PROJECT FINAL REPORT

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Project acronym: BIOMAGSCAR

Project title: Biodegradable Magnetic Stent for Coronary Artery Luminal Regeneration

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Period covered: **from** 01/01/2012 **to** 31/12/2016

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4.1 Final publishable summary report

Executive Summary

The project 'Biodegradable Magnetic stent for coronary artery luminal regeneration (Biomagscar)' started on 01/01/2012 and finished 31/12/2016. The project was a collaborative effort of a Consortium that included 4 academic partners (UCL, QMUL, Yale, UEF) and 2 companies (Magnus and Qmed) combining expertise in medical device development, biomechanical engineering, advanced imaging, stem cell research and intellectual property exploitation. The project had the ambitious aim to develop a novel biodegradable, magnetisable stent to be evaluated in patients indicated for coronary angioplasty. The ambitious aim was to dial-in magnetism to deliver novel biological therapy offering regenerative medicine solutions to the coronary artery vessel.

Therefore, the first main objective of the Biomagscar project was to evaluate different stent materials and designs to identify a platform that would support the magnetism concept. This would serve as the basis for a novel biodegradable stent capable of restoring natural vessel anatomy within 12 months of implantation.

The second main objective of the Biomagscar project was to explore the use of magnetism to deliver advanced therapeutics to the coronary artery. This would be accomplished with the use of magnetisable particles that would be embedded into the polymer of the stent. Once magnetised, the implanted stent can hold stem cells at the site of injury through magnetic forces.

The above objectives were reached by combining the expertise of the Consortium partners who have extensive experience in biochemical engineering, device design and manufacturing, and clinical expertise. These objectives were tested using several bench testing and animal research models to demonstrate the feasibility of the novel concept, before a decision was made to proceed with a preclinical evaluation of the novel stent platform.

The preclinical research experts performed animal research using pigs to evaluate stent performance in a flowing blood of a pig coronary artery, and other experiments included injecting iron-labelled stem cells into the coronary artery of a pig to evaluate visualization and homing properties of the magnetisable stent. This set of experiments demonstrated the conditions of cell preparation and cell number to allow translation into human studies.

Other key results of the project are the development of a novel non-magnetisable stent platform (i.e. without the magnetisable particles) that will be taken forward into human trials once the remaining analyses of the preclinical studies, and preparation of the regulatory dossier is complete. These were performed by Qmed. A human trial of the Biomagscar product (stent-stem cell combination) is intended to be performed after the completion of the Biomagscar project. As the trial could not be carried out during the project, a joint venture with some of the project partners is being considered as a mechanism in which to carry out the trial post project. A joint venture would explore further fundraising in order to carry out the clinical trial, and exploit the patents arising from the Consortium.

Although the Biomagscar project confirmed that a biodegradable magnetisable stent prototype can attract and hold stem cells to the deployed device, in an animal model we are confident that the conditions used provide confidence that the results will be reproduced in a clinical setting. These results, along with protocols developed for cell isolation and preparation for use in human studies, have provided the foundation for a novel and important therapeutic advance.

Biomagscar project context and objectives:

The Biomagscar project had two main objectives:

