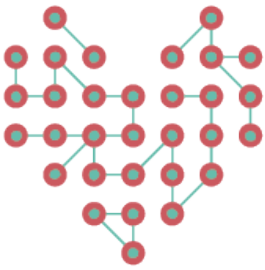
Final publishable summary report



AMCARE

ADVANCED MATERIALS for CARDIAC REGENERATION

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Table of contents

Executive Summary

Transformative therapies for Myocardial Infarction: The **A**dvanced **M**aterials for **CA**rdiac **RE**generation (AMCARE) project is a consortium of academic and industrial partners, which has received funding from the European Union (EU) to develop regenerative therapeutics for the treatment of a heart attack or myocardial infarction. The project began in November 2013 and finished in October 2017 and was tasked with the advancement of novel and transformative therapeutic strategies to address the significant healthcare burden posed by myocardial infarction.

The AMCARE partners derive from 5 countries within the EU. The consortium consisted of:

- 4 academic/research partners - The Royal College of Surgeons in Ireland (RCSI), Trinity College Dublin (TCD), the Eberhard Karls University, Tübingen (EKUT) and the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB)
- 5 small and medium enterprises (SMEs) – Celyad (formerly Cardio3 Biosciences), Explora Biotech, Adjucor, Innova and Contipro
- 1 multinational partner - Boston Scientific Limited

Each partner possessed a specific skill-set and expertise which contributed towards fulfilment of our common goal. More details on the partners and their roles in the consortium can be found at the 'partners' section on the AMCARE website www.amcare.eu .

The new approach proposed in AMCARE.

Myocardial infarction (MI), more commonly known as a heart attack, occurs when a blood vessel feeding part of the heart muscle becomes blocked, depriving the muscle of blood leading to heart degeneration. If a person is overweight or obese, they are at a high risk of developing cardiovascular disease. **MI represents an enormous source of mortality and morbidity globally.** According to the European Society of Cardiology (ESC) one in six men and one in seven women in Europe will die from MI. According to the Irish Heart Foundation, heart disease or cardiovascular disease (CVD) is the most common cause of death in Ireland, accounting for 33% of all deaths. The largest number of these deaths are mainly due to myocardial infarction - at 5,000. 13% of premature deaths (under age 65) are from CVD.

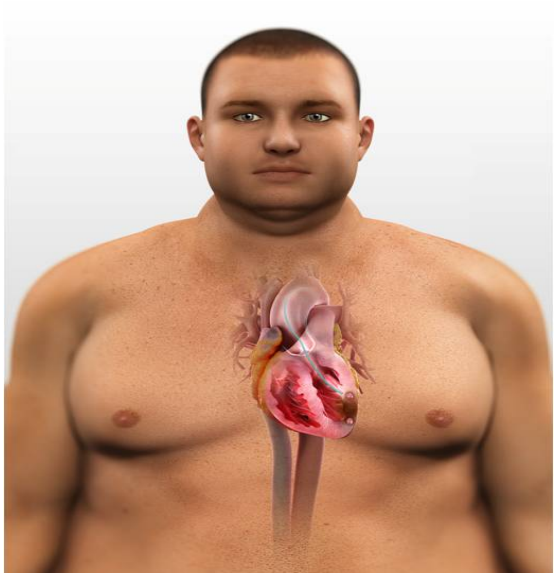


Figure 1. Patient undergoes hydrogel injection procedure. *A patient who has suffered a myocardial infarction undergoes a gel injection from the inner surface of the heart chamber. The catheter device (blue) can be seen entering the heart through a blood vessel. The infarcted region appears as dark and discoloured. Two small gel beads have been injected at the edges of the infarct.*

The AMCARE project developed therapeutic technologies which aim to overcome some of the limitations of common treatments, with a view to maximising the effectiveness of regenerative therapies so that they can be used to treat MI patients in the clinic. **AMCARE's results** have generated new knowledge with respect to treating the effects of myocardial infarction. The AMCARE technologies can be translated to the clinic and have positive impact on patients after having an MI.

Summary description of project context and objectives.

Regenerative Medicine deals with replacing, regenerating or engineering human cells and tissues to restore normal function. The AMCARE project **rationale was to use biomaterials to deliver stem cells to the heart to increase repair and regeneration and impove outcomes for patients.** People are often unaware that every adult has stem cells within many tissues of their bodies such as in the skin, bone marrow, fat tissue and the brain to name a few. Stem cells can be isolated from the body with relative ease and cultured in the lab. These cells have the ability to become a more specialised cell type when cultured in specific conditions. They can subsequently be returned to the body to regenerate lost tissue/muscle. Biomaterials are substances that are compatible and interact with living tissue and so are very often used and/or adapted for medical applications. **By using biomaterials, stem cells are retained more effectively within the heart tissue and the cells are in a protective environment. This will help enhance stem cell survival in the harsh conditions of the heart following a heart attack.** By combining regenerative stem cells within a biomaterial, the consortium aimed to capitalise on synergistic effects between both therapeutics to maximise regeneration in the heart.

The AMCARE project has developed two major biomaterial delivery approaches – a patch to be placed on the outside of the heart (epicardial approach) and an injectable gel to be delivered from the inside of the heart (endocardial approach). Both the patch and injectable hydrogel provide a protective and retentive environment for cells so that therapeutic effects can be produced for a long time. Our goal is to deliver our biomaterial therapies in a minimally invasive way, using custom medical devices to access the heart while maximising patient wellness. Such an approach is much less invasive than traditional open heart surgery. For the injectable gel, the AMCARE project has designed multiple new catheters (C-Cathgel & VisCath) which allow gels to be injected into the heart wall from the inside of the heart chamber (**Figure 2.a**). The epicardial patch is deployed and spread on the outside surface of the heart using a novel medical device (SPREADS). The patch will be delivered in a minimally invasive way through a small incision between two ribs, without the need for open heart surgery (**Figure 2.b**). The attached patches or injected gels contain regenerative stem cells or the gels can also be used to carry other therapeutic cargoes.

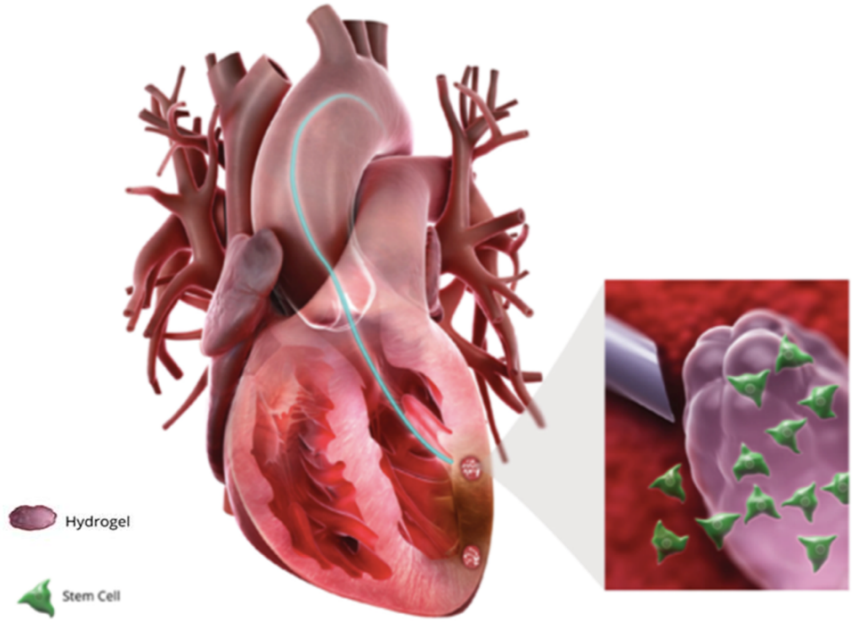


Figure 2.a. Gel injection into infarcted heart. *An infarcted heart undergoes a gel injection from the inner surface of the heart chamber. The catheter device (blue) can be seen entering the heart through a blood vessel. The infarcted region appears as dark and discoloured. Two small gel beads have been injected at the edges of the infarct. The injected gel contains regenerative stem cells The stem cells are able to migrate out of the gel to help regenerate the infarcted tissue (inset).*

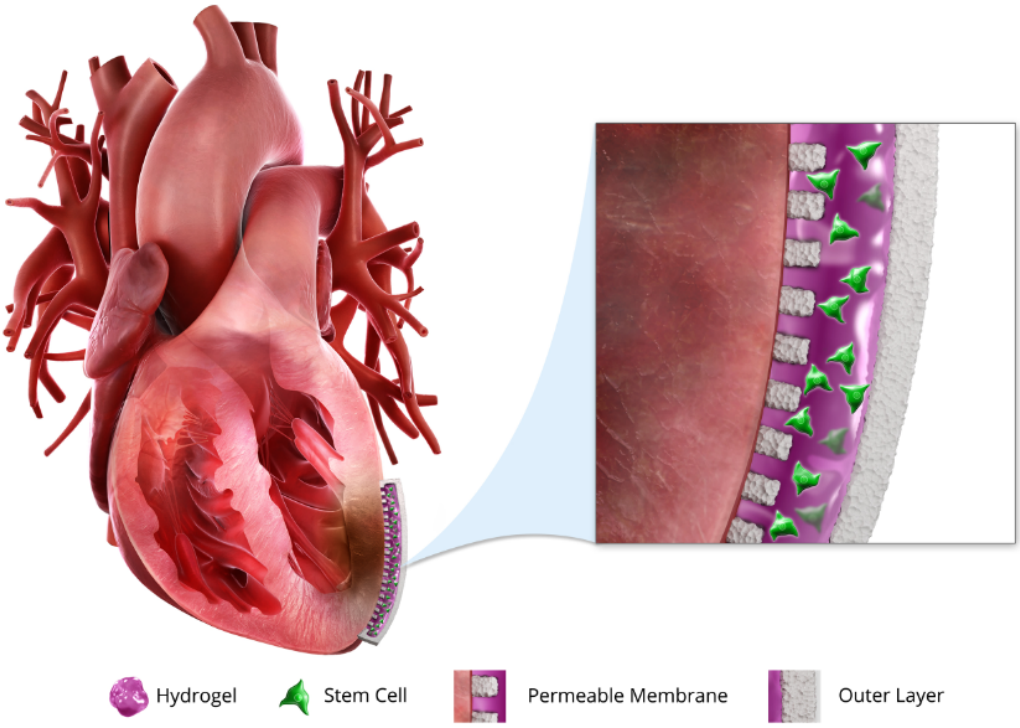


Figure 2.b Attachment of patch onto infarcted heart.
An infarcted heart has a patch (SPREADS) attached to the infarcted region of the heart's surface. The infarcted region appears as dark and discoloured. The patch contains regenerative stem cells. The stem cells are able to migrate out of the patch to help regenerate the infarcted tissue (inset).

Main Objectives and Results Achieved in AMCARE's Workpackages (WP)

Advanced Materials Development (WP2)

The objectives of this work package was to develop a gel (Cardiogel) with specific properties and applications. Cardiogel is injected directly into the heart wall for the endocardial approach or injected into the patch for the epicardial approach.

Contipro have developed a material for Cardiogel which is now at an advanced stage of pre-clinical assessment and scale-up production. The required materials have been sent for *in vivo* trial experiments in Rome (Explora) and Tuebingen (EKUT). Material and hydrogel properties of each batch were determined in Contipro before *in vivo* testing. **RCSI** have worked closely with Contipro to identify the optimal materials in terms of compatability with cells and delivery devices. **Fraunhofer IGB** have tested the materials used in Cardiogel to make sure they are safe to use in the human body. They have carried out cytotoxic tests to regulatory standards on heart cells in order to ensure the cells can survive and thrive once they are placed onto the materials and placed into the body. CardioGel was shown to be fully cytocompatible with no cytoxicty picked up in testing.

Minimally Invasive Device and Biomaterial Testing (WP3)

Adjucor has developed a breakthrough technology. They have constructed and manufactured an epicardial patch and a minimally invasive delivery device called 'SPREADS'. It is a novel, single-stage therapeutic strategy to apply biologics or therapeutic cells to the surface of the beating heart in a minimal-invasive, closed chest intervention. The reabsorbable material enables a one-time procedure to be performed on patients with a myocardial infarction. Completion of phase III of the large pre-clinical study for all SPREDS groups was the central activity of the reported period, including assisting Explora in implantation, explantation and sample extraction. Characterization of SPREDS material has taken major focus to move in the direction biocompatibility and material characterization due to chronic implantation. The preliminary analysis of the pre-clinical trial showed promising data regarding safety and efficacy of the SPREDS device. The survival rate was 100%. In addition, when compared the gold standard (medical therapy), subjects treated with the SPREADS device showed significant improvement in heart function (restoration of Left Venticular Ejection Fraction). **Celyad** (formerly Cardio3Biosciences) provided the adult stem cells that are loaded in the CardioGel. **Celyad**, **Boston Scientific** and **TCD** have designed and developed two novel catheter delivery devices to deliver stem cells embedded in Cardiogel into the inner surface of the heart. One of these devices, C-Cathgel, has undergone pre-clinical assessment. C-Cathgel has shown innovative aspects to assist with delivery of viscous materials like hydrogels and anchorage into tissue, and has shown to improve improving delivery. TCD & Boston Scientific's Viscath 3.0 injection catheter was redesigned from recommendations from the acute trial of Viscath 2.0. Viscath 3.0 design was downselected from several designs built and was tested using simulated testing in a heart model and visualized with fluoroscopy using a C-arm. The system was used with and extended version of the Zurpaz steerable guide catheter in an acute trial in Explora in Oct 2017. The use and delivery of the catheter was successful. **TCD and RCSI** assessed the effect of Cardiogel preparation techniques (SPREADS, catheter prototypes versus standard in vitro gel preparation method) on stem cell viability. RCSI have also shown that the stem cells can migrate out of the Cardiogel in vitro, enabling them to regenerate infarcted tissue in vivo.

Contipro have assessed the effect of preparation techniques (catheter prototypes versus standard in vitro gel preparation method) on the properties of Cardiogel.

