreover the effect of the induced magnetic field allow to change, control and predict the architecture of the regenerated tissue.

34) Mathematical model of loading and transfer of BIOAGs under influence of Magnetic Field into MagS (Magnetic Scaffolds).

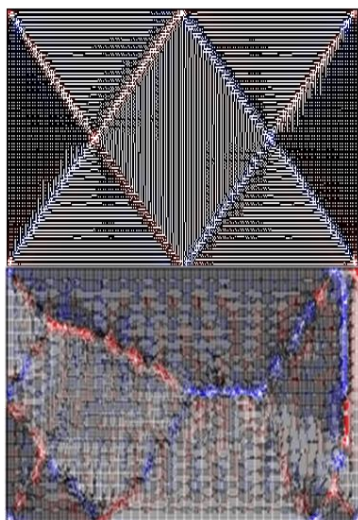


Description: The model gives a possibility to calculate the parameters of the magnetic fields (MF) as well as the processes of BIOAGs distribution and transfer leading to the creation of a definite BIOAGs density in local compartments of scaffolds. According to mathematic modelling results, the magnetic scaffold can be saturated by BIOAGs resulting in the creation of extended zones of high concentration of these agents, able to stimulate the formation of vessels and layers of osteogenic cells, which was practically proved by colleagues from WP4.

Scientific Impact: A novel mathematical model which describes approaches to magnetic scaffold saturation has been elaborated.

Application Impact: Novel computerized methods for optimization of the parameters of magnetic field, magnetized scaffolds and Bioags. Prediction of distribution of BIOAGs (magnetically labelled cells and particles) in local compartments of scaffolds provides new possibility for tissue engineering applications and cell base analysis.

36) Magnetic Nanoshuttles



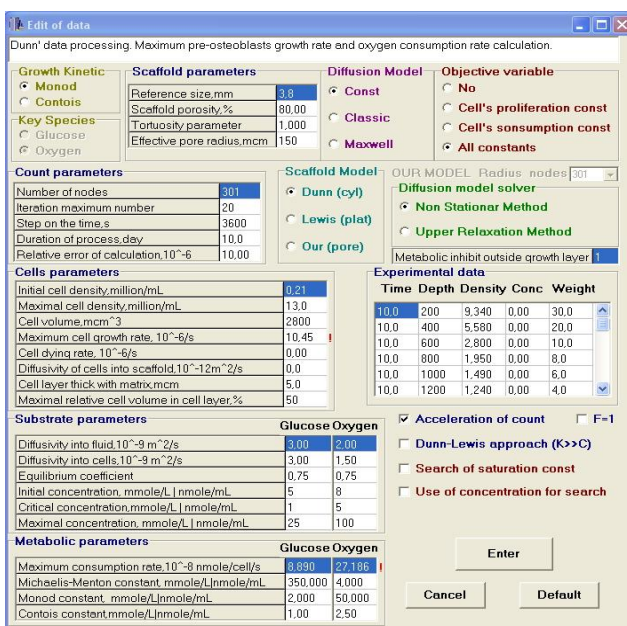
Description: Mathematical simulation of nanostructured shuttles for the transport of active factor (AF) in vivo. The presence of pillars influences the magnetization dynamic response irrespective of the configuration of the pillars, especially in systems characterized by a very thin film. Magnetic domains at zero field are also influenced by the nanopillars: Pillars affect remanence of the base while their contribution to the overall magnetization is negligible

Scientific Impact: A mathematical simulation of a regular nanostructure of columns of controlled diameter, height and period has been realized.

Application Impact: The mathematical model enables to predict conditions for higher loading of active factors to be delivered in an individual and controlled way to the area to be healed. The approach can be used for a wide range of tissue-engineered applications.

37) Software for computer simulation of tissue development

Description: Software for computer simulation of tissue development in magnetic and non-magnetic scaffolds depending on their inner microarchitecture and external environment in vitro and in vivo.



