



<p style="text-align: center;">37-48 Months Periodic Report</p>	 <p style="text-align: center;">MAGISTER Magnetic Scaffolds for in vivo Tissue Engineering NMP3-LA-2008-214685</p>	 <p>Project funded by the European Commission Seventh Framework Programme (2007 -2013)</p>
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PROJECT PERIODIC REPORT

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Project acronym: **MAGISTER**

Project title: Magnetic Scaffolds for in vivo Tissue Engineering

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1 Publishable summary

1.1 Executive summary

The Consortium work in the period 36-48 Months has significantly moved to *in vivo* experimental part as well as it has proceeded by finalizing various *material* and *in vitro* issues.

The material related work has proceeded along two main lines: providing all necessary materials for *in vitro* and *in vivo* experiments in progress (service work) and the development work related to injectable scaffolds. The former, while established conceptually in the previous reporting periods, requested significant efforts considering both person-months and consumable costs. Among these we can list (1) a significant number of scaffolds delivered to the Consortium by ISTE, IMCB, FINCERAMICA and (partially) ISMN; (2) bare, shelled and functionalized magnetic nanoparticles of highest magnetic and structural quality delivered by USC and UoB; (3) lots of engineered cells for magnetization process and various experiments have been provided by UHB as well as by joint UniBo-ISMN-UHB efforts; (4) fully sufficient for the planned experiments amounts of VEGF-MNP aggregates were provided by UoB, USC and partially by Explora; (5) powders of the outstanding newly established bioresorbable magnetic material were provided by ISTE to FZD and USC for advanced characterizations and other.

The work on injectable magnetic scaffolds, which represented a fully innovative line, has successfully achieved the fixed objectives and represents a significant step ahead in this direction in general (non only on magnetic side).

In vitro experiments have successfully and actively moved from more general, although very challenging, proof of principle style objectives to strategy selection and to the development of strategies for the most efficient transfer of knowledge and know-how from the *in vitro* into the *in vivo* fields.

The latter culminated in a joint MULTIPARTNER *in vivo* experiment involving a number of partners and consisting in (1) sample taking from rabbits at partner UniBo, (2) cell engineering at UHB, (3) cell magnetization at ISMN (with the support of BSMU and UEDIN) by using functionalized MNP provided by USC/UoB, (4) magnetic loading of magnetized cells by ISMN/BSMU of magnetic scaffolds provided by ISTE/IMCB, (5) implantation to same rabbits of magnetically pre-loaded MagS by UniBo, (6) data analysis by UniBo, UEDIN, CITO.

In a parallel multipartner *in vivo* experiment BIOAGs of VEGF-MNP provided by UoB/USC where injected *in vivo* after the scaffold implantation at UniBo and magnetically guided towards and inside the scaffold.

This work lead to the successful fulfilment of the “in vivo bone regeneration and neoangiogenesis through MAGNETIC SCAFFOLDS, MNPs-VEGF (BIOAGG) delivery and MAGNETIC GUIDING”, undoubtedly representing the most challenging deliverable result of MAGISTER, 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.

In addition, noteworthy, 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 work on thermo-sensitive magnetic materials in the reporting period had its culminating point via the successful demonstration of the proof of principle establishing the possibility of VEGF to be released from BIOAGs by the generation of a controlled thermal stimulus that is derived from the energy loss of magnetic nanoparticles under specific magnetic field conditions. It was 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.

The magnetic fixation, a line moving in the project in parallel to the magnetic guiding, had passed important if not crucial tests during this last period – the *in vivo* validation. Remarkably the evaluation of *in vivo* fixation UniBo/ISTEC/ISMN/FINCERAMICA 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.

Modelling efforts in this period have been concentrated in two directions: modelling of the bone tissue development and modelling (in parallel to complex experimental work) of fully innovative nano-shuttle objects, representing samples of large surface-to-volume magnetic able to carry each a large amount of selected bio-agents.

For the latter, extensive calculations and simulations together with experimentation by Partners UPVLC/BIOVAC have lead to the real fabrication of the demonstration object. 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.