Therapeutic Efficacy (WP4) and **Achieving Clinical Reality (WP5)**

Explora: The study design for pre-clinical experimentation has been evaluated and approved by the appropriate channels by both **Explora** and **EKUT**. **EKUT** have performed pre-clinical testing of Cardiogel in a small pre-clinical model of acute myocardial infarction (AMI). In the reported period, the experiments for the biocompatibility study ("*Biocompatibility of CardioGel 2%: in vivo Assessment by Myocardial Injection")* were executed. **Explora** have carried out the pre-clinical testing of all of the AMCARE technology in a large AMI pre-clinical model. 5 treatment groups were complated in 5 blocks over a 12 month period, with data demonstrating the superior outcomes were when SPREADS were used to delivery CardioGel to the epicardially surface of the heart.

IPR Monitoring and Exploitation Management (WP6)

INNOVA have performed patent literature studies to identify possible overlaps between existing patents and planned project developments, consistently with the AMCARE's IP management and exploitation strategy. INNOVA have set up the final Exploitation plan in close contact with all project partners and in particular with the industrial partners. It provides the AMCARE partners with useful feedbacks on the marketability of AMCARE technologies and provide a comprehensive background for each partner's individual exploitation and business plan.

Dissemination and Training (WP7)

During the project INNOVA and the Project Coordination team at RCSI, have jointly:

- maintained the AMCARE website with relevant news, events and achievements
- maintained the contents of the AMCARE website and social media pages
- published scientific papers in peer reviewed journals
- attended several outreach events
  delivered training sessions (e.g. H2020 grants applications & successful exploitation of project results).

Talks and project showcases were given at a number of international conferences during the AMCARE project including at the **Controlled Release Society** meeting in Boston, July 2017. The AMCARE team used CRS as a platform to showcase research coming out of the project. Garry Duffy gave an invited talk entitled Development of Biomaterials Carries and Delivery Systems to Improve Cellular Retention for Cardiovascular Regeneration, and Laura Gallagher won one of 6 prizes at the conference for her work on the AMCARE project. At the **EuroScience Open Forum**, Manchester; July 2016, Garry presented on the AMCARE project in a session entitled 'Biomaterials for Healthy Hearts'. At the **Matrix Biology Ireland,** the 4th Annual MBI meeting was hosted by Trinity College Dublin from December 2017, the theme was "Learning from Development to Engineering Therapeutics". Garry gave an invited talk entitled "Advanced Materials for Cardiac Regeneration" which showcased the main outcomes of the AMCARE project.

Training

Training sessions and exchanges of researchers involved in AMCARE have taken place on several occasions throughout the 48 months. INNOVA delivered a training session in **April 2017** in Stockholm (Sweden) where Tommaso Foglia (senior expert of EU grants applications) and Carmela Canonico (junior expert of EU grants applications) have provided insights to consortium partners on "the main instruments of the Horizon 2020 programme" and "How to build a successful proposal for Horizon 2020". INNOVA has also delivered a 2-days training

and coaching session on **September 11-12th 2017** in Rome (Italy) where Aleardo Furlani (CEO of Innova) and Tommaso Foglia (senior expert) have provided insights to consortium partners on the routes for a successful exploitation of AMCARE's results. Two external experts were invited: one, Mr. Alfredo Poicano, with a strong experience with SME instrument scheme of the H2020 program, which represents one of the financial instruments that could support the next steps needed to Adjucor and Contipro to bring the SPREADS system on the market. Another one, Mrs. Paola Antonini, with experience with regulatory aspects for ATMPs. The participants were asked to prepare a brief presentation (structured as an "elevator pitch") of their exploitable result.

Description of the main S&T results/foregrounds

AMCARE Technologies

Therapeutic Cargo 1

CardioGel

Highlights:

- A novel hyaluronic acid hydrogel was developed that could be coupled with percutaneous and minimally invasive delivery devices
- The CardioGel was biocompatible to ISO standard XXX, and also showed promising acute biocompatibility in a rodent model of MI.
- The gel properties were in line with other clinically tested technologies to promote ventricular stabilisation after a myocardial infarct and limit negative remodelling
- A scale up manufacturing process as achieved to 30g batches
- Stability assessment is ongoing

Overview: HA-PH-RGD derivative with low Mw (60–90 kDa, DS 2–4 %) was the final biomaterial used to produce CadioGel, and we used in all subsequent development steps for the AMCARE therapeutic cargoes and delivery technologies.

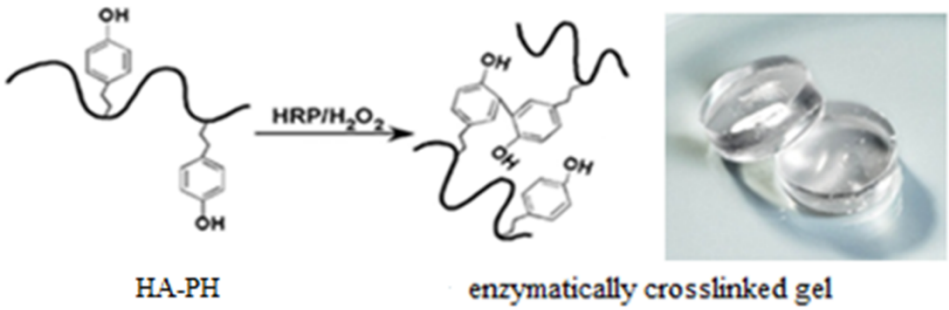


Figure 3: Scheme of crosslink reaction of HA-PH derivative which leads to the formation of hydrogel

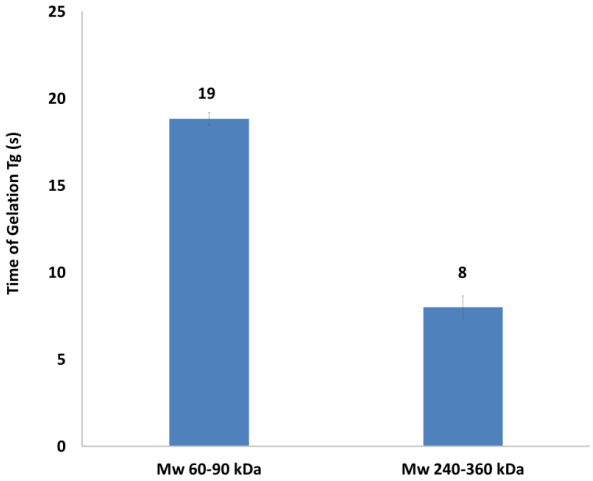


Figure 4: Time of gelation (Tg) of different molecular weights of 2% HA-PH-RGD.

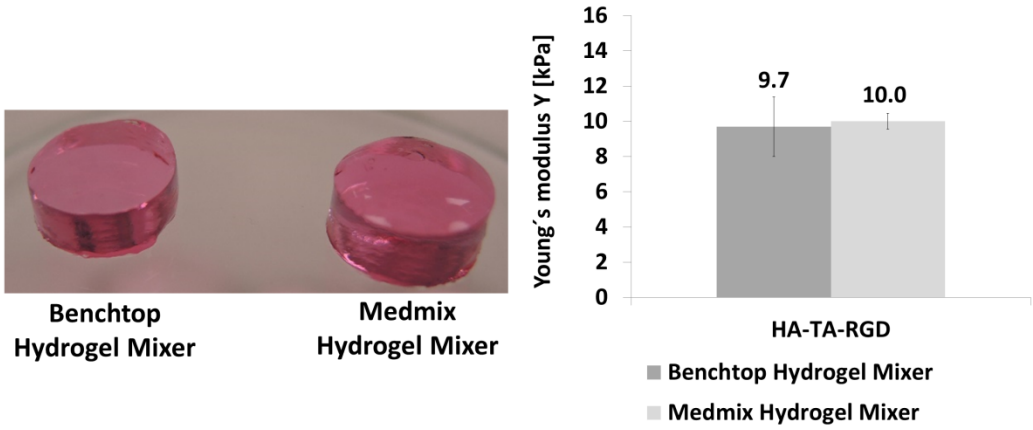


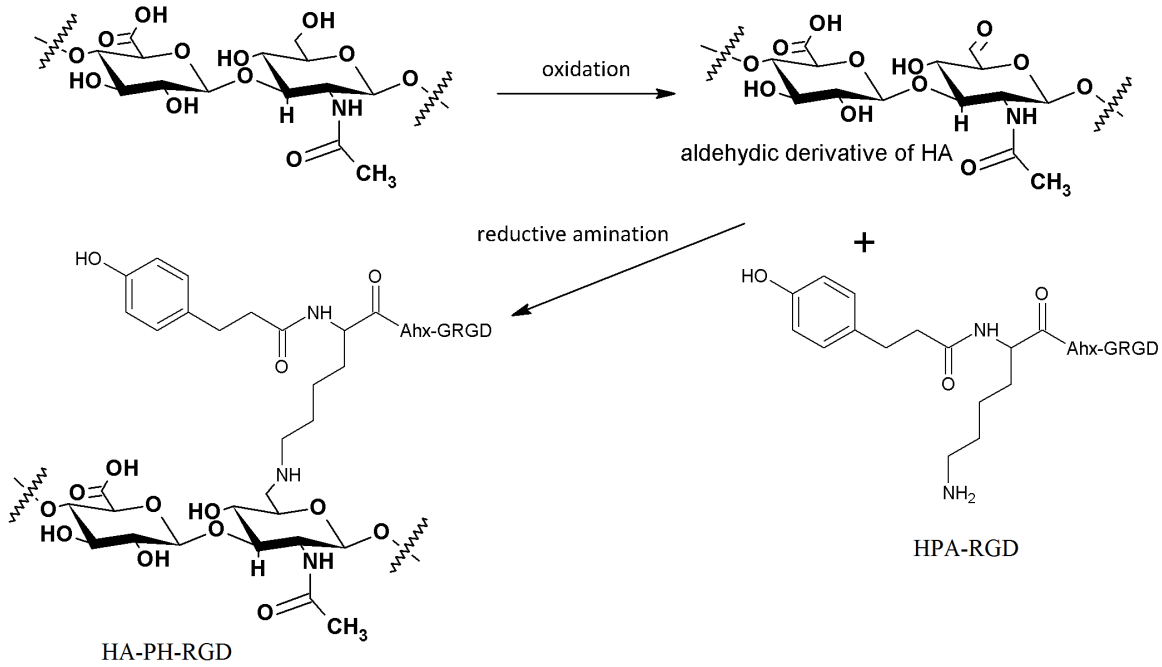
Figure 5: Hydrogels prepared by Benchtop Hydrogel Mixer (Contipro) and Medmix Hydrogel Mixer (commercially available). There was no statistical difference in Young's modulus of the gels prepared with either mixer

During AMCARE the scale-up of HA-PH-RGD production and verification of reliability of the production process was performed.

Scale-up and verification of CardioGel

Synthesis of hydroxyphenyl derivative of hyaluronic acid bearing RGD adhesive peptide sequence (HA-PH-RGD) was developed in the earlier stages of this project. Scale-up of HA-PH-RGD production and verification of reliability of production was performed. First step of HA-PH-RGD synthesis involves oxidation on hyaluronic acid by a TEMPO mediated process which leads to hyaluronic acid derivative partially oxidized in the position 6 of *N*-acetylglukosamine. Result of this process is a polyaldehyde of hyaluronic acid (HA-CHO) with a degree of substitution 3 - 10 %. Process of production of HA-CHO was earlier developed and transferred to pilot plant scale by Contipro.
In the next step of the process HPA-K-AHA-GRGD (RGD) oligopeptide sequence is synthesized by solid phase synthesis using a Fmoc-SPPS protocol. Briefly, *N*-termini of the well-known RGD adhesive peptide was modified by subsequent attachment of lysine, 6-

aminohexanoic acid (Ahx) and glycine. Furthermore α-amino group of lysine was acylated by 3-(4-hydroxyphenyl)propionic acid (HPA). This peptide sequence (HPA-K-Ahx-GRGD) is purified by RPC and characterized by ^{1}H NMR and Mass Spectroscopy.
Production of HPA-K-Ahx-GRGD was transferred to pilot plant scale and the peptide is produced in the amount of 30 g per batch as AMCARE ends. The new synthesized HPA-K-Ahx-GRGD sequence is further conjugated with hyaluronan polyaldehyde (HA-CHO) via reductive amination.



Scheme 1: General description of HA-PH-RGD synthesis.

Reliability of process of HA-PH-RGD productions was confirmed by preparation of six batches in the scale of 20 g of product. Contipro is able to produce two types of derivatives which differ in molecular weight (60-90 kDa or 240-360 kDa).
In the present time stability studies are in the progress. Two different studies of shelf stability of raw HA-PH-RGD materials were started (long term (2 years; 5 °C) and short-term study (6 months; 25 °C, 60 % RH)). The short term studies should serve as accelerated temperature studies of the derivative. These studies should establish shelf life of the raw material and aid in commercial translation of the CardioGels. Kinetics of gelation and viscoelastic properties has been established as the methods for hydrogel properties to be evaluated at the end of the stability studies.

Therapeutic Cargo 2

CardioGel loaded with Cardiopoietic Stem Cells

Highlights:

- A novel hyaluronic acid hydrogel was developed that could be loaded with up to 20 million cells per mL
- The stem cells proliferated over time and were viable into the gel for up to 7 days
- The viability of the stem cells was not compromised after injection through the catheter

Overview: GMP grade cells were manufactured and loaded in the CardioGels described above. 20 million cells could be loaded my mL of gel, which allowed for the delivery dose to be clinically relevant. The cells were compatible with the gel and spread in response to the presence of the RGD peptide.

Cell manufacturing

Tissues were processed as detailed below to extract the ADSCS. The process to generate cardiopoetic stem cells was the same than the established one for CQR-1, the lead product of Celyad. Once isolated mesenchymateuse stem cells (MSC) were put in culture, and expanded and treated with Celyad properitary cardiogenic cocktail of growth facts, per Celyad'SOP (see figure below).

The manufactured adipose derived cardiopoietic cells were evaluated for the following parameters used as release criteria for C-Cure® cells: Homogeneity, Purity, Identity & Genetic stability.