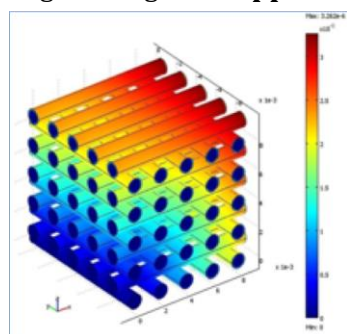
Simplified (reduced) interface (2 forms) for calculation of the basic parameters of a cell system inside a porous scaffold presented at the internet (www.biodevicesystems.cz) by limited beta testing mode. A special diagram for creation of request for full-featured version can be used is offered.

Scientific Impact: Principally new innovative mathematical approaches, used in the program, allow to make calculations of cell proliferation and tissue development in any tissue-engineered scaffold-based systems both in vitro and in vivo.

Application Impact: Method of efficient

optimization of a wide range of cell-based methodologies.

Novel computerized tool with commercial application in cell-based screening and tissue engineering – **the application HAS BECOME a commercial product of Biodevicesystems.**



Example of scaffold/osteogenic tissue elastic properties simulation. (Example of a simulation of the elastic properties of scaffolds with osteogenic tissue) As the scaffold containing osteogenic cells is an anisotropic object, the program gives provides the possibility to define the axes (pl.) with biggest and lowest Young's modulus. Hence, the program gives a recommendation concerning the appropriate orientation of the scaffold inside a host bone to provide the most suitable distribution of biomechanical stimulation.

The potential impact and the main dissemination activities and exploitation of results

MAGISTER has promoted and consolidated a pioneering role in endorsing new magnetic materials for biomedical applications, going beyond the pre-MAGISTER state of the art characterized by the presence of only magnetic nanoparticles. Pioneering papers of the consortium have promoted significant citation events and are nowadays certainly promoting the establishment of a new field of multi-functional macroscopic but nanostructured magnetic materials for tissue engineering. In addition to the RTD work, significant efforts have been dedicated to patent search and dissemination issues. A training exchange between some partners was performed.

Regulatory aspects (essential requirements) were also considered in order to list the international standard or guide lines applicable and to follow to carry out pre-clinical and clinical validation of devices developed in the project.

The work performed confirmed the expectations credited to MAGISTER. The successful realization of magnetic guiding objectives indicate significant benefits towards the development of conceptually innovative therapy techniques as well as it indicates the possibility of establishing new research&production units able to bring to the market these new tools and materials. The Consortium has generated a significant amount know-how which was already partially patented and will be subject of further patenting practices.

The successful realization of most of the objectives promise significant benefits for the fields of therapy and surgery promoting conceptually novel techniques. Most of the achieved results are considered by the consortium as clearly and sometimes easily enough exploitable, requiring strong research&production collaboration and exchange.

Most of results achieved require significant investments to perform clinical trials and to obtain the regulatory approvals needed before the commercialisation of the products for clinical use. This is in line with the expectations of MAGISTER whose aim was to develop and pre-clinically validate new concepts (MagS, BIO-AGs, Scaffolds fixation and Magneto-thermosensitive effects) and new materials (Fe-HA biocompatible magnetic materials), leaving the subsequent clinical validation as a follow up activity to be carried out after project conclusion.

The **social implications of the project achievements** lie generally upon possible benefits for a large number of orthopedic patients and new applicable know how able to promote the formation of future companies (spin-off or start-up companies), most probably of SME size.

In the previous section the main results achieved have been described together with their relevance, not only in terms of scientific impact, but also describing the possible applications of each result.

A very interesting result that could be pursued beyond MAGISTER project is a class of BIO-AGs based on functionalised Fe-HA magnetic nanoparticles. Such a class of products could potentially substitute the traditional functionalised MNPs in all the applications that foresee their injection in human body (e.g. drug delivery, magnetic labelling, Cancer treatment).

Economical impact – main reference market

Related to the **tissue engineered products**, MAGISTER market potential resides in the sector of **bone graft** in the **orthopaedic field (as well as in the maxillo-facial one)**. Through the systems and integrated approach developed, MAGISTER could potentially act in Europe as a major player in these fields offering a competitive advantage over established non-European manufacturers. Improvements in competitiveness will arise through long-term dynamic

framework solutions (both horizontal and vertical in nature) by which European industries have the best chances of competitive success.