As far as bone tissue development is concerned a (1) model was developed by BIOSYS/BSMU providing the possibility to calculate an elasticity modules of the porous scaffold taking into account parameters of cell growth with their influence on matrix formation and ossification. This new model allows to consider the scaffold porosity and time-dependent effects of biogenic mineralization on biomechanical properties of the bone substitute in whole.

Next (2) a new software was developed by BIOSYS providing the possibility to predict substantial features of cell proliferation and bone maturation in porous Magnetic Scaffold. Processing of experimental data together with definition of osteogenic tissue development indices allows to forecast most important parameters of bone tissue developing. This model represent a FINAL PRODUCT which is under commercialization now by partner BIOSYS.

Continuous biocompatibility and biotoxicity tests in this last period. The main efforts have been successfully applied to the finalization of two important protocols: (1) Protocol for evaluation biomaterials based on population dynamics in mixed cell type 2D co-cultures and (2) Protocol for evaluation biomaterials based on cell outgrowth out of multiple cell type tissue like 3D cell clusters.

An important achievement has been realized on the investigation of toxicity of the static magnetic fields. It was found that the effects of static magnetic field on HBC, HBMC differentiation is not straight forward and may be dependent on the unknown factors. No effect of static MF was observed on HDF cells as presented in the previous report.

Good biocompatibility was found for late project materials, like (noteworthy) magnetic materials for injectable scaffolds, latest thermosensitive materials and latest variants of magnetically doped hydroxyapatite.

All the important objectives of this period, including most important and challenging ones, have been successfully fulfilled. Some marginal deviations have occurred (see paragraph below) without influencing nevertheless the overall successful work in the last 12 Months.

1.2 Main project objectives for the reporting period:

- ⇒ 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).
- ⇒ **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.

1.3 Deviations from DoW in the period 36-48 Months:

In the reporting period of 36-38 Months only deviations related mainly to quantitative criteria rather than qualitative ones have happened. These deviations did not really influence the realization of the project Objectives, which was extremely successful leading to the fulfilment of ALL the Deliverables.

WP1 - no deviations

WP2 A little significant QUANTITATIVE deviation has been detected during the realization of the magnetic shuttles. The magnetization of these objects resulted higher than planned in DoW – let us mention that too high magnetization would prevent a controllable operation due to possible magnetic aggregation of shuttles. Nevertheless it was found out that at useful concentration of such shuttles in the body and hence at possible working distances the remaining magnetization is not dangerous for aggregation and the objects can be successfully used for promised applications, after having passed the bio-compatibility test which were not planned in MAGISTER. So a good step ahead was done in this direction and this step has the length corresponding to that assumed by DoW.

WP3 - no deviations

WP4 - no deviations

WP5 - There was a deviation by used method and not by achieved result. Deliverable 5.4 claimed In vivo Imaging of tissue differentiation and neoangiogenesis through RMI and angiographic techniques. The results were achieved bringing a convincing evidence for the neo-angiogenesis in the bone formation, but it was achieved histological characterization and not claimed technique for reasons of opportunity, availability and especially much lower costs. It was supposed in DoW that the size and quality of vessels may not be sufficient to detect them by simple microscopy, instead it was! So the very good result also allowed to use simple observation method.

WP6 A deviation has happened for the task dealing with D 6.6: Morphological/biochemical characterization of medium/long-term controlled temperature increase on the overall regenerative process. This work was only partially fulfilled providing nevertheless interesting and also useful results and indications. The work plan underpinning D 6.6 was to produce a 3D culturing of endothelial cells in a hydrogel system mimicking the osteoid formation during the process of bone regeneration and the study of the effect of VEGF released by mild hyperthermia from the aggregates. A protocol was optimised to this purpose, but a series of logistical hurdles mainly related to limited equipment configuration prevented its full implementation. The consortium hopes to finish the work after the end of the project by using available local funding.



WP7 - no deviations

WP8 - no deviations

WP9 - no deviations

WP10 - no deviations