After a process validation on a reduced scale process to validate the process with that new source of MSC, a large scale production of cardiopoietic cells was performed from the donor samples, leading to the production the cells that will be used all along the AMCARE consortium.

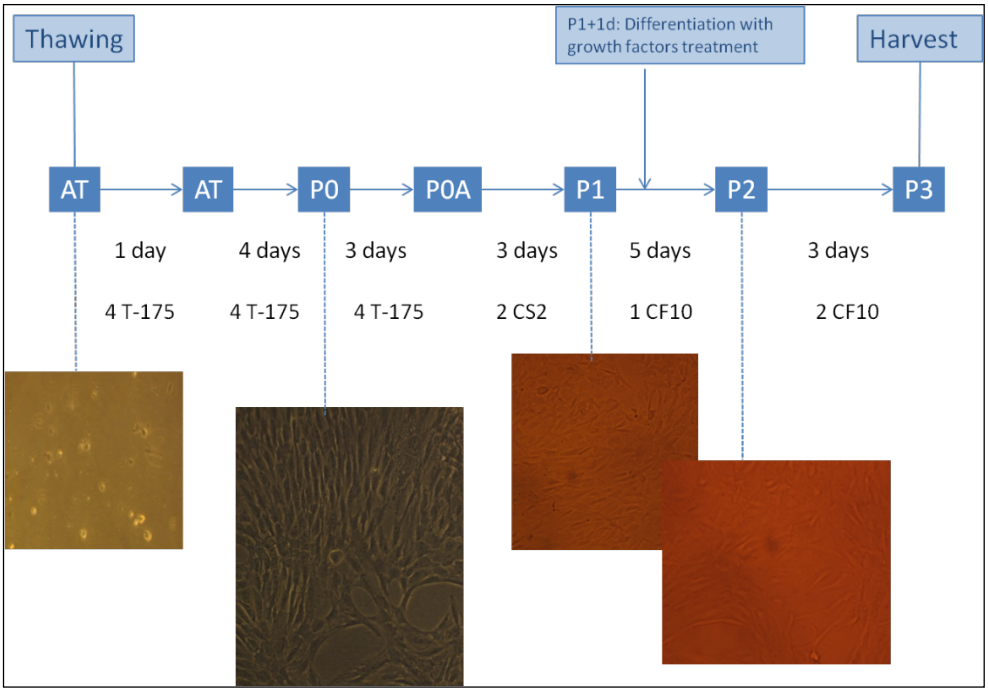


Figure 6: Cardiopoietic Cell Production schematic.

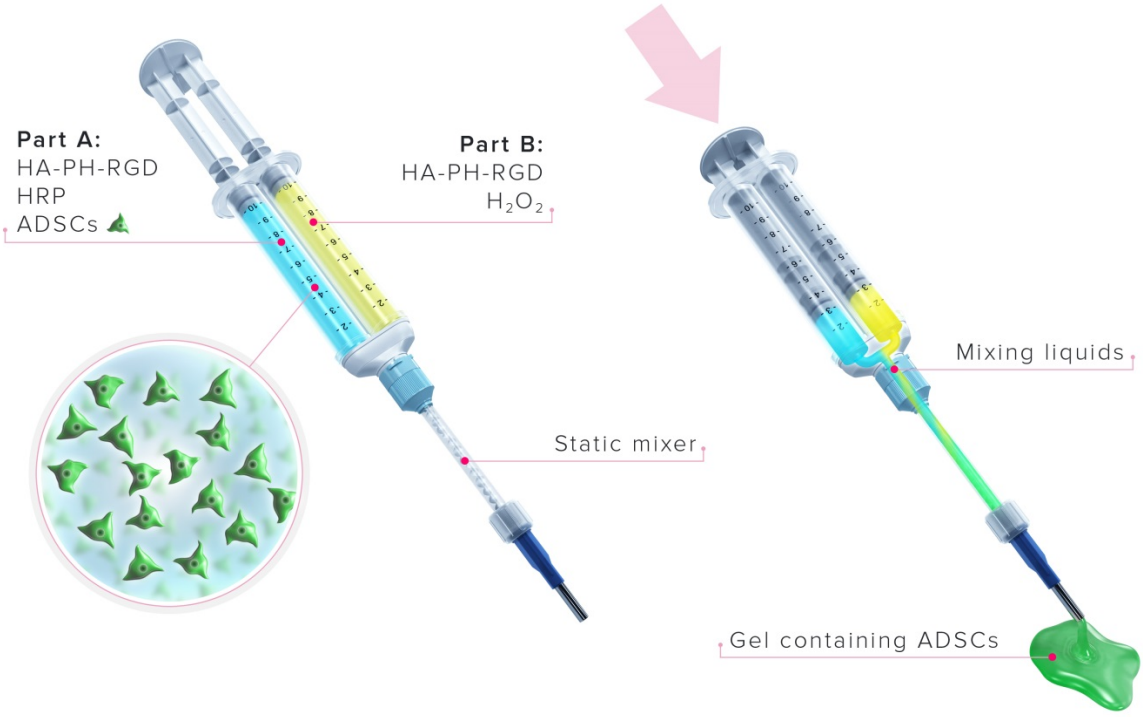


Figure 7: HA-PH-RGD Hydrogel Preparation using Benchtop Hydrogel Mixer – The components and precursor solutions used to prepare the HA-PH-RGD hydrogels are included the solution where the stem cells are added is detailed.

Stem Cell viability was not affected by 4h incubation in HA-PH-RGD+HRP precursor solution
There was no significant loss to cell viability when 40 million ADSCs cells/mL were stored in HA-PH-RGD+HRP precursor solution for 4 hours, while a significant decrease was seen for storage longer than 4 hours ($p<0.05$), independent of the storage temperature.

Figure 8: Stem cells loaded in RGD modified CardioGels spread out in response to the adhesion molecules.

Increasing the crosslinking concentration when 20 million ADSCs/mL are present maintains the mechanical properties
To maintain the hydrogel mechanical properties when 20 million cells/mL were incorporated, it was necessary to increase the crosslinking. There was no statistical difference between the Young's modulus (9.7 versus 8.2 kPa) or toughness (8.2 versus 8.0 J/dm^3) for the hydrogels prepared at crosslinking concentration 1 without cells and

crosslinking concentration 2 with cells.

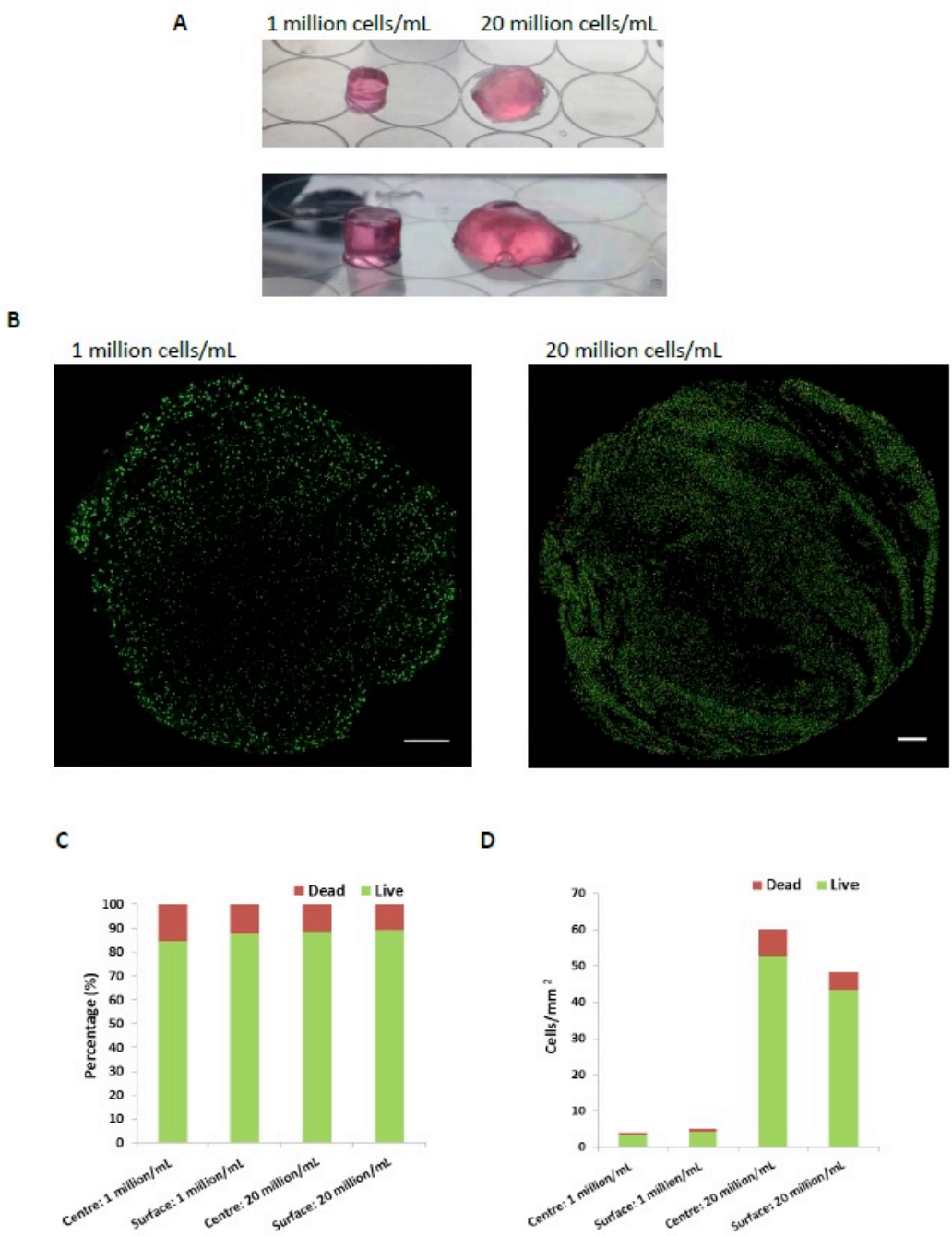


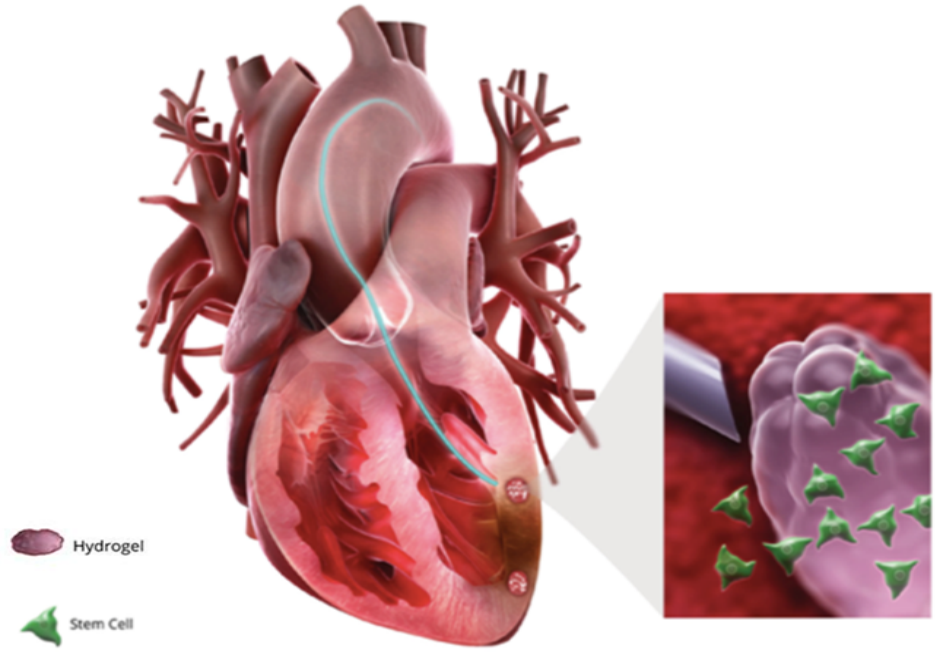
Figure 9: Loading of 20 million cells per mL did not affect cell viability C & D, but an increased level of crosslinkers were required to maintain structure (A&B).

Regulatory Status: The committee for Advanced Therapies (CAT), following consultation with the European Commission, has adopted at its plenary of 6 October 2017 a scientific recommendation of the classification of CardioGel, according to according to Article 17 of Regulation (EC) No. 1394/2007.

The EMA/CAT considers that CardioGel, falls within the definition of tissue engineered product (Combined Advanced Therapy Medicinal Product) as provided in Article 2 of Regulation (EC) No 1394/2007.

Delivery Devices

Therapeutic Route of Administration - Endocardially using femoral access



C-CathGel

Highlights:

- A novel catheter to deliver hyaluronic acid hydrogels to the myocardium was developed
- A novel catheter to deliver cell-loaded hyaluronic acid hydrogel to the myocardium was developed
- The pre-clinical feasibility was demonstrated by accessing the left ventricle using femoral access and injecting up to 10 times in the myocardium
- The hydrogel was retained in the myocardium after injection demonstrating efficacy
- The viability of the ADSCs was not compromised after injection through the catheter
- LVEF ejection fraction was not negatively affected following delivery in a porcine model of MI (28 days after treatment)

Overview: A minimally invasive endocardial catheter (C-CathGel) for the delivery of stem cells embedded in fast-gelling hyaluronic acid hydrogels was developed. An experimental technique to measure the force required to inject the hydrogels was carried out and the mechanical properties of the resulting hydrogels post-injection through C-CathGel were also determined. Viability of cells embedded in the material pre- and post-injection was investigated. Finally, the pre-clinical feasibility of C-CathGel was determined in a large pre-clinical model *in vivo*. The cumulative objective of our experiments was to demonstrate that biomaterial hydrogels with living cells could be injected via minimally invasive catheter technology, that potentially has the ability to be translated to everyday cardiology practices.

A patent application to protect this final design was introduced in July 2016 under the **Gel mixing catheter** title by Celyad.