Bone graft (supplemental bone material to replace existing natural bone that has been damaged by trauma or disease) have been used in surgical procedures for many years, and offers to this new generation of products high potential to expand treatment options, improve patient quality and reduce healthcare costs.

Bone grafting is a big business, generating sales of more than **\$2.5 billion a year**¹. Of the more than 3 million musculoskeletal procedures done annually in the United States, about **half involve bone grafting with either an autograft** (bone grafting utilizing bone obtained from the same individual receiving the graft) **or an allograft** (the bone graft is harvested from an individual other than the one receiving the graft).

Worldwide, autografts or allografts are used in approximately 2.2 million orthopaedic procedures annually². Although autografts are considered the gold standard for repairs of bone defects, their usage is limited by donor-site morbidity and supply.

In Europe Autograft is used in nearby half of the number of BGS (Bone Graft Substitute) procedures, as shown in the figure below:

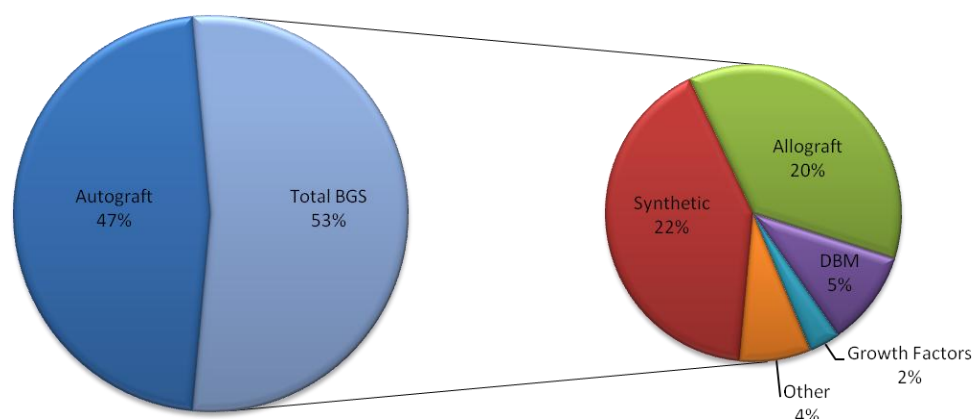


Figure 2: 2012 EU No. of BGS Procedures³

Limitations of using allografts include immunogenic response by the host to the foreign tissue of the graft and the potential for disease transmission. Another problem inherent with allograft procedures is the screening of human tissues used in bone grafts and transplantations across Europe. As mentioned, the European market of bone graft substitutes is driven by an increasing awareness of the advantages offered by synthetic bone graft substitutes over allografts. The future of this segment is expected to grow at an expected CAGR equal to 14,7% (7 times higher than Allograft CAGR).

It's worth to stress that also the segment of Growth Factors in the bone replacement market, addressed by MAGISTER solution, is rising at a very high CAGR between 2012 and 2016 (CAGR: 15,2%) and is expected to achieve over 30 million € in 2016.

¹ Desai BM: Osteobiologics. *Am J Orthop* 2007;36:8-11.

² By A. Alex Jahangir, MD; Ryan M. Nunley, MD; Samir Mehta, MD; Alok Sharan, MD; and the Washington; Health Policy Fellows; AAOS Now January 2008 Issue

³ Source: SmartTRAK Data Base

Finally, it's important to recall which are the main MARKET DRIVERS and MARKET LIMITERS related to MAGISTER main outcomes:

MARKET DRIVERS

- Aging European Population
- Innovative Biomaterials and New Approvals
- Surgeon Acceptance - Growing acceptance by surgeons that BGS products provide increased efficacy, decreased morbidity
- Reduction in the use of Autograft
- Patient Pressure
- Clinical success with GF