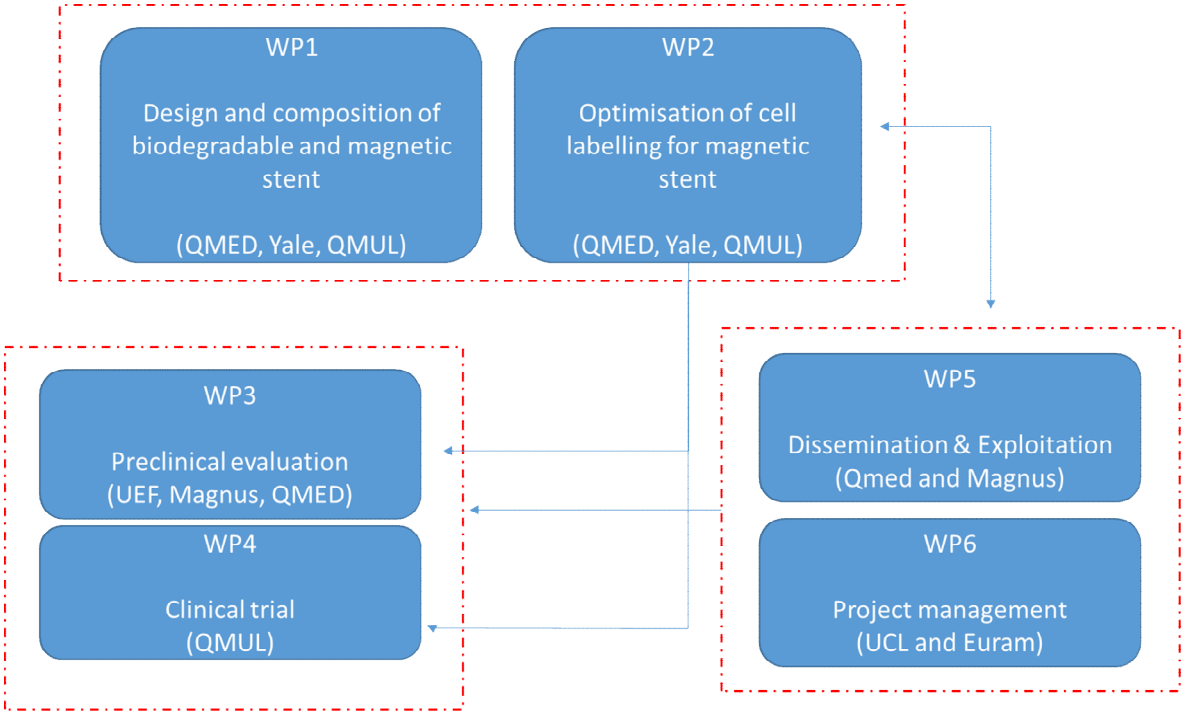
The first main objective was to complete a clinical trial of a novel biodegradable, magnetisable stent that could deliver biological therapies that would offer regenerative medicine solutions to the coronary artery vessel wall. The second main objective of the Biomagscar project was to explore the use of magnetism to deliver advanced therapeutics to the coronary artery. This would be accomplished with the use of magnetisable particles that would be embedded into the polymer coating of the stent.

To achieve the two main objectives, several work plans were devised that included evaluating many different stent designs and materials. The aim was to identify the appropriate stent design and material that would provide the sufficient radial strength to deliver the novel concept of magnetism. The main objectives were achieved through several sub-work packages that included the following:

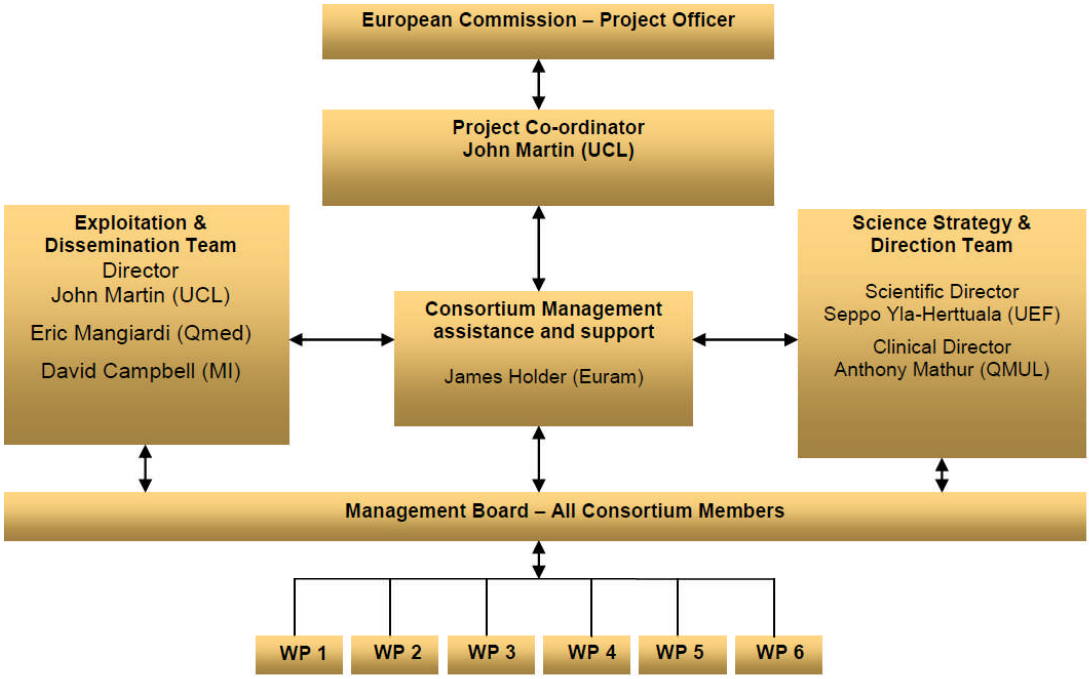
- Evaluation, design and composition of novel biodegradable, magnetisable stent
    - This broadly focused on identifying the right materials and designs that would enable the magnetism concept to be achieved. Key considerations were given to radial strength and degradation profiles.
- Magnetisation of the stent
    - This work focused on how to enable magnetism in a clinical setting, and considerations were given safety, feasibility and adoption.
- Evaluation of the conditions for cell preparation and for the human trial
    - This work package focused on developing a cell isolation and preparation protocol for use in human trials
- Preclinical evaluation of the stent prototype
    - This work package centred on the safety and performance of the novel stent platform in large animal models. Results from this work package will be included in a regulatory dossier that be used to support a human study.
- Clinical evaluation of the novel stent platform
    - A clinical trial of the novel stent to be performed by QMUL, a Consortium partner in the Biomagscar project.