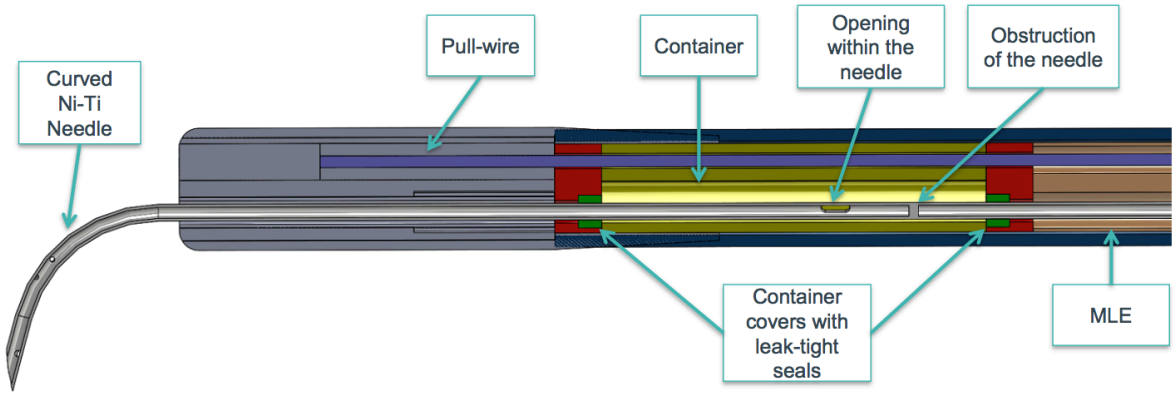


Figure 9: A cross-section of C-CathGel tip which demonstrate the reservoir for gel mixing at the distal end of the catheter.

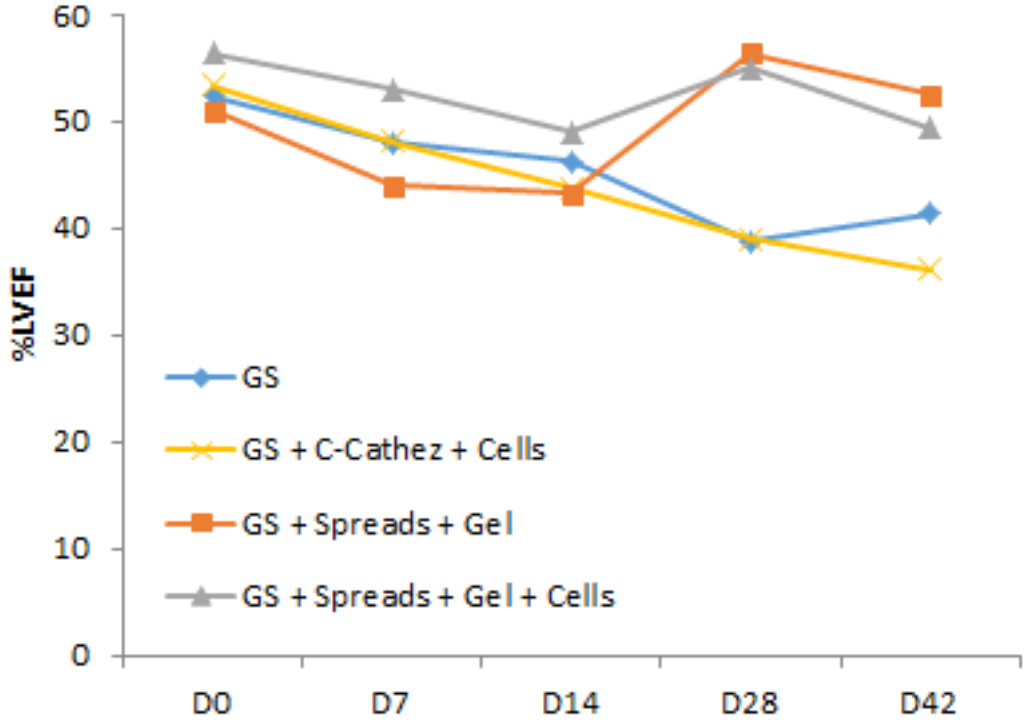


Figure 10: Left Ventricular Ejection Fraction (LVEF) following the induction of myocardial infarction over a 42 period in a large animal model of MI. Treatment occurred at D14. Treatment groups including gold standard clinical pharmacological treatment (GS), GS + stem cells injection endocardially using commercially available C-Cathez, GS + Cardiogel injection endocardially using C-CathGel, and GS + Cardiogel loaded with stem cells injection endocardially using C-CathGel. There was no improvement in heart function measured by ejection fraction in the C-CathGel groups. This may be because of the low technical replicates carried out due to the commercial pivot of Celyad.

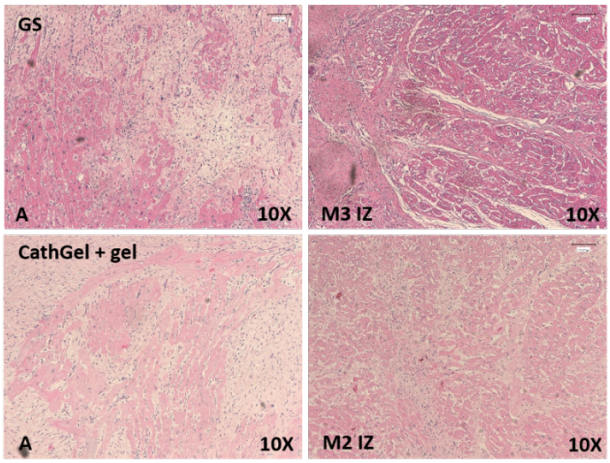


Figure 11: Representative histology images of the heart following 28 days after treatment and 42 days after induction of a myocardial infarction.

Due to a commercial pivot of Celyad, the development of C-CathGel was stopped. The consortium worked to develop a second gel delivery catheter called VisCath.

Viscath 2.0 & 3.0

Highlights:

- A novel steerable catheter to deliver hyaluronic acid hydrogels to the myocardium was developed
- The viability of the stem cells was not compromised after injection through the catheter
- Bench top testing demonstrated that it was possible to steer the injection needle to defined areas of the myocardium
- The pre-clinical feasibility was demonstrated by accessing the left ventricle using femoral access and injecting up to 10 times in the myocardium
- The hydrogel was retained in the myocardium after injection demonstrating efficacy
- LVEF ejection fraction was not negatively affected following delivery in a porcine model of MI (28 days after treatment)
- Higher viscosity biomaterials were compatible with VisCath which may be useful for ventricular stabilisation

Design Features of Endocardial Catheter

- VisCath, a minimally invasive endocardial catheter for cells embedded in fast-gelling hyaluronic acid hydrogels, was developed. The full assembly can be seen in Figure 2A. The device consists of:
- A) The inner component: 1.2 meters of dual-lumen tubing (outer diameter 1.5 mm), each lumen having an inner diameter of 0.5 mm which is connected to a 5 µL reservoir at the distal tip. A needle (diameter 0.24 mm ± 0 .01) is connected to distal tip of the reservoir. The hydrogel precursor solutions pass through the individual lumens and mix in the reservoir (crosslinking reaction initiated) before being ejected through the needle.

- B) The outer sheath: An Oscor deflectable sheath (Oscor Destino Twist® Deflectable Steerable Guiding Sheath, 6.5 French and 9 mm curve configuration) which can be actuated to 180° is the main component of the outer sheath, allowing the device to be steerable and deflectable. This is connected to a tip (Figure 2B) comprising of a PTFE insert attached to the distal end of the Oscor sheath to allow for the needle to be retracted.
- C) The Handle: The primary function of the handle is to extend/retract the needle a specified distance. It consists of several parts as in Figure 2C. A torque lock at the top prevents the dual-lumen inner component from slipping and is fixed to the cap. The cap actuates up and down and locks to a threaded trigger mechanism that controls the extent of the caps actuation. This can be user defined by manipulating the dial at the centre of the device. A spring near the base of the device allows the cap to return to its starting position when the trigger is released. A torque lock at the bottom of the catheter anchors the PTFE sheath that passes through the Oscor sheath.
- D) The adapter housing in Figure 2D allows a simple assembly and activation of the syringes containing the precursor solutions.

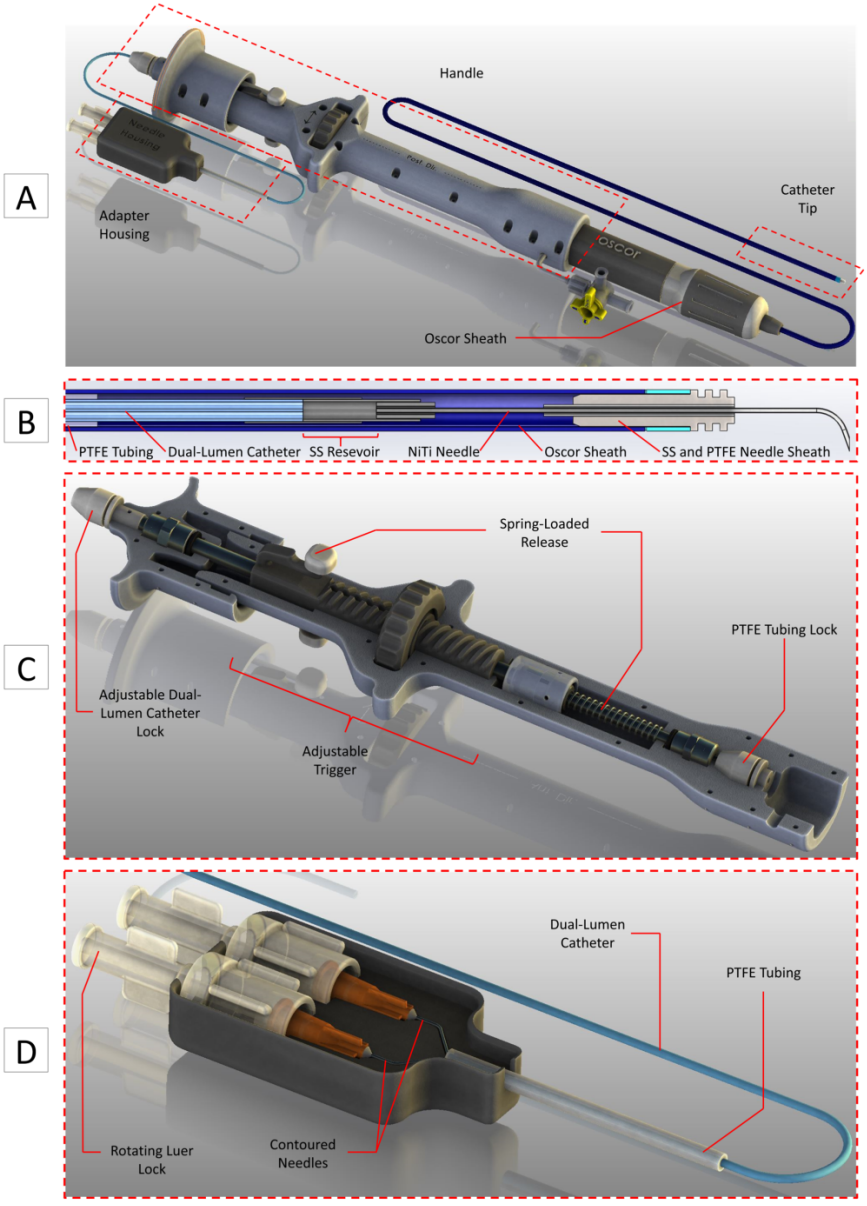


Figure 12: VisCath - The inner component (A), the outer sheath (B), the handle (C) and the adapter housing (D) of the catheter described in Section 2.2.

Injectability and Gelation of CardioGels

The force required to inject the hydrogel precursor solutions through VisCath remained below the maximum male and female pinch strength (70N and 50N, respectively). The force increased up to 3 minutes between each injection, and then became constant at ~43N, Figure 3A. At this point the polymerised hydrogel in the VisCath reservoir resulted in slightly higher injection forces to inject the material. If >3 minutes passed between each injection the polymerised hydrogel could still be ejected from VisCath with <50N force (female pinch strength) due to the small size profile of the reservoir. This result is in agreement with the gelation curve which shows that gelation occurs between 0-3 minutes, Figure 3B. This result also informed our injection procedure described in Section 2.7 whereby the needle was kept in position after each injection for 30 seconds to ensure gelation was initiated. Additional information regarding the properties of the hydrogel can be found in Supplementary Figures 8, 9 and 10, where we show the viscosity as a function of shear rate, zero rate viscosity of 0.4 Pa.S, gelation time of 19 seconds, and the gelation curve of 2% HA-PH-RGD (60-90 kDa).

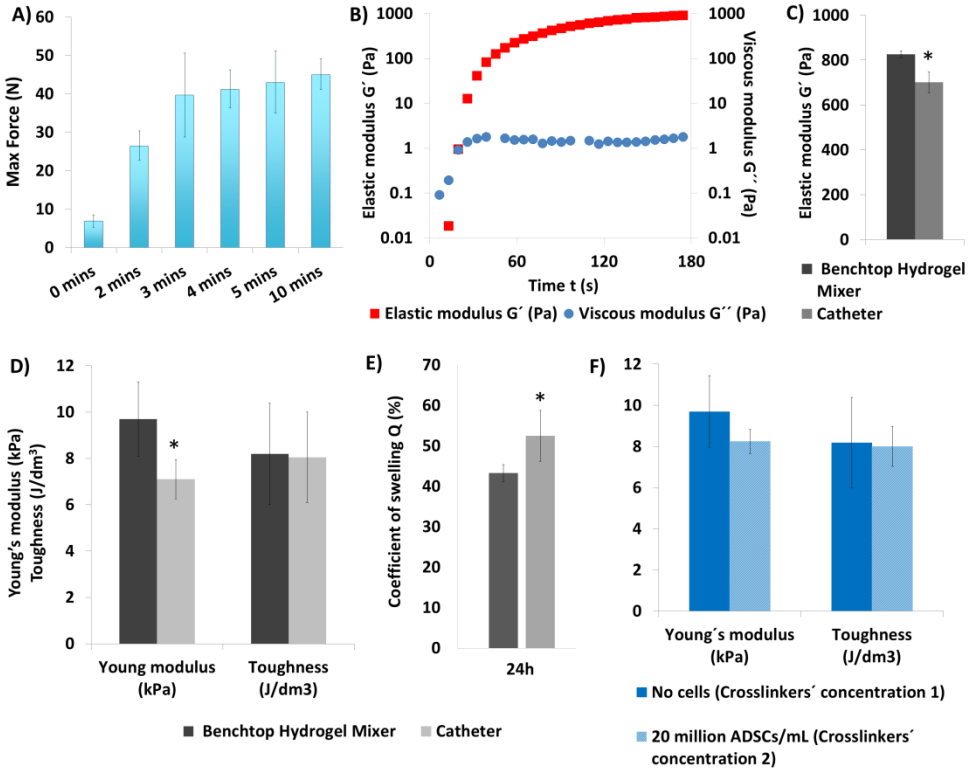


Figure 13: Characterisation of CardioGels – A) Injection Force and B) Gelation curve of HA-PH-RGD hydrogel at crosslinking concentration 1, C) Elastic modulus, D) Young´s modulus and toughness and E) Swellability of HA-PH-RGD hydrogel at crosslinking concentration 1 prepared by BHM and VisCath, F) Young´s modulus and toughness of HA-PH-RGD hydrogel prepared with BHM with/out 20 million cells/mL.