MARKET LIMITERS

- Economic Situation: slow market growth
- Reimbursement - Reducing reimbursement or price freezes will slow market expansion as governments utilize this to reduce spending
- Hospital Tenders
- Brand Differentiation
- Price Competition - New market entrants utilize price to try and gain market share from established competitors driving down ASPs.
- Adverse Events with BMPs
- Sourcing Allograft

Economical impact – Other related applications and markets

Even though bone graft represents the main reference market related to MAGISTER integrated approach (which covers the “*Magnetic Scaffolds*”, “*Cell populated Magnetic Scaffolds*”, and “*Magnetic Fixation*” macroproducts), other market sectors are suitable for other MAGISTER macroproducts such as the *Magnetic Nanoparticles* (MNP) and the *Bio-Aggregates* (BIOAGs), depending on their potential and possible applications.

In fact, functionalized Magnetic nanoparticles can be used in a wide variety of biomedical applications⁴⁵, ranging from contrast agents for **magnetic resonance imaging** to the **deterioration of cancer cells via hyperthermia treatment**. Most of these promising applications require well-defined and controllable **interactions between the MNPs and living cells** (Figure 3).

⁴- Q.A. Pankhurst, J. Connolly, S.K. Jones, J. Dobson, “Applications of Magnetic Nanoparticles in Biomedicine,” *Journal of Physics D: Applied Physics*, 36, pp. R167-R181, 2003.

⁵ C. Xu, S. Sun, “Monodisperse Magnetic Nanoparticles for Biomedical Applications,” *Polymer International*, 56, pp. 821-826, 2007

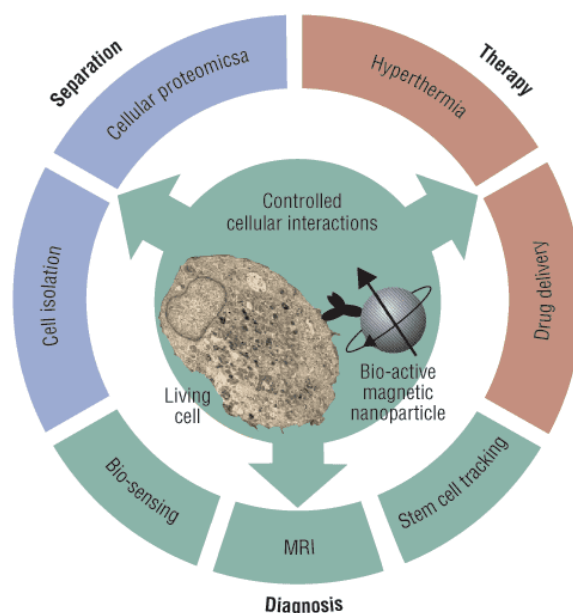


Figure 3 **Biomedical applications based on the controlled interactions between living cells and biologically activated magnetic nanoparticles**

Biomedical applications:

MRI. A well-known application in the **field of diagnosis** is the use of MNPs as contrast agents for magnetic resonance imaging (MRI), which is used to better differentiate healthy and pathological tissues and to visualize various biological events inside the body. Due to their low toxicity, iron oxide MNPs have received US Food and Drug Administration approval to be used as MRI signal enhancers.

Magnetic labelling. Another **diagnostic application** is the magnetic labeling of all kinds of biological entities, such as cells, DNA, and proteins. An interesting application is the labeling of stem cells to noninvasively monitor the distribution and fate of transplanted stem cells in the human body. Furthermore, MNPs show great promise as magnetic labels in biosensing with many advantages compared to conventional labels such as enzymes, fluorescent dyes, chemiluminescent molecules, and radioisotopes. For example, magnetically labeled cancer cells can be purified, transported, and detected on a single chip surface, enabling simple and cost-effective cancer screening in a lab-on-a-chip approach.