These work packages were refined as the project progressed, and were intended to culminate in the initiation and completion of a clinical trial. However, it was not feasible to initiate the clinical trial before the end of the project. Therefore the consortium partners are exploring carrying out the clinical trial after the end of the project, financed through funds raised by the commercial partners involved in the project.

A chart to highlight the interdependency of the work packages:



The project management structure, including key names involved in the project, are as follows:



Project partners included institutions from Germany, UK, Finland and the United States.

Key Results

- Evaluation of different stent materials; a magnesium alloy was chosen as a metal-based platform is believed to be more biocompatible and offer few adverse effects to patients with coronary disease. Several strut thicknesses were considered and different types of polymer coatings were also evaluated. The optimal thickness and polymer coating should not be bulky to avoid issues with implantation issues in humans, but enough to provide adequate radial strength to avoid stent collapse. The optimal prototype (magnesium alloy with poly-l-lactide coating) was chosen after completing a battery of tests and then was manufactured for preclinical evaluation. This prototype also served as the foundation for the biodegradable, magnetisable stent platform, i.e. the Biomagscar product (stent-stem cell combination).
- A cell isolation and preparation protocol was developed by the Consortium. This protocol used human CD34+ cells isolated from bone marrow and processed with a system that allows them to be iron-tagged and then re-injected into the patient. This protocol provides the conditions for magnetism to be achieved in a human trial. This protocol was implemented in the animal models with much success.
- A preclinical evaluation of the non-magnetisable stent, with interim results suggested that our novel platform performs as well as other bioabsorbable stents that are on the market. The data is currently being analysed and it is expected that these data will be used to support a human trial of the non-magnetisable scaffold.
- A prototype of the Biomagscar product; this prototype was tested in different animal models suggesting that it exhibits the features that would support further investment and development as a novel biodegradable, magnetisable stent.

Science and Technological results – foreground

Proof of concept experiments to demonstrate that human stem cells tagged with iron can be attracted to a magnetised stent prototype

Design and composition of a biodegradable stent

A fundamental output from the Biomagscar project is the design and development a biodegradable stent that would support regenerative solution. The aim was to have a biodegradable, magnetisable stent that can attract iron-labelled cells and that can biodegrade in 6-12 months *in vivo.* Several platforms were considered and tested by the Consortium, and the Consortium decided that a magnesium alloy coated with a biodegradable polymer would be sufficient as the polymer could act as a coating to deliver magnetism. The magnetisable particles were dispersed into the polymer and then coated on the magnesium stent.