Viscoelastic and Mechanical Characterisation of Hydrogels

Injection through VisCath reduced the properties of hydrogels

There were statistical differences between the elastic modulus (825 versus 701 Pa p=0.0002), Young's modulus (9.7 versus 7.7 kPa p=0.0007) and swelling coefficient (43.3 versus 52.5% p=0.0077) for the hydrogels prepared by the BHM compared to VisCath, no significant difference was observed in the toughness (8.2 versus 8.0 J/dm^3).

Viability of Encapsulated ADSC

ADSC viability and metabolic activity was not affected by injection through VisCath

Representative confocal images of the cross-sectional area of the cell-loaded hydrogels can be seen clearly in Figure 4B. No significant difference was seen in cell viability or metabolic activity in the hydrogels injected through VisCath compared to the BHM, Figure 4C-E.

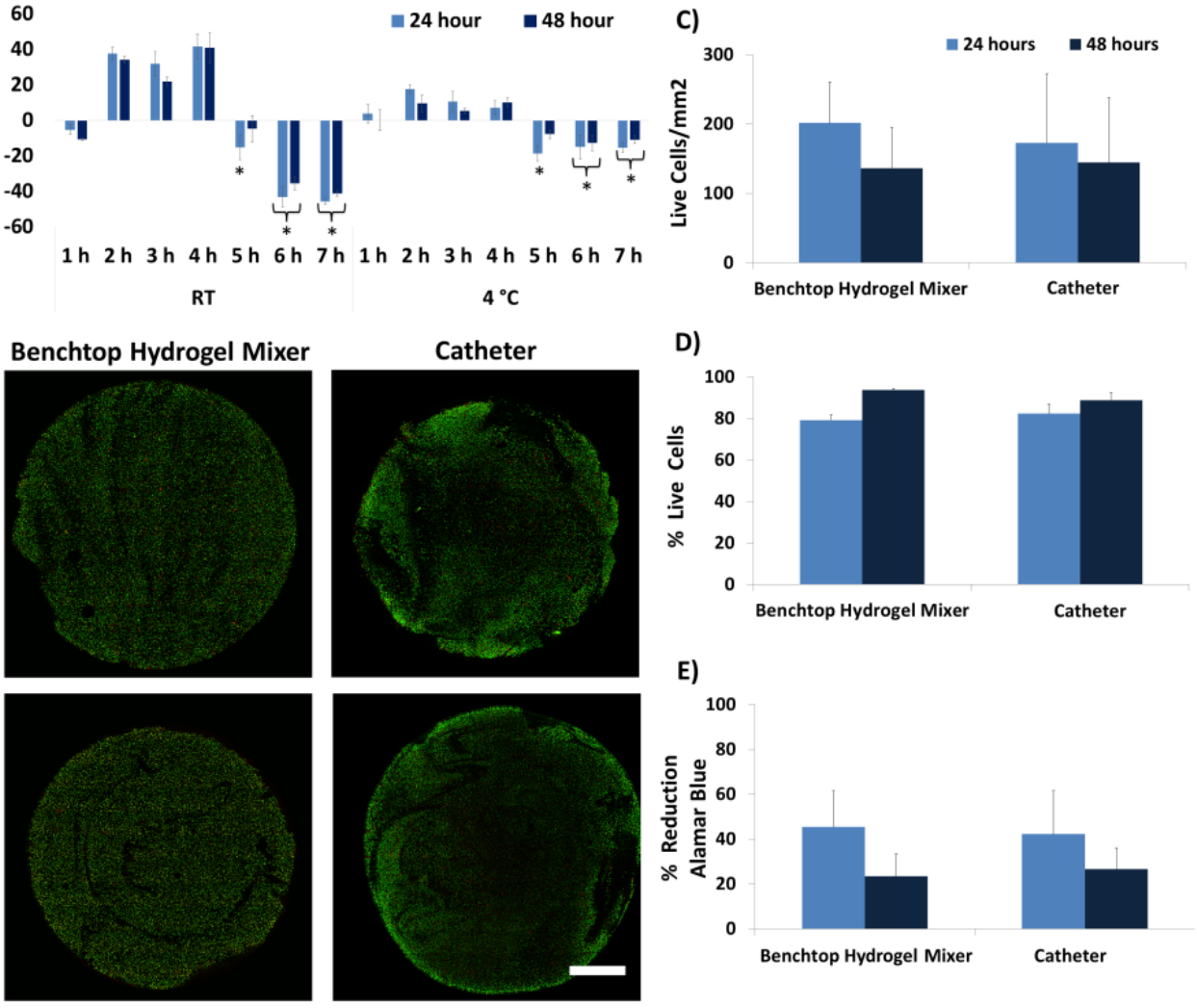


Figure 14: Viability of Encapsulated ADSC – A) Prolonged incubation of ADSCs in HA-PH-RGD+HRP precursor solution, B) Confocal scans (scale bar=1 mm), C) Number and D) Percentage of live cells and E) Metabolic activity of 20 million ADSCs/mL of HA-PH-RGD.

Assessment of pre-clinical Feasibility of Catheter *In Vivo*

Under fluoroscopic guidance VisCath 2.0 was advanced to the aortic arch and into the LV by abdominal aortic access, see Figure 5A-F. This site was chosen due to a length restriction in the VisCath prototype, ordinarily femoral access would be preferred. Once in place in the

LV, the hydrogel was successfully injected into the LV wall as demonstrated by MRI, Figure 5G-I.

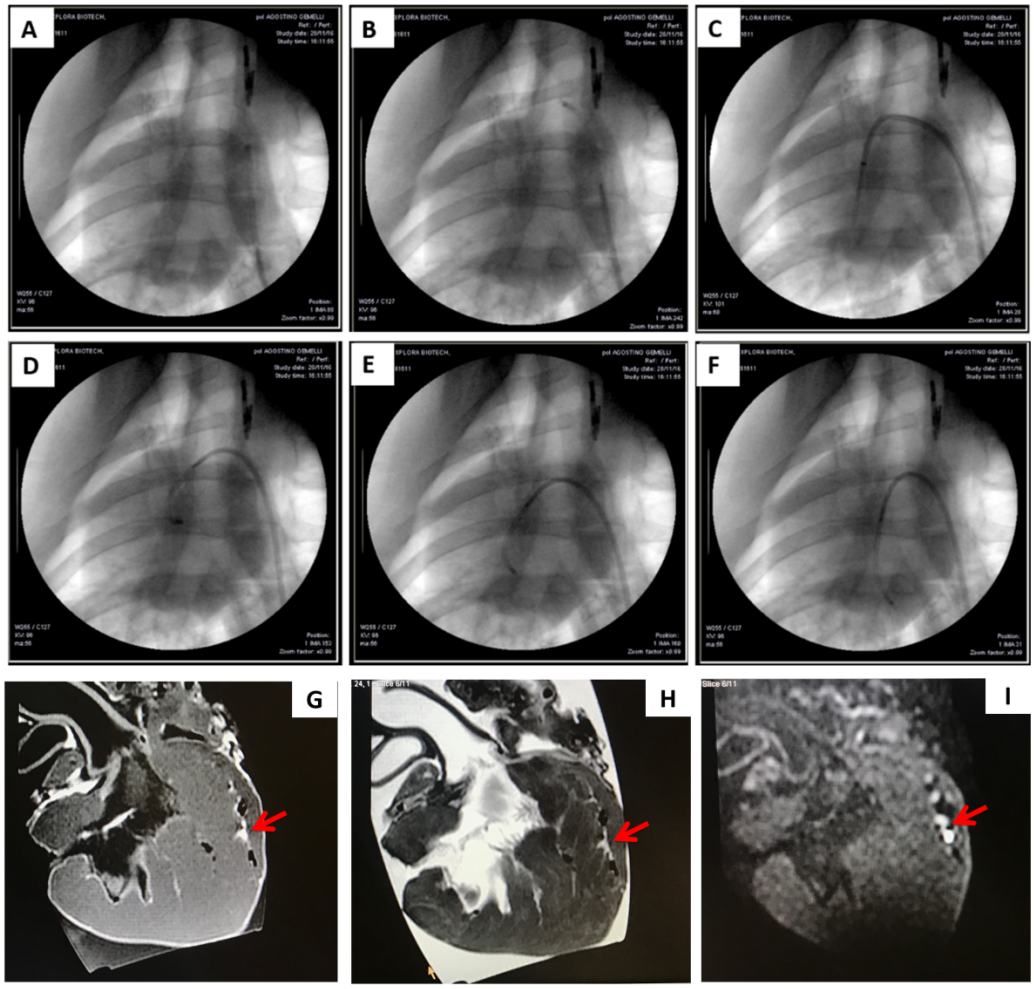


Figure 15: Pre-clinical Feasibility of VisCath 2.0 - VisCath was advanced into the abdominal and the descending aorta (A-B), through the aortic arch(C) and mitral valve (D) into the LV (E). The device was positioned to the target site of injection (F). MRI of explanted heart showing retention of injected hydrogel in T1 (G), T2 (H) and diffusion scan (I).

Viscath 3.0 injection catheter was redesigned from recommendations from the acute trial of Viscath 2.0. Viscath 3.0 design was downselected from several designs built and was tested using simulated testing in a heart model and visualized with fluoroscopy using a C-arm. The system was used with and extended version of the Zurpaz steerable guide catheter.
Simulated testing was performed with the Viscath 3.0 and modified Zurpaz; the model (had simulated pumping and the aortic valve to help understand access and placement. This testing showed that delivery of the catheter to the LV was possible.

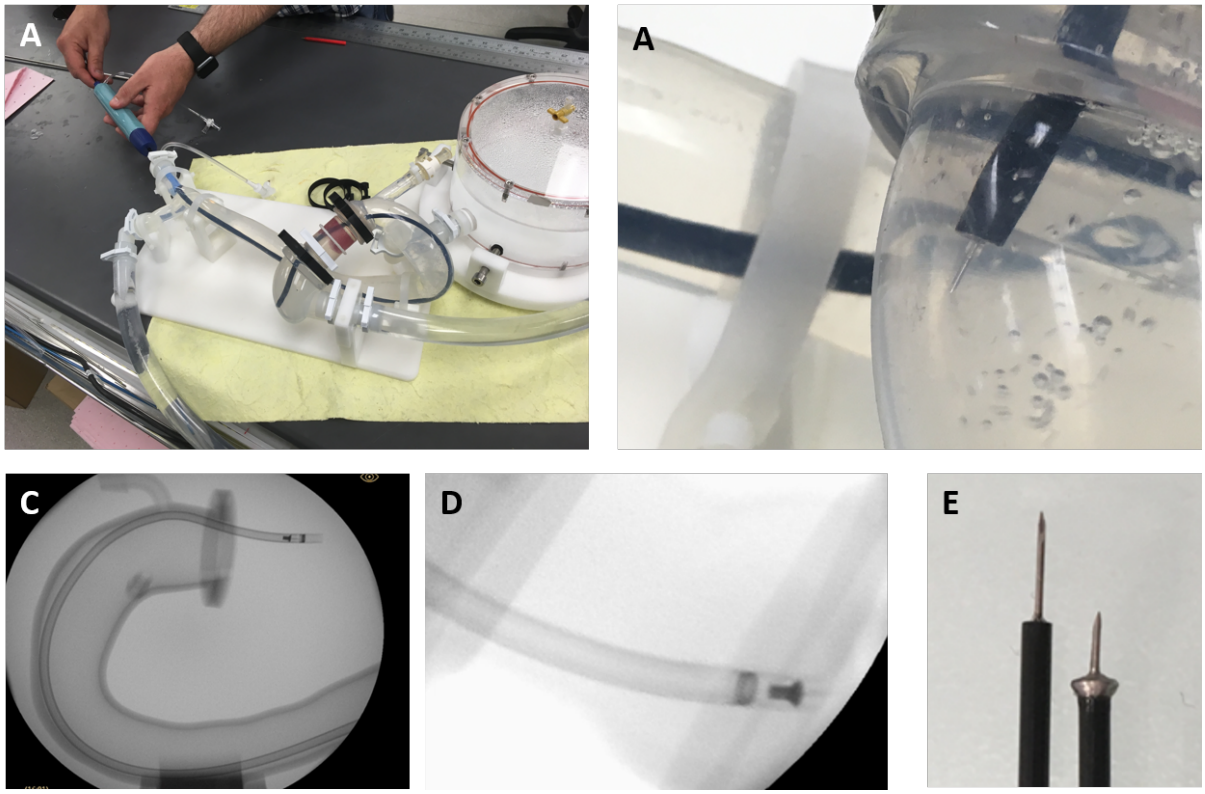
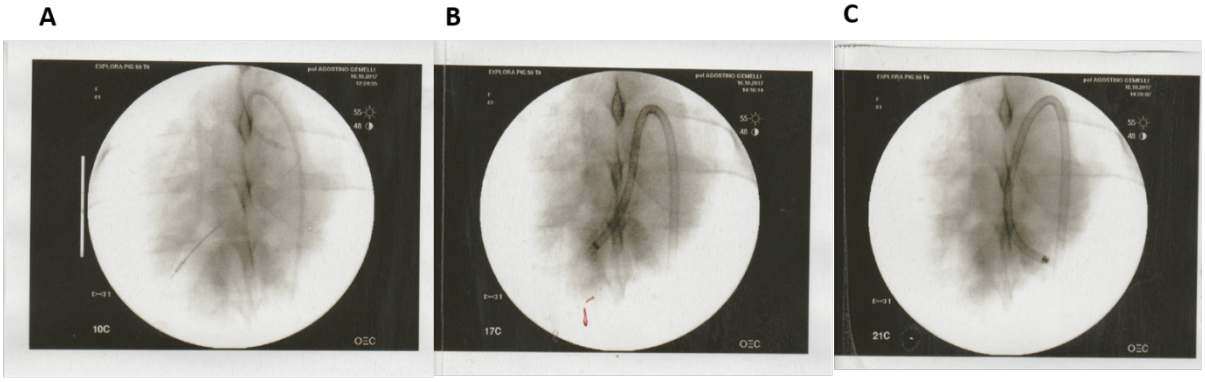


Figure 16: A & B Heart delivery Simulation model, C & D Fluoroscopy image during simulated testing of the Viscath3 inside the Zurpaz guide catheter. This shows clear visualization of RO marker and distal tip of the injection catheter, E Picture of distal tips of final catheter designs with the downselected Viscath 3.0 on the right

Assessment of pre-clinical Feasibility of VisCath 3.0 *In Vivo*

Under fluoroscopic guidance VisCath 3.0 was advanced to the aortic arch and into the LV by abdominal aortic access, see Figure XXA-C. Once in place in the LV, the hydrogel was successfully injected into the LV wall at 10 sites.