Controlled drug release. Next to their small size and low toxicity to humans, MNPs have the advantage that they can be transported through an external magnetic field gradient, penetrating deep into the human tissue. In this way, controlled transport of drugs to target sites can be achieved. The latter usage is realized by attaching a drug to a biocompatible MNP carrier, injecting the ferrofluid into the bloodstream, and applying an external magnetic field to concentrate the **drug/carrier** complexes at the target site. As one example, this principle is used with cytotoxic drugs in cancer treatment.

Hyperthermia. Another interesting therapy is based on the ability of MNPs to be heated when a time-varying magnetic field is applied. This characteristic is used to burn away cancer cells (hyperthermia), often in combination with chemotherapy. It is in fact known that cancer cells

are more sensitive to temperatures in excess of 41°C than their normal counterparts. Both applications present a bright future for **targeted therapy**, which can specifically destroy a desired target without deteriorating healthy surrounding tissue.

Cell isolation. Finally, the attraction between an external magnet and the MNPs enables separation of a wide variety of biological entities. Examples are the isolation of cancer cells in blood samples or stem cells in bone marrow to allow for improved diagnosis and the removal of toxins from the human body. Furthermore, MNPs can be biologically activated to allow the uptake of cells via endocytotic pathways, thereby allowing certain cellular compartments to be specifically addressed. Once taken up, the desired cellular compartments can be magnetically isolated and accurately studied using proteomic analysis.

Other potential markets

Below a short summary related to other market segments in which some of the exploitable results might fall, depending on their flexibility for possible applications.

Drug Delivery

Not easy to estimate. According to GBI Research, the overall drug delivery market is forecasted to grow to to \$199 billion in 2016 from \$101 billion in 2009, at a CAGR of 10.3%.

Biologics Manufacture Market

The biologics manufacture market was estimated of 4\$bn in 2009 with a CAGR of 13.4% over 2010-2016 [GBI Research. 2009]

Pharmaceutical And Medical Device

The technology is exploitable across a number of market sectors, from pharmaceuticals to medical devices. However, the cell The global cell-based assay market has been estimated at \$6.2 billion in 2010 (BCC Research, May 2011) and is expected to grow at a CAGR of 11.6% to reach nearly \$10.8 billion in 2015.

Recombinant Protein Market

The worldwide recombinant protein market has been steadily growing since 2003 grossing 32 billions US\$ to reach 52 billions US\$ in 2010 (Source:Marketresearch). Recombinant proteins for therapeutic applications (high-segment market) account for the 64.3% of the market, whereas recombinant proteins for research applications (low-segment market) account for the rest. Both market segments are forecasted to grow in the next five years (Source:Datanomitor).

Hyperthermia Cancer treatment Market

Market for local hyperthermia solid cancer treatment using MNPs is still difficult to estimate because this technology represents an emerging technology. However, potential applications in solid tumour treatment i.e. a) brain solid tumours, b) prostate cancer (200.000 men in US diagnosed in 2007), pancreatic and oral cancers which are common in developing countries.

Oral cancer, which is common in developing countries as well, is the eighth most common cancer worldwide. Approximately 1 - 10 cases per 100,000 people (depending on country) are reported (World Cancer Report, 2010). With these already high incidence rates, the World Cancer Report (2010) suggests that these rates could further increase by 50% to 15 million new cases in the

year 2020. In United States there are 36,000 people suffering from oral/lip cancer in the each year.

The reduction of side effects as compared with traditional treatments could lead to hyperthermia becoming an acceptable treatment for various types of cancer therefore hyperthermia technology with conventional treatments should increase the size of the hyperthermia market.

The soft tissue tumour ablation therapy world market potential is estimated to exceed \$2 billion. Hyperthermia may provides significant advantages over currently available cancer therapies that will allow us to capitalize on this rapidly expanding market. Above considerations may therefore predict a fast growing market for Hypertermia.