Magnesium is an attractive metal for biodegradable material because of its low thrombogenicity and well-known biocompatibility. The use of magnesium as a biodegradable stent material is a natural evolution as it is a structural constituent of the tissue and an essential element in the living organism. Magnesium is a substantial intercellular cation which is involved in more than 300 biological reactions of the cell. Magnesium is also regarded as a non-carcinogenic element. However, magnesium has a rapid degradation and implants results in tissue overload with degradation products and this can lead to neointimal formation. Therefore, magnesium is alloyed with other elements such to decrease the degradation rate. The Consortium identified a suitable composition (patent protected) that would be carried further into development as a platform for delivering regenerative solutions.

Several factors are considered when determining the optimal configuration, and these include strut thickness, polymer coating and tube configuration. Several tests are also completed as part of the design and prototype evaluation and these include the following:

- Visual inspection of the bare and coated stents before crimping
- Measurement of strut thickness and strut breadth
- Evaluation of the crimpability
- Measurement of crimp profile
- Expansion to nominal pressure (NP) and rated burst pressure (RBP) with a standard balloon (2.75mm x 20mm) catheter
- Visual inspection regarding any cracks or flakes of the coating as well as breaks in the stent struts after expansion
- Measurement of stent foreshortening
- Measurement of stent recoil
- Measurement of radial strength and deformation force (standard measuring method by plates)
- Further expansion on a 4.0mm catheter to its nominal pressure (NP)
- Visual inspection regarding any cracks or flakes of the coating as well as breaks in the stent struts after further expansion to NP
- Further expansion on a 4.0mm catheter to its rated burst pressure RBP
- Visual inspection regarding any cracks or flakes of the coating as well as breaks in the stent struts after further expansion to RBP

Based on the above, a novel prototype was selected that was based on a magnesium alloy platform and a polymer coating containing magnetisable particles. The precise composition and details remain confidential as several patents and peer-review publications are pending. Publishing this data ahead of these events would compromise commercial and academic viability of the Biomagscar project.

Cell Isolation and preparation

A key goal for the Biomagscar project is to develop a stent platform to enable attraction of stem cells, such as endothelial progenitor cells, that are tagged with iron *in vitro.* To achieve this goal a protocol for cell isolation, preparation and cell magnetic labelling was developed. This protocol would be tested in human trials. Using human blood samples, endothelial progenitor cells were further isolated from mononuclear cells by positive selection using a commercial system (immune-magnetic separation system, Miltenyi Biotech). The methods for isolation were based on the manufacturer's recommendation with minor modifications. In short, immunomagnetic positive selection of CD34+ or CD13+ cells were performed using paramagnetic microbeads conjugated to specific monoclonal antibodies (anti-human CD34 or anti-human CD133). Labelled cells were then enriched on a column placed in a magnetic field. The column provides a strong magnetic field, allowing a selection of cells carrying only a few specific antigens on their surface. The retained labelled cells were eluted from the column, and the purity of the CD34+ and CD133+ cell fractions was determined by flow cytometry using anti-human CD34 or anti-human CD133 specific antibodies.

On average, $9.00x10^5$ CD34+ cells from a 100ml of human cord blood. The mean viability was 77% for CD34+ and CD133+ cells, and this was in-line with values published in the literature. The functionality of the cells was evaluated by incubating the cells with acetylated low-density lipoprotein and determining DilAc-LDL uptake. After 2 weeks of culture, CD34+ cells displayed more elongated morphology and stained positively for Ac-LDL, anti-vWF, anti-VE-cadherin, and anti-CD34. This protocol could be used in a human trial, where isolated stem cells from human bone marrow could be magnetically labelled and re-injected into the patient as a biological treatment.

Magnetisable particles

Since biodegradable magnesium stents do not possess the intrinsic ability to be magnetised, the Consortium chose to coat a stent with a biodegradable FDA approved polymeric coating embedded magnetisable particles that consists of a proprietary composition of iron and a transition element. The aim of these experiments was to identify the concentration of magnetisable particles needed as a percentage of the polymeric coating that would be needed to induce a sufficient magnetic field for iron oxide labelled endothelial cell attraction to occur. Several iterations were considered but a proprietary composition of iron and a transition element offered unique ferromagnetic properties, allowing the implanted stent to have a magnetic retention of at least 60 days which will provide sufficient time for the attraction of labelled endothelial progenitor cells. The exact composition and the optimal percentage of the polymer coating remain confidential as there is a pending patent application and peer-reviewed publications planned for 2017.