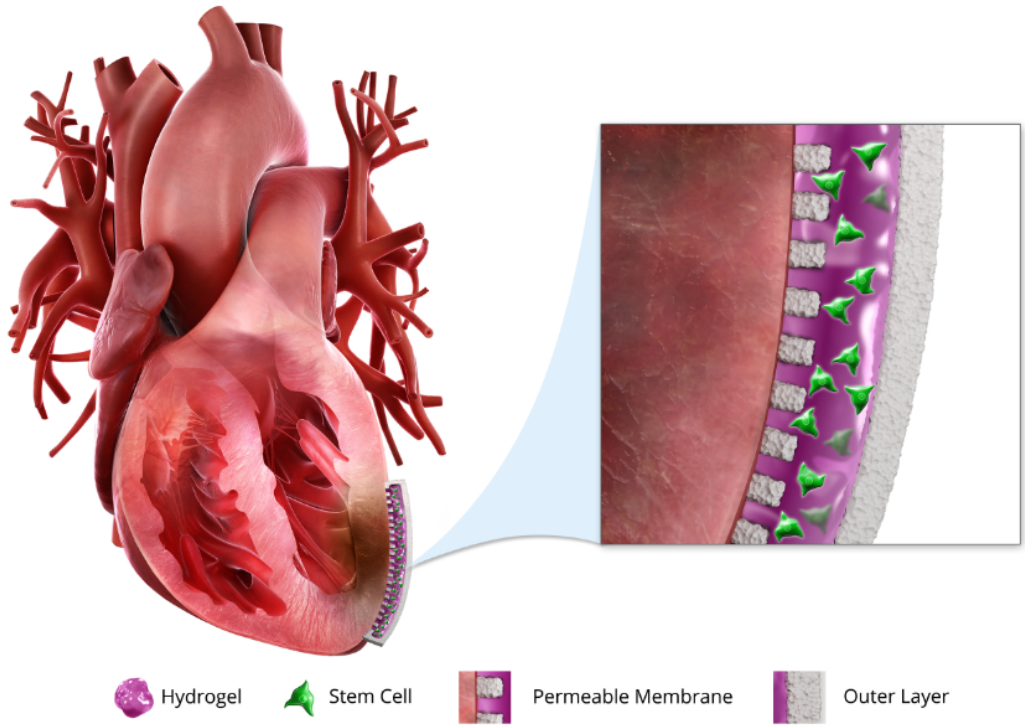
A: AMI induction; B-C: two sites of injections in antero-posterior view

Figure 17: Coronary Artery Occlusion (A) & 2 CardioGel injections at various locations are demonstrated in B&C.

Conclusion

We have demonstrated that a carefully chosen cross linkable cell embedded biomaterial can be successfully delivered using a 1.2 meter length, small bore lumen catheter technology. In these experiments the biomaterial maintains appropriate physical properties, viable cells and does not possess any properties that inhibit successful material delivery – such as too high a maximum push force or blockages of the catheter lumen. Furthermore, the VisCath was successfully used in large pre-clinical experiments and we demonstrated that the biomaterial could be delivered and was retained in the myocardium of a beating heart. In summary these experiments demonstrate that careful biomaterial choices and parallel catheter design activities can result in a potentially new clinical strategy to repair damaged myocardium.

<u>Therapeutic Route of Administration – Epicardially using a mini-thoracotomy</u>



SPREADS

Highlights:

- A novel epicardial reservoir (SPREADS) to hold CardioGel on the surface of the heart was developed with biocompatible materials and saleable manufacturing procedures
- A novel design feature to allow bioglue to be applied around the circumference of the reservoir to adhere the device to the epicardial surface was developed
- The reservoir was compatible with gel alone and gels loaded with stem cell
- A procedure was developed to allow minimally invasive delivery of the reservoir to the epicardial surface in a large animal model
- A procedure and tools were developed to allow minimally invasive filling of the reservoir with Cardiogels to the epicardial surface in a large animal model

- The pre-clinical feasibility was demonstrated by accessing the left ventricle epicardial surface using a mini-thoracotomy
- The hydrogel was retained on the epicardium surface after reservoir filling demonstrating efficacy
- LVEF ejection fraction was statistically improved following delivery in a porcine model of MI (28 days after treatment versus gold standard treatment)

Overview:
SPREADS is a breakthrough technology. It is a novel, single-stage therapeutic strategy to apply biologics or therapeutic cells to the surface of the beating heart in a minimal-invasive, closed chest intervention. The reabsorbable material enables a one-time procedure to be performed on patients with a myocardial infarction. Despite carrying a specific treatment to the surface of the heart, it also serves as a passive stabilization device supporting the weakened (infarcted) myocardial region. This resulted in improved LV chamber geometry and preserved myocardial function over 28 days in a large animal model. This treatment strategy is a disruption to current treatment including revascularization and medical therapy. This is important to a large number of patients that survive myocardial infarction and subsequently develop cardiac remodelling and heart failure.

From the results achieved with the SPREADS device during the AMCARE project it is proposed by members of the Consortium to apply for further funding from H2020 call with the primary goal to reach CE-mark for the SPREADS device as and get it to the market a medical product.

An iteration of the SPREADS device occurred in the latter half of the AMCARE programme
The following noteworthy changes/improvements occurred compared to the cardiac patch manufactured using HA:

- In order to decrease the risk of positional instability once implanted, a design iteration of the SPREADS was performed to include the **addition of a biological adhesive**;
- A **biological adhesive available on the market**, i.e. with regulatory approval, was chosen that is suitable for application on the heart surface in the small amounts required for the SPREADS design, i.e. < 0.5ml; Furthermore, a suitable interface with the SPREADS supply lines (female Luer) was established and sourced;

Testing of 1st generation SPREADs
The in-vitro tests consisted of pre-wetted glass-plate tests, where the SC was pressed against the bottom of the plate, revealed adequate intrinsic adhesion of the SC. Furthermore, filling of the gel cavity with a dummy gel did not compromise the adhesion, as shown below.

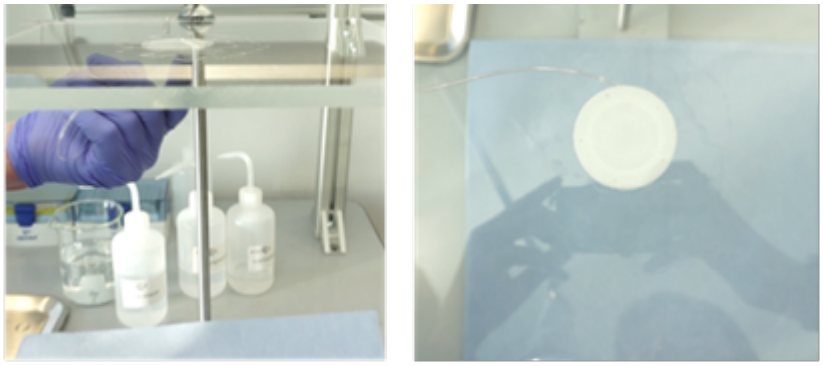


Figure 18: Pre-wetted glass-plate tests – adhesion prior to filling

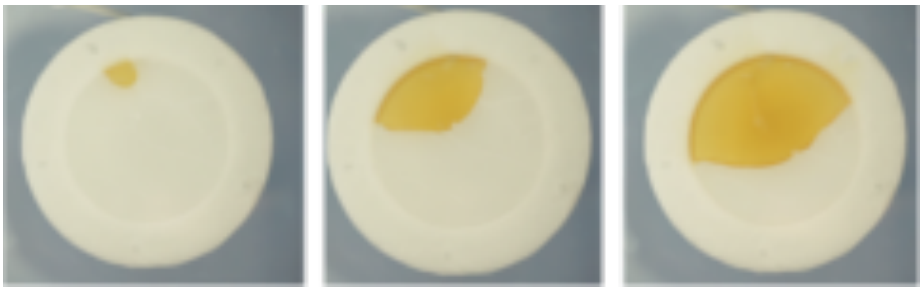


Figure 19: Pre-wetted glass-plate tests – filling of dummy gel while adhering to glass-plate

The ex-vivo tests consisted of applying the SC on a porcine heart (obtained from a local abattoir). The tests revealed sufficient adhesion to the non-planar surface of the heart (anterior ventricle). Furthermore, filling of the gel cavity with a dummy gel did not compromise the adhesion and was evenly distributed, as shown below.

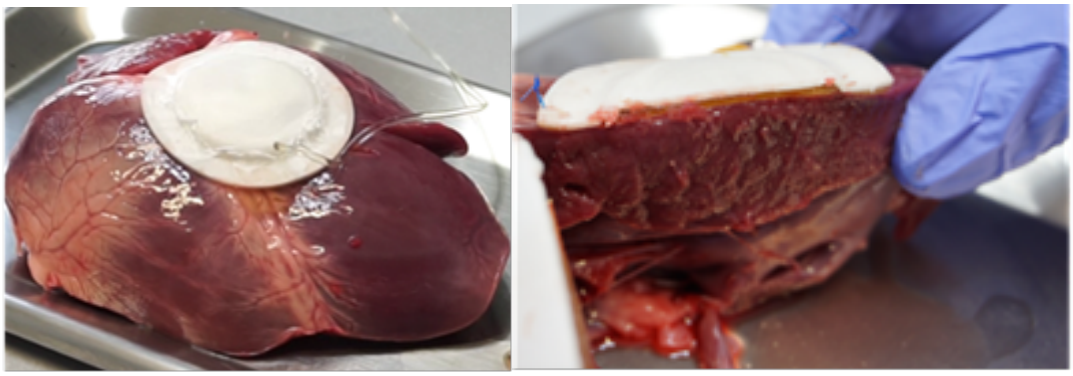


Figure 20: Ex-vivo tests on porcine heart – adhesion prior to filling (left) and cross-section showing distributed filling of dummy gel (right)

Initial acute in-vivo tests revealed sufficient adhesion to the epicardial surface, even after the chest wall was closed for approximately 60 seconds, as shown below.

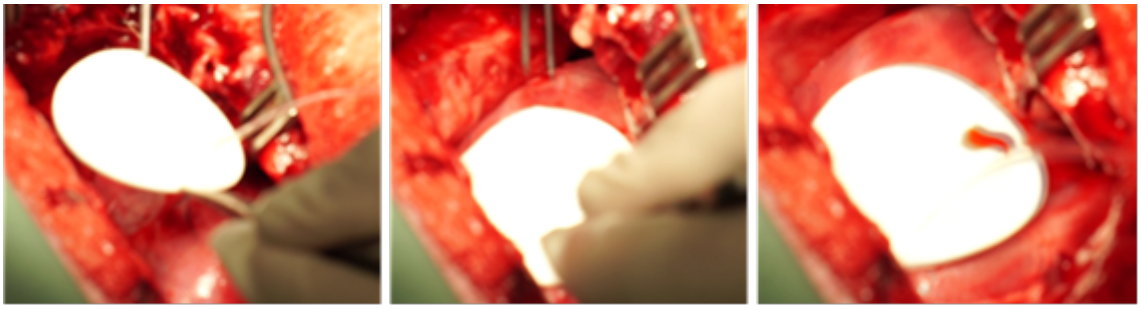


Figure 21: Acute in-vivo tests on porcine heart – showing placement on the beating heart

Design iteration SPREADS

As mentioned above, to improve adhesion in-vivo, an extra channel for the addition of a biological adhesive per extra-corporeally communicating supply line was included in the design, as shown below. In-vitro tests to follow.

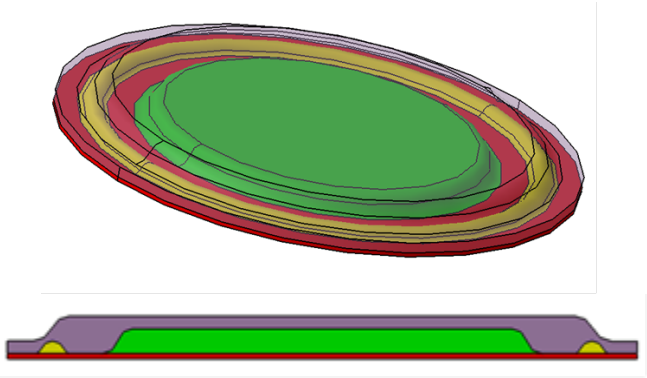


Figure 22: 2nd generation SPREADS – isometric (left) and cross-sectional (right) views, respectively

Chronic preclinical trial to assess safety and efficacy of the SPREADS device including Gel and Cells

In preparation of the chronic animal study phase, two additional test procedures were utilized in an acute setting to perform minimal-invasive closed chest implantation and positioning of the SPREADS device. These experiments showed excellent feasibility of applying and positioning of the SPREADS device via a small incision on the beating heart. Subsequently, SPREADS devices were applied on 10 large animals in the chronic setting. All animals survived the procedure and the follow-up time thereafter.