Dissemination activities

The main dissemination activities carried out during project implementation can be summarised as follows:

1. Publication of 33 scientific papers, 15 of which have been jointly published by different project partners (in particular by ISTEC, ISMN, IMCB, USC, UNIBO, FINCER, HZDR). Several joint articles are under development and are expected to be published after project conclusion
2. 74 presentations made at scientific conferences
3. Participation with an own booth in 2 international Conferences on Research&Innovation and on Health (Euronanoforum 2009 and SANIT2011).
4. Creation of a LinkedIn group named "MAGISTER - Magnetic Scaffolds for in vivo Tissue Engineering"

Patents

1 patent application dedicated to the development of the First ever magnetic bioresorbable material has been submitted; inventors/authors: ISTEC-CNR, FINCERAMICA, USC, UniBo (four partners). It is expected to be enriched in the future by adding or patenting separately the part dedicated to fabrication of mangetic nanoparticles from this material

2 more patents are in preparation: one on the magnetic shielding effect (partner FZD) and one on magnetic scale module (ISMN-CNR)

A few more patent possibilities are in discussion and it is expected that final number of patents caused by MAGISTER could exceed the number of 5.

Outline of the project exploitation plan

As previously shown, MAGISTER project carried out a significant number of R&D activities and produced a good amount of outstanding results. Specifically, the market placement analysis of MAGISTER main results gave evidence of the real possibilities of MAGISTER solutions to be promoted and implemented as advanced biomedical tools addressing needs and opening further opportunities.

The following figure reports a positioning of the main results of MAGISTER in terms of potential economic impact vs. further investments needed. A very interesting result that could be pursued beyond MAGISTER project is a class of BIO-AGs based on functionalised Fe-HA magnetic nanoparticles. Such a class of products could potentially substitute the traditional functionalised

MNPs in all the applications that foresee their injection in human body (e.g. drug delivery, magnetic labelling, Cancer treatment) and has therefore been positioned on the top-right of the figure 4 (and put in brackets since this represents a future result).

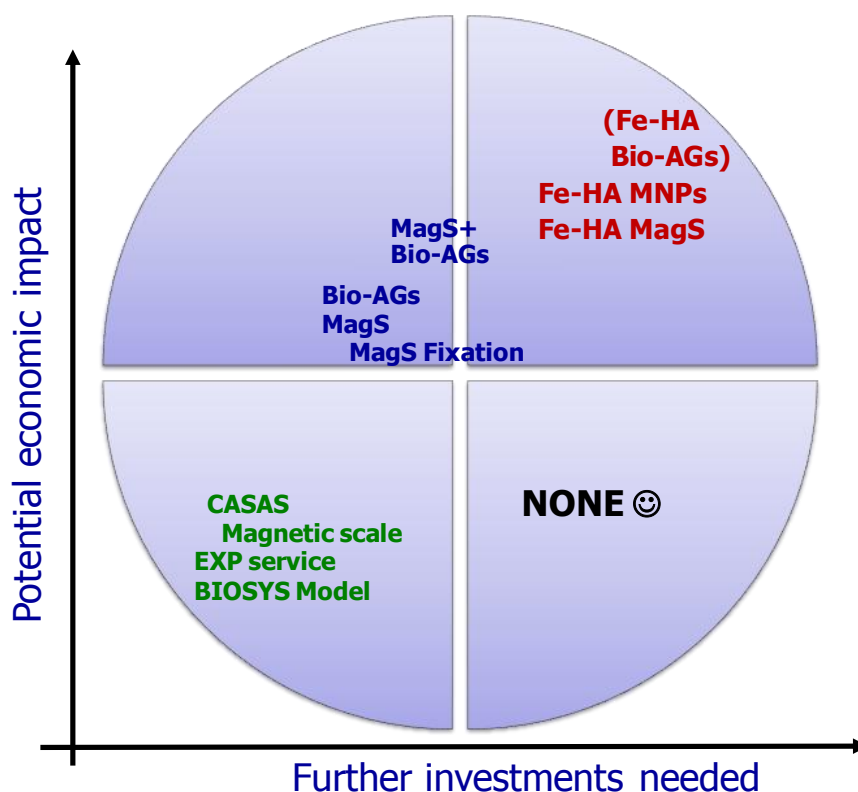


Figure 4: MAGISTER's results placement

Most of results achieved require significant investments to perform clinical trials and to obtain the regulatory approvals needed before the commercialisation of the products for clinical use. This is in line with the expectations of MAGISTER whose aim was to develop and pre-clinically validate new concepts (MagS, BIO-AGs, Scaffolds fixation and Magneto-thermosensitive effects) and new materials (Fe-HA biocompatible magnetic materials), leaving the subsequent clinical validation as a follow up activity to be carried out after project conclusion.