Magnetisation of stent

An important success of this project is the manufacture of the magnetisable particles (composition of iron and a transitional element) in a stable format that is amenable to dissolution in a biodegradable polymer solution. The manufacture of magnetisable particles was developed based on methods identified in the literature, and further modified by Consortium partners. This process and know-how has been patented and remains confidential until the patent is published.

A key output has been the successful *in vitro* and *in vivo* demonstration that magnetisable particles can be attract, and hold, stem cells under conditions that would mimic a flowing artery. Using a bioreactor system, various flow conditions were tested to examine the capture efficiency of the magnetised stent. Stents were mounted to plates in a closed tube system. Human CD34+ cells were labelled with iron-oxide (as above) and fluorescently labelled so they could be tracked and later quantified for capture efficiency by flow cytometry. Initial testing examined continuous flow rate of (25ml/min), or stasis flow conditions (start for 3 mins and stop for 3 mins). This *in vitro* set of experiments showed that stents embedded with magnetisable particles captured iron-oxide labelled human CD34+ cells when compared with stents not embedded with magnetisable particles. The higher magnetisable concentration, the more cells were captured. Both continuous flow (steady infusion of stem cells) and stasis flow conditions were amenable, and potentially applicable in the clinical setting. The data on this experiment is being compiled and prepared for a peer-review publication. The figure below describes the preparation of the magnetisable stent.