The surgical procedure under sterile conditions consisted of a small, median sub-xyphoideal incision and pericardiotomy. Thereafter the SPREDS devices were positioned under direct vision onto the infarct area at the anterior aspect of the heart near the apex. The CardioGel was applied followed by a biodegradable glue to hold the patch in position. Thereafter the incision was closed and the animals were allowed to wake up and recover. Fig. 8 ADJ shows some aspects of the intraoperative procedure.

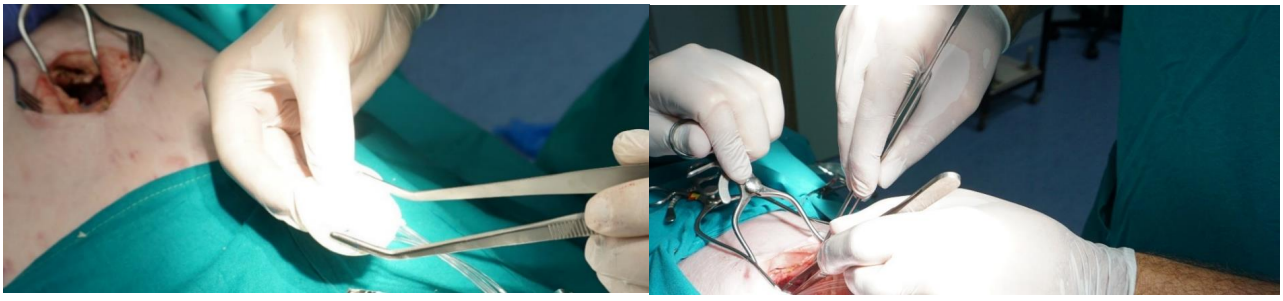


Fig. 8 ADJ: Implantation and positioning of the SPREDS device under sterile conditions. The procedure was safe and effective and lasted around 20-30 minutes. After gel and glue administration, tubings were removed leaving only reabsorbable material behind.

After completion of the follow-up period, animals were euthanized on day 42. Prior to euthanasia, all functional parameters were collected. Hearts were excised and inspected. Macroscopic evaluation revealed partial absorption of the SPREDS patch. All patches were found where they were initially positioned and were encapsulated by thin fibrous tissue. No active inflammatory reaction was observed.

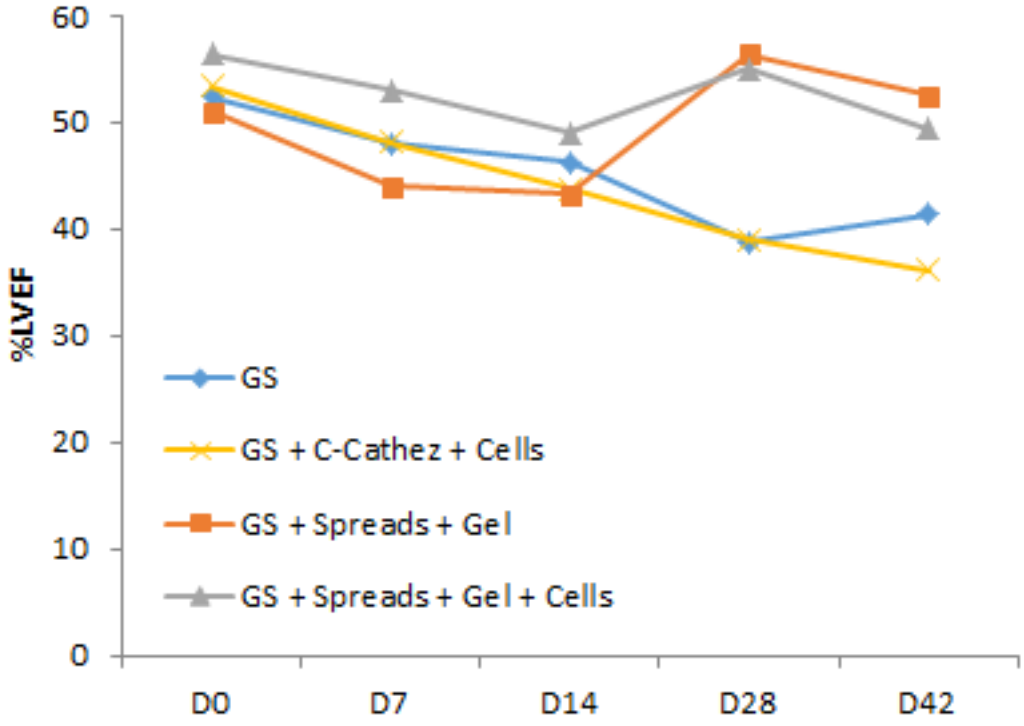


Figure 23: Left Ventricular Ejection Fraction (LVEF) following the induction of myocardial infarction over a 42 period in a large animal model of MI. Treatment occurred at D14. Treatment groups including gold standard clinical pharmacological treatment (GS), GS + stem cells injection endocardially using commercially available C-Cathez, GS + SPREADS filled with Cardiogel, and GS + SPREADS filled with Cardiogel loaded with stem cells. There was a significant improvement in heart function measured by ejection fraction in the SPREADS groups at day 28 and day 42.

The preclinical assessment demonstrated that SPREADS shows promise as a therapeutic strategy in treating post-MI negative remodeling. Apart from safety, efficacy and biocompatibility considerations for the preclinical study, also in-vitro tests, gel mechanics and cell-viability tests were performed prior to start of preclinical study.

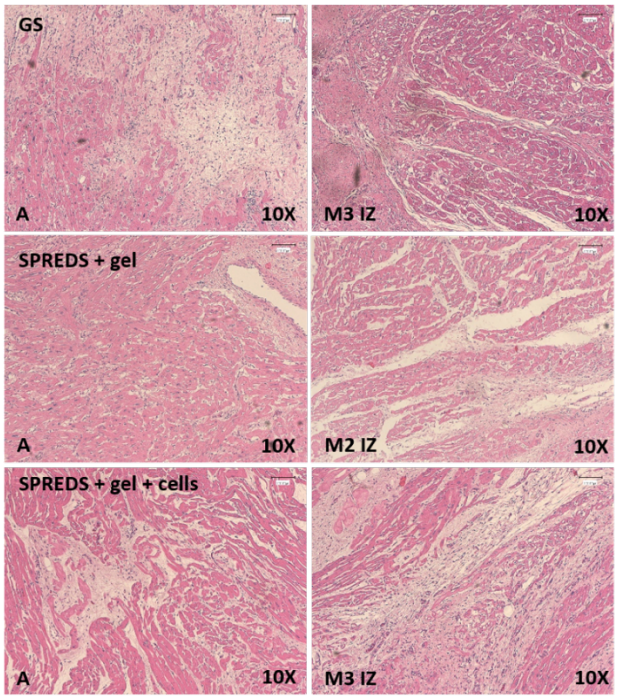


Figure 24 demonstrates representative histology images from the myocardium following implants of the devices on the epicardial surface.

AMCARE Ancillary Technologies

Highlights:

- A small animal model of CardioGel delivery was developed to assess biocompatibility in the mycadium
- A large animal model of myocardial infarction using ischemia and reperfusion was developed and a chronic study was completed with a 100% survival outcome measure
- Histological assessemnts tools were developed to interrogate the outcomes seen in the preclinical trials
- A cardiac specific ISO biocompatibility test was established and is currently being accredited
- IGF particles were formulation and advanced drug release techniques were developed

Impact of AMCARE results

At the time the AMCARE (Advanced Materials for Cardiac Regeneration) project was about to take off – November 2013 –, cardiovascular diseases (CVDs) represented one of the main causes of morbidity and mortality both globally and at the European level.

About four years later, the incidence of CVDs within the European Union borders keeps showing a steadily increasing trend (EHN 2017), with obvious consequences for national

healthcare systems (see Figure 25). It thus goes without saying that the research for new therapies for these diseases keeps being an extremely topical issue.

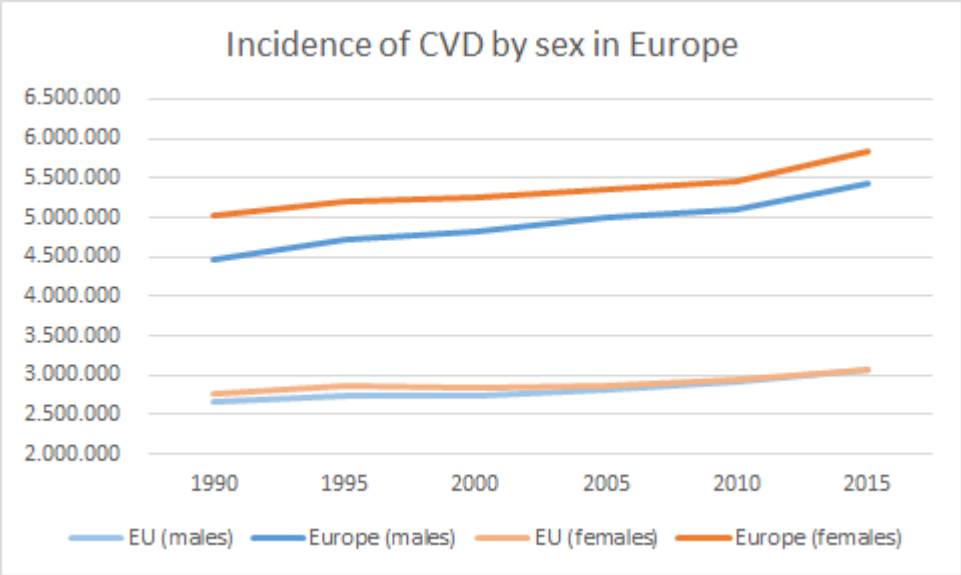


Figure 25: Incidence of CVD by sex in Europe

For many years, scientific research and medical treatments in this field assumed that myocardial death process was irreversible due to the supposed incapability of cells composing the myocardium to proliferate or renew after AMI. Subsequently, therapeutic approaches to these conditions ended up having mainly a palliative function rather than a properly curative one, in that they failed to address the fundamental post-MI loss of myocyte and the persistent damages to myocardium (Pascual-Gil et al. 2015; Oh et al. 2016; Saludas et al. 2017; Silvestre & Menasché 2015; Behfar et al. 2014).

By treating the underlying myocardial damage post-MI with unique and effective biomaterials and delivery systems, harnessing the potential of regenerative medicine, AMCARE offers a novel therapeutic modality to treat acute MI patients.

- ✓ AMCARE developed innovative biomaterials and medical devices that enable successful minimally invasive and cost effective delivery of new Advanced Therapeutic Medicinal Products (ATMPs).
  The ATMPs are based on innovative new HA-based biomaterials that were developed within the consortium. Based on the results of the in-vitro and of the pre-clinical experiments, this new suite of ATMPs and new medical devices can provide significant healthcare benefits to the aging EU/Global population particularly after an MI. We have seen increases of heart function go back to baseline with our lead SPREADS technology. After the AMCARE project the therapy is expected to advance towards a first in man clinical validation phase.

- ✓ AMCARE can improve the quality of life: the outcome that AMCARE developed has the potential to progress healthcare away from commodity products such as devices

that retain remaining function but do not restore cardiac function e.g. stents. Those extended healthcare benefits and improvements to quality of life will be sustainable over a longer timeframe post intervention with this intervention.

✓ AMCARE contributed to the success of European biomaterials industries.
The AMCARE SMEs (ADJUCOR, CELYAD, CONTIPRO and EXPLORA) and the MNC (BOSTON SCIENTIFIC) gained a competitive advantage in the following ways:

- Developed new knowledge and technologies
- Decreased the time-to-market of their technologies bringing them from TRL3 to TRL5

Commercially exploitable results from the AMCARE programme include the following:

1. CPC (cardiopoietic cells) GMP grade production **(Celyad)**
2. Biomaterial (Hydrogel) and nanoparticle drug carriers **(RCSI, Contipro)**
3. A medical device for the epicardial delivery of the hydrogel **(AdjuCor)**
4. 2 different catheters, C-Cath$_{Gel}$ and Viscath, for endocardial delivery of hydrogel **(Celyad, TCD, Boston Scientific)**
5. a large animal model of acute myocardial infarction that is currently marketed in Europe by **EXPLORA** (www.aurumlaboratories.com). To date, EXP was contacted by several possible clients (academics and private companies) and the service is expected to generate revenues ca. 300.000 euro in the next 2 years.

Each exploitable result (medical devices, biomaterials and ATMPs) has been patented and will be exploited by the industrial Partners of the Consortium by licensing to 3rd parties manufacturers or further developing the technology for rapid bench to first in man and subsequent market transition.