The consortium has developed a confidential exploitation plan with the aim **to analyze the project's potential to create revenues and/or provide social benefits. The exploitable results** are considered **products, processes, methods, services, etc. resulting new, improved or less costly.**

MAGISTER exploitable results were jointly defined by MAGISTER beneficiaries through a first identification drafted within an Exploitation Strategy Seminar (ESS) organized in January 2012, followed by a final definition carried out during MAGISTER final meeting.

The identification of the project exploitable results represents the starting point of MAGISTER **exploitation methodology** (Figure 5) which follows an analysis of each selected result in terms of:

- Identification of the market and market needs and the product intended use (possible applications and related customers);
- Analysis of the market size and players;
- Market analysis for each potential product in terms competitiveness, innovativeness, time to market, further investments needed, price range(cost-benefit assessment);

- SWOT analysis in order to assess the whole prospective and potential achievement of the market targets.

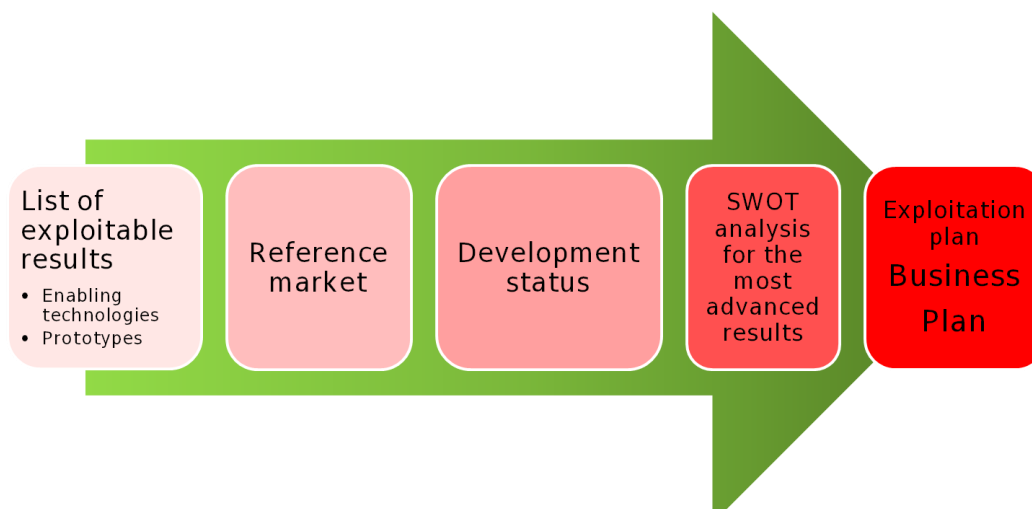


Figure 5: **Exploitation methodology**

The elaboration of the information collected for each exploitable result allowed to define the **exploitation potentialities** of the most advanced results and the **exploitation strategies** that MAGISTER consortium, composed by a variety of companies and organizations having heterogeneous core activities, missions and business focus, should undertake.

As shown in the table 1, MAGISTER partners can be grouped in 4 main categories: Academic, Clinical, Industrial and a Seed Capitalist. Among the 20 MAGISTER partners, 10 of them are Academic/Research, 3 are Clinical, 6 are Industrial Partners and 1 is a Seed Capitalist.