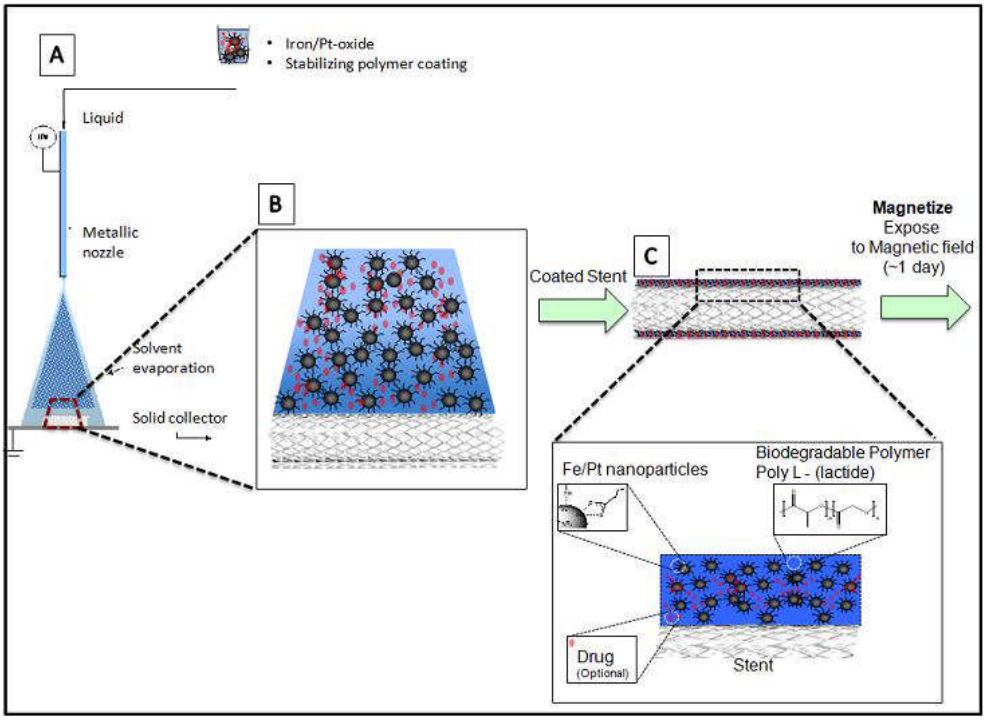


Figure 1. Preparation of magnetisable particles coated onto stent

Following these *in vitro* experiments that that magnetisable particles dispersed into a polymer and then coated onto a stent could attract and hold stem cells, animal models were used to test the capture efficiency of the magnetisable stent in flowing blood in the pig coronary artery. In these experiments, a protocol was developed to label cells with a magnetic and radiolabel to effectively show the delivery and retention of cells to the magnetised stent. Human CD34+ cells were labelled with iron-oxide and then a small portion of the injected cells were labelled again with a radiolabel tag. The radiolabel tag is required so the cells could be visualized with advanced CT imaging techniques. A stop-flow stasis technique was adopted (cell infusion of 1ml/min for 3 minutes during a 3-minute occlusion, done 3 times). In conclusion, the Consortium demonstrated that human stem cells tagged with iron-particle can be attracted to the magnetised stent in flowing blood in the pig coronary artery. The conditions of cell preparation and cell number have been established to allow translation into human studies. The conditions of magnetism that we have developed have been demonstrated to be efficacious in a pre-therapeutic situation. It should be noted that the pig coronary artery is the size of a human coronary artery and the conditions of blood flow are similar. This successful experiment lays the foundation for a clinical trial. These results are currently being written up for publication.

Toxicology of magnetisable particles

The polymer surface embedded with magnetisable particles and tested for potential toxic effects. Viability of endothelial progenitor cells cultured with magnetisable particles embedded in the polymer was tested, also the biodistribution of the magnetisable particles was tested in mice to understand what organs were impacted by the magnetisable particles. Our results showed that cell viability did not indicate any significant decrease below 100% viability under normal concentrations. The biodistribution data for the magnetisable particles showed no long-term accumulation after 7 days as most of the magnetisable particles had been cleared by the kidneys. Our results indicate that we do not anticipate any toxic effects of the magnetisable particles in the clinical setting.

Preclinical evaluation of the stent prototypes

Restenosis due to an increase in cell proliferative behaviour is a primary factor in the failure of percutaneous coronary revascularization, occurring within three to six months in 40–50% of patients who undergo angioplasty. The incidence of restenosis has been reduced with the use of stents, although neointimal hyperplasia and in-stent restenosis remain significant problems associated with coronary stent implantation. The recent introduction of drug-eluting stents has provided the interventional cardiologist with a new tool for the treatment of patients with coronary artery blockage. However, it has been proposed that the long-term effects associated with the permanent implantation of a metal scaffold and durable polymer for drug release cause further injury to the vascular wall as well as prolongs the inflammatory responses in the target artery. Our proposed solution of a biodegradable stent would provide mechanical support needed for the artery immediately after balloon angioplasty but would be completely degraded to leave the vessel without a chronic irritant.

The purpose of the preclinical evaluation is to evaluate the biodegradable magnetisable stent (i.e. the Biomagscar product or described as the stent-stem cell combination). A pig model was chosen as this is in-line with regulatory expectations, and the pig coronary artery is similar to a human coronary artery. The main objective of this study was to determine the characteristics of these new devices against with conventional, commercially approved stents (bare metal and commercially available biodegradable stents). Stents were implanted in naïve pig coronary arteries and follow-up data is collected for acute (seven days), sub-acute (1 month), and chronic (six months) time points. Stents were implanted into the left anterior descending and left circumflex coronary arteries. The right coronary artery was used in case of tortuous anatomy or too small or too large vessels.

The primary objective was:

- To evaluate and compare angiographic lumen loss between treatment and control groups over the six-month period

Secondary objectives:

- Evaluate the ability to access the target location with the delivery system
- Evaluate the handling and visualisation of the delivery system and visualisation of the stent
- Verify the accuracy and efficacy of deployment
- Characterize the ability to withdraw the delivery system
- Evaluate the appropriateness of the stent sizing
- Assess the positon, integrity and functionality of the stent
- Assess local biological responses (e.g. thrombus deposition, inflammation, endothelisation, necrosis, aneurysm formation) and downstream systemic effects (i.e. embolism, infarction) through an evaluation of histology and pathology of explants and tissues/organs

Six different devices were evaluated. Three magnesium based stents with a poly-L-lactide acid (PLLA) coating. The PLLA coating has been shown in preliminary tests (in vitro) to slow down the biodegradation of the device. The coating also enables inclusion of magnetisable particles into the device to improve radio-opacity of the stent. The PLLA coating can alternatively be used to carry an anti-mitotic medication to the vessel wall or a combination of medication and radio-opacity improving nanoparticles. The degradation process of a biodegradable implant will likely induce an inflammatory reaction that facilitates the removal of the stent material from the body. On the other hand, a robust inflammatory process can increase restenosis and therefore studying the effects of a well-established anti-mitotic drug are warranted.