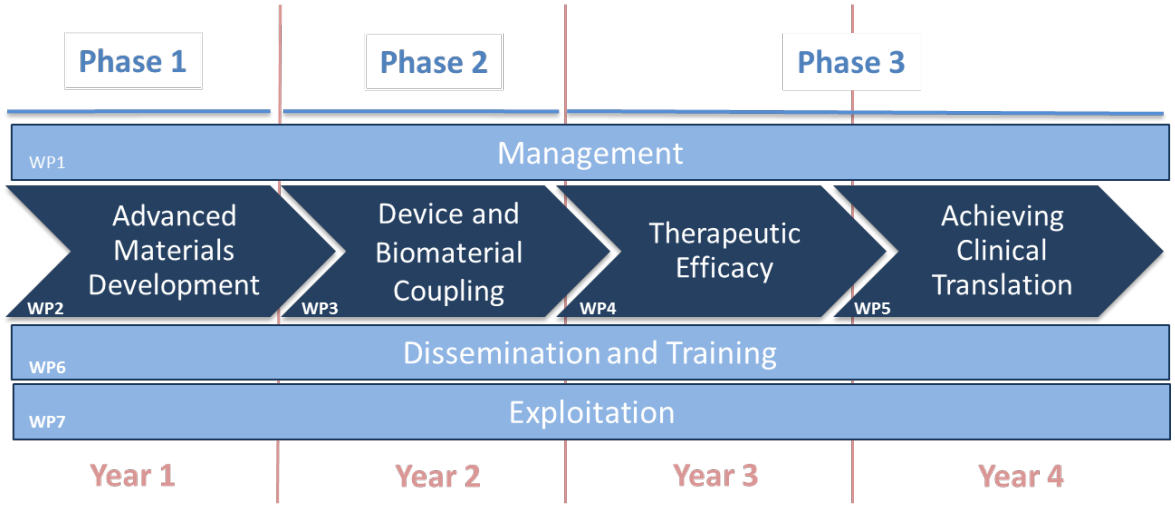
✓ AMCARE contributed to the leadership of European Universities and R&D centres.
The **academic and research partners** took advantage of the AMCARE programme by:

- Strengthening and commercially focusing their research
- Developing novel methods and therapeutics.
- Deepening academic and industrial collaborations

Actions completed to speed up the beginning of the clinical phase

To maximise the impact of the action and speed up the beginning of the clinical phase, the AMCARE project has encompassed the following steps:

- the RTD programme covered all the research steps from materials development, down to the achievement of clinical translation as schematised in the next figure;



- regulatory aspects have been adequately considered and the related activities started early in the project lifetime;
- IP literature (patents) has been properly monitored and any IP was timely secured as it was produced during the project;
- dialogue with citizens and patients has been part of the dissemination work (WP7) so that any problem related to public perception was addressed at early stages.

Economic and Social Impact

According to the World Health Organization (WHO), cardiovascular diseases (CVD) are the leading cause of death throughout the world – more people die from CVDs per year than any other cause. The risks of re-infarction and adverse events with current therapies are high, particularly in elderly populations (Chugh 2012, Orn 2009). Current therapies are ineffective in restoring full cardiac function post-MI, which highlights the need for new therapeutic strategies to aid in reducing the impact of CVD in Europe and globally. The necessity of new strategies demonstrates a clear need for the novel formulations and delivery methods. The progression of heart failure post-MI leads to significant morbidity and mortality, so the formulations and delivery methods developed by AMCARE consortium aim to halt or even reverse this damage. Our novel and comprehensive technologies will harness the potential of regenerative medicine and offer a new therapeutic modality to effectively treat acute MI patients in the future.

AMCARE *established a translational research program to develop advanced multimodal regenerative therapies using smart biomaterials that effectively treat damage caused by myocardial infarction*. Specifically AMCARE achieved these objectives by:

- Optimising cardiac stem cell therapy (endogenous and induced) using smart biomaterials and advanced drug delivery
- Coupling these therapeutics with minimally-invasive surgical devices

AMCARE contribution is particularly remarkable for the following indicators/items:

- ***Reduce Years of Life Lost***. Years of life lost (YLL) is a measurement assessing the burden of mortality for a condition, and weighs mortality, age at death, and the population

structure. In Europe, ischaemic heart disease costs 21,1% of all YLL (source: WHO 2016).

- ***Reduce Disability-Adjusted Life Years (DALYs) Associated with CVD***. DALYs are the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability. In 2015, IHD (Ischaemic Heart Disease) was the cause of 13% of all DALYs in Europe, and all CVDs were the cause of 23% of all DALYs in the region.

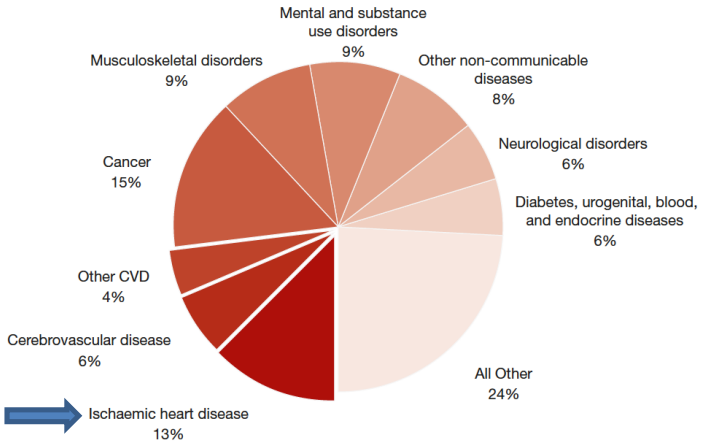


Figure 26: Disability-adjusted life years lost by cause, 2015, Europe (source: European Cardiovascular Disease Statistics 2017)

- ***Decrease the Economic Burden***. CVD cost the health care systems of the EU just under €111 billion in 2015.This represents a cost per capita of €218 per annum, 8% of the total health care expenditure across the EU. The cost of inpatient hospital care for people who have CVD accounted for over 50% (€57 billion) of these costs, and that of drugs for 25% (€28 billion). Almost one-fifth (17%) of health care expenditure on CVD in the EU is due to IHD. IHD cost the health care systems of the EU just under €19 billion in 2015.

	CVD		IHD		Cerebrovascular disease	
	€ thousands	% of total	€ thousands	% of total	€ thousands	% of total
Direct healthcare costs	€ 110,809,465	53%	€ 18,875,775	32%	€ 20,058,318	44%
Productivity loss due to mortality	€ 31,631,317	15%	€ 13,783,879	23%	€ 5,440,593	12%
Productivity loss due to morbidity	€ 22,635,461	11%	€ 6,031,162	10%	€ 3,983,874	9%
Informal care costs	€ 45,088,142	21%	€ 20,636,600	35%	€ 15,855,181	35%
Total	€ 210,164,386		€ 59,327,415		€ 45,337,965	

Figure 26: Total cost of CVD, IHD and cerebrovascular diseases, 2015, EU (source: European Cardiovascular Disease Statistics 2017)

- ***Improve the Health and Survival of the Workforce in Europe***. For those under age 65, the economically active work force of Europe, IHD was determined to be the cause of death in 16% of men and 11% of women (source: European Cardiovascular Disease Statistics 2017). Moreover, the WHO notes that chronic diseases deprive individuals of their health and productive potential, as well as of their income, savings, and investments. With the reduction of economic productivity associated with chronic diseases such as CVD, labour forces are depleted. Workforce productivity can be

increased by preserving health, particularly post-MI as AMCARE aims to do, and reducing incapacity, disability and workdays lost.

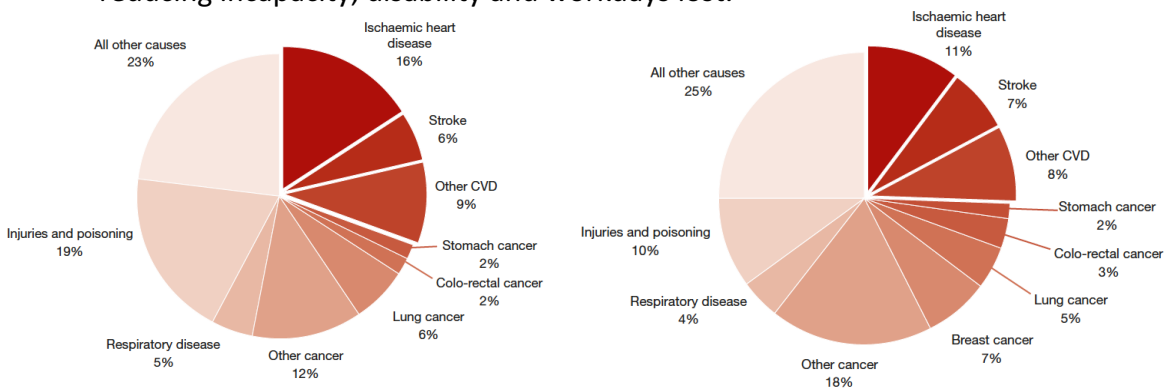


Figure 27: Deaths under 65 years by cause, males (left) and females (right), latest available year, Europe. (source: European Cardiovascular Disease Statistics 2017)

Scientific Impact

- ***Novel Delivery and Therapeutic Systems***. The development of a distinct Cardiac Progenitor Cell (CPC) biomaterial delivery system allows to treat small focal areas of myocardial damage using endocardial gel delivery and large areas of myocardial damage using epicardial placement, tailoring our approach to the patient specific clinical need.
- ***New Restorative Treatment Modality***. The combination of these novel medical devices and advanced biomaterial formulations creates an entirely new restorative treatment modality for patients with acute-MI – one that can drastically impact morbidity and mortality by harnessing the potential of regenerative medicine and biomaterials to stop the progression of heart failure.
- ***Creating a Multidisciplinary Team***. Numerous areas of materials science were integrated, including biomaterials, stem cell biology, drug delivery and medical devices.
- ***Bringing Europe to the Forefront of CVD Research***. The biomaterial utilisation and unique delivery methods developed can provide a new standard of care for MI patients in Europe and abroad.

Clinical Impact

- ***Reducing Mortality Post-MI***. The WHO estimates that by 2030, CVDs will *still* be the primary cause of death, and almost 23.6 million people will die from CVDs, including heart disease and stroke. Up to half of patients suffering an acute MI or stroke die before they reach medical attention, and for those acute MI patients who survive the first thirty days, there is a 10% risk of dying in the first year and a 5% risk of dying in subsequent years. Acute MI survivors with congestive heart failure (CHF) are also a group at high risk for death, with a five-year mortality of 26–75% overall (Gazian et al., 2007). The ESC estimates that "every sixth man and every seventh woman in Europe will die from myocardial infarction" (McMurray et al., 2012). The AMCARE consortium can reduce these devastating mortality rates by providing a new treatment for MI patients.
- ***Reducing the Risk of Re-infarction***. With the current standard of treatment, re-infarction is still a tangible and dangerous risk. In the three-year follow-up, 4.9% of the patients experienced severe cardiovascular events such as death from cardiac causes, cardiac arrest, or myocardial infarction (Stone et al., 2011). By utilising AMCARE's outcomes to

treat damaged myocardium tissue post-MI, we aim to prevent re-infarction.

- ***Protecting the Health of the Elderly***. According to the European Society of Cardiology, approximately 1–2% of the adult population in developed countries has heart failure, with the prevalence rising to ≥10% among persons 70 years of age or older (Chugh 2012). In younger populations, preventative interventions and interventions that prolong survival have been instrumental in declining age-adjusted death rates for most causes of CVD. However, the elderly population is growing older and surviving longer, and the average age of death from CVD continues to rise, thus affecting a larger elderly population (De Gregorio et al., 1998). Furthermore, short- and long-term outcomes of elderly patients undergoing coronary artery stenting are poorer than those of younger patients, and elderly patients have significantly higher rates of procedural complications compared to younger patients (Chugh 2012).

Exploitation of project results and management of IPRs

The **AMCARE** consortium have demonstrated a clear commitment towards exploitation and protection of intellectual property. The consortium ensured that the results of the project were disseminated in an appropriate and useful manner. A clear IPR Agreement and strategy of innovation protection and transfer reflecting the differing stakeholder priorities was established at the beginning of the project. Commercially exploitable results were protected by patents.

Each of the industrial partners has developed a proprietary IP that could significantly impact on its own business. The outlook of the IP and commercialization rights of the envisaged materials (*CardioGel*), devices (*SPREDS, C-Cath*$_{Gel}$ and *Viscath*) and services (large animal model of acute MI) are reported hereinafter.

1) ***CONTIPRO*** owns the Patent and the IPRs related to the *Cardiogel*
2) ***ADJUCOR*** owns the Patent and the IPRs related to the SPREADS device
3) ***CELYAD*** owns the Patent and the IPRs related to the C-Cath$_{Gel}$ device and the CPCs
4) ***EXPLORA*** developed and validated the large animal model of acute myocardial infarction that is currently marketed in EU through a dedicated website (www.aurumlaboratories.com). To date, EXP was contacted by several possible clients (academics and private companies) and the service is expected to generate revenues ca. 300 keuro in the next 2 years.
5) ***BOSTON SCIENTIFIC*** has developed Viscath, an injection catheter successfully tested in an acute animal trial, which needs further validation in pre-clinical studies before beginning clinical trials.

Furthermore the Coordinator has shown interest towards the creation of a **start-up company** to get a license from Celyad for the further development and subsequent exploitation of project results related to the C-Cath$_{Gel}$ device. Negotiations with Celyad have been started and will be continued after project conclusion.

Competition analysis

Currently, the treatments adopted for myocardial infraction consist of pharmaceutical therapy, medical device implants (e.g. stents), and organ transplants, all of which characterized by severe limitations such as thrombosis or stenosis of devices, high invasiveness and scarcity of donor organs, immune rejection, and prolonged hospitalization time.

AMCARE proposed an alternative approach to MI treatment by combining the concept of stem cells therapy for tissue repairing and injectable hydrogels as a promising solution for in situ cardiac tissue repair in infarcted hearts after MI. AMCARE has investigated the potential of a number of HA-based biomaterials platforms for the delivery of stem cells to the heart which would provide protection during delivery and cohesion at the infarct site post-delivery. Taking into account the objective of AMCARE, we can consider that the potential competitors deal with either biomaterials (e.g. HA-based hydrogel) or stem cell therapy.

BIOMATERIALS

Despite a variety of injectable biomaterials have been used for the in vivo delivery of stem cells to damaged myocardium as reported in Table 1, only few materials have progressed to clinical trials (Table 2).

Table 1: Selected injectable biomaterials for myocardial infarction

Material	Other therapeutic	Species	Injury model	Delivery method	Time of treatment after injury	Time of assessment	LV dimensions	Function (EF, FS)	Neovascularization	Other
Peptide nanofibers	BM-MNCs	Pig	Permanent occlusion of LAD	Direct epicardial injection	Immediate	4 wk		Increased systolic, diastolic dP/dt	Increased capillary density	Decreased infarct size, increased cell survival
Gelfoam	BM-MSCs or adenovirus	Pig	Embolization coil in LAD	Intrapericardial injection	2 days	1 wk				Cells engraft to infarct sit pericardial delivery is safe
Peptide nanofibers	VEGF	