#	Partner name (short name)	Type of organization
1(C)	Consiglio Nazionale Delle Ricerche (ISMN; ISTEK; IMCB)	A
2	Alma Mater Studiorum universita di Bologna (UniBo)	CL
3	Fin-Ceramica Faenza S.p.A. (FINCERAMICA)	I
4	Ruprecht-Karls universitaet Heidelberg (UH)	A
5	Universidad Politecnica de Valencia (UPVLC)	A
6	Bio-Vac España, S.A. (BIO-VAC)	I
7	Universidade de Santiago de Compostela (USC)	A
8	Helmholtz-Zentrum Dresden-Rossendorf (HZDR)	A
9	Eidgenoessische Materialpruefungs- Und Forschungsanstalt (EMPA)	A
10	The University of Edinburgh (UEDIN)	A
11	Sveuciliste U Zagrebu, Medicinski Fakultet (UniZAG)	CL
12	Universitaetsspital Basel (UHB)	A
13	Invent Srl (INV)	SC
14	I+ Srl (I+)	I
15	Biodevice Systems Sro (BioSys)	I
16	Belarussian State Medical University (BSMU)	A
17	Explora S.r.l. (Explora)	I

18	Avanticell Science Ltd (ACS)	I
19	Central Institute of Orthopedics and Traumatology Of Russia (CITO)	CL
20	University Of Brighton (UoB)	A

Table 1: Partners type of organization defined as follows:

(A) Academic & Research Organisations; (CL):Clinical (I) Industrial; (SC) Seed Capitalist;

The figure 6, reported below, gives a synthetic overview of partners' exploitation strategy grouped by categories.



Figure 6: MAGISTER partners' exploitation strategy

The main exploitation guidelines emerging from the study are confidential and have been reported in the Exploitation Plan of MAGISTER, divided in two main sections:

- The consortium exploitation strategy, which gives a major outline of the exploitation of MAGISTER results based on a coordinated and integrated approach in which the whole consortium is involved
- The partners' exploitation strategy, developed in collaboration with each related partner, describes the approaches that the industrial partners will possibly undertake in order to bring MAGISTER products into the market.

Contacts

Project Coordinator: Dr. Valentin DEDIU

Organisation: ISMN-CNR, VIA GOBETTI 101, 40129 BOLOGNA, ITALY

e-mail: V.Dediu@bo.ismn.cnr.it

phone: +39 051 6398507

fax: +39 051 6398540

Project website address: <http://www.magister-project.eu/>

List of beneficiaries

Beneficiary #	Organisation	Contact Person	Email
1	CNR-ISMN	Valentin Dediu	v.dediu@bo.ismn.cnr.it
1	CNR-ISTEC	Anna Tampieri	anna.tampieri@istec.cnr.it
1	CNR-IMCB	Roberto De Santis	rosantis@unina.it
2	UNIBO	Alessandro Russo	a.russo@biomec.ior.it
3	FINCERAMICA	Daniele Pressato	dpressato@finceramica.it
4	UHEI	Amin Rustom	amin.rustom@urz.uni-heidelberg.de
5	UPVLC	David Busquets Mataix	dbusquets@mcm.upv.es
6	BIO-VAC	Jose F Moreno	jfmoreno@biovac.es
7	USC	Jose Rivas	farivas@usc.es
8	HZDR	Thomas Herrmannsdoerfer	t.herrmannsdoerfer@hzdr.de
9	EMPA	Arie Bruinink	arie.bruinink@empa.ch
10	UEDIN	Donald Salter	Donald.Salter@ed.ac.uk
11	MEDICINSKI	Mislav Jelic	mislav.jelic@mef.hr
12	UHB	Andrea Banfi	abanfi@uhbs.ch
13	INVENT	Tommaso Foglia	t.foglia@innova-eu.net
14	I+	Cristiano Paggetti	c.paggetti@i-piu.it
15	BIOSYS	Igor Balykin	biodevicesystems@gmail.com
16	BSMU	Vitaly Goranov	vitgoranov@gmail.com
17	EXPLORA	Davide De Lucrezia	d.delucrezia@explora-biotech.com
18	ACS	Colin Wilde	colin.wilde@avanticell.com
19	CITO	Anton Kurpyakov	kurpyakov@mail.ru
20	UoB	Matteo Santin	m.santin@brighton.ac.uk