The utility of the magnesium prototypes has been demonstrated through photographs and scanning electron microscopy (SEM) images of coated stents that do not show cracking or delamination before and after expansion. The stents will be 2.75-3.00 mm in diameter and 18-19 mm long, representing a clinically relevant size for a coronary artery stent. Due to the similarities between the coronary arteries of man and pig these devices are of the appropriate size for the experimental setting. All stents are mounted on regular CE certified coronary balloon catheters which have a nominal balloon diameter of 2.75mm and a nominal balloon length of 20mm. The balloon is at the distal end of the catheter providing a platform for mounting, delivering and deploying the stent.

Preliminary results indicate that the magnesium stent prototypes perform similarly to the commercially available controls. Advanced imaging (optical coherence tomography) analyses at one month after stenting show both comparable rates of restenosis, an indicator of the stents overall performance, as well as comparable results for the radial strength of the stents compared to commercially available stents. Below is a figure of the advanced imaging (optical coherence tomography) from a magnesium prototype (without magnetisable particles) compared with two commercially available controls (one metal stent and one biodegradable stent). These stents were deployed and show good performance at day 7 and day 28.

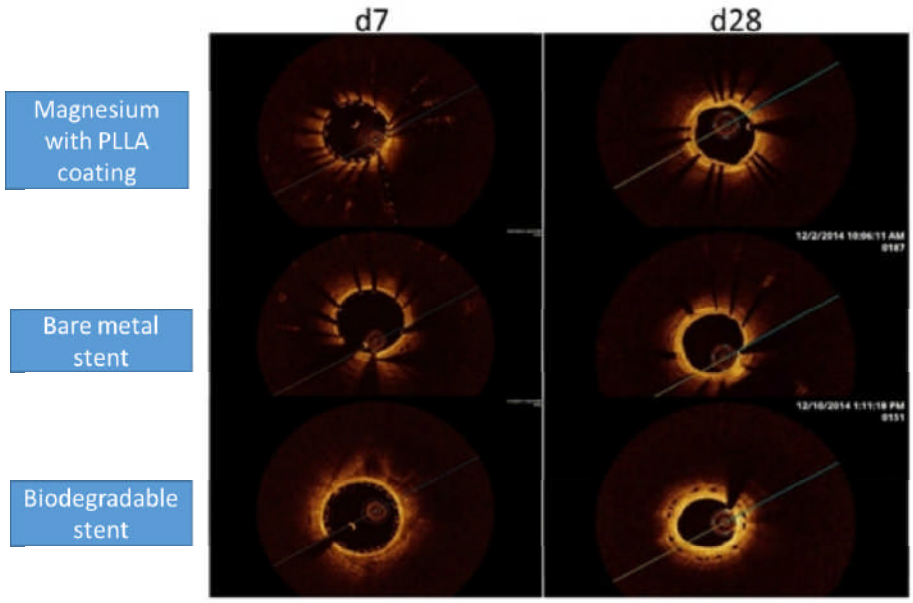


Figure 2. Optical coherence tomography images for a magnesium prototype, and compared with two commercially available controls (bare metal stent and a biodegradable stent). No thrombus or other signs of pathology were observed.

Predictably the bare metal stent offers strong support for the artery and changes very little over follow up. The Mg- stent core offers comparable strength to the commercially available biodegradable stents one month after stenting. As our stent is expected to be more flexible and biodegrade sooner than other biodegradable platforms, and we are confident our product is on track to be the next generation platform to deliver a biodegradable and biomagnetic stent capable of delivering advance therapeutics.

The data from this set of experiments is under review. The data is being, analysed in preparation for a regulatory submission and peer-review publications. Therefore, only limited data can be shown in this report.

Amendment and minor modifications to the stent

Since this was an ambitious project, the Consortium sought a time extension to achieve its goal of developing a novel biodegradable magnetisable stent (i.e. Biomagscar product, or known as the stent-stem cell combination). The rationale for this time-extension is because the regulatory environment for using a medical device as a tool for regenerative medicine has evolved considerably since the project started. At the outset, the Consortium's overarching milestone centred on delivering a biodegradable arterial stent as a tool to deliver stem cells (i.e. Biomagscar product). The Consortium took the position that the quickest and economical way to the clinic was to test the stent-stem cell combination as a first in man clinical trial. However, after extensive consultation and meetings with regulators, industry experts and advisors, it was decided that the safety and performance of the individual components of the novel platform should be investigated independently in animal models and human trials before the stent-stem cell combination could be tested in a human trial. Therefore, the clinical trial was suspended from the Biomagscar project the aims of the Consortium were then to focus resources on milestones and deliverables that would enable Consortium partners to prepare for a clinical trial of the Biomagscar product (i.e. stent-stem cell combination) as soon as possible after the end of the project.

Potential impact & Dissemination

Impact & perspectives

Scientific impact and perspectives

The Biomagscar project has provided interesting new information on biodegradable stents and how it could be used as a tool to deliver advanced therapeutics such as stem cells to the coronary artery.

This information will be useful for the development of new tools and approaches for addressing restenosis and thrombosis associated with stent implantation for coronary angioplasty.

Overall the Biomagscar Consortium results demonstrated that a medical device such as a biodegradable device can be modified and used as a tool to deliver regenerative medicine solutions. These protocols developed by the Consortium for cell isolation, preparation and labelling show that human CD34+ cells can be tagged with iron-oxide and used as part a therapeutic solution. Separately, the Consortium modified a biodegradable device to embed magnetisable particles into the polymer coating. Once magnetised and implanted into a pig coronary artery, it was demonstrated that it could attract and hold human CD34 +cells tagged with iron-oxide. This provides the foundation for a future medical solution that would combine a medical device and regenerative medicine for tackling the issue of restenosis and thrombosis. Additional work needs to be completed to demonstrate that such an approach would promote endothelial healing following stent implantation.

The Biomagscar project results are consistent with published data on biodegradable stents where the short and mid-term performance of biodegradable stents are in-line with standard of care (drug-eluting bare metal stents). These results are promising as the current standard of care, while effective, prevents the restoration of natural vessel function. Patients also require long-term dual anti-platelet therapy to reduce the risk of thrombosis. Biodegradable stents dissolve over time leaving behind a natural vessel, whereas bare metal stents are permanently embedded in the vessel wall. Further, vulnerable plaques and complex scenarios such as coronary artery disease in patients with diabetes or patients with extensive multivessel disease would also benefit from a platform that dissolves over time, and potentially from a regenerative medicine solution that would accelerate endothelial healing.

The prototypes, protocols, preliminary insight, and foreground intellectual property will be exploited for future use by the Consortium partners and will generate further investment and collaboration opportunities for the SMEs involved in the Biomagscar project.

Business impact and perspectives

Future commercial opportunities arising from the results of the Biomagscar project are driven by several factors.

Even though a clinical trial was not initiated or completed as part of the Biomagscar project, preclinical results highlight that human stem cells tagged with iron-particle can be attracted to the magnetised stent in flowing blood in the pig coronary artery. The conditions of cell preparation and cell number have been established to allow translation into human studies. The conditions of magnetism that we have developed have been demonstrated to be efficacious in a pre-therapeutic situation. It should be noted that the pig coronary artery is the size of a human coronary artery and the conditions of blood flow are similar. This successful experiment lays the foundation for a clinical trial.

In addition, the results obtained in the preclinical, toxicology and manufacturing work confirm that a prototype can be designed and manufactured and scaled for human trials. Thus, the potential of the platform to enter human trials is de-risked and attractive for future funders. It is expected that the SMEs in the project will continue to invest in the project through a joint venture, with an aim to deliver a human trial of the Biomagscar product.

Dissemination activities

- Two abstracts were also presented at conferences in the final reporting period (January to December 2016):

    a. An abstract describing the interim preclinical results of the non-magnetisable stent were submitted to the European Association of Percutaneous Cardiovascular Interventions (EAPCI) in May 2016.
    b. An abstract describing the different materials considered in the development plan was also submitted for consideration to the European Association of Percutaneous Cardiovascular Interventions (EAPCI) in May 2016.

Expected publications in 2017 by the Consortium include:

- preclinical evaluation study (non-magnetisable platform)
- proof of concept of the stent stem cell combination (bioreactor work, cell labelling, task 3.5)
- clinical trial protocol of the Biomagscar product (stent-stem cell combination)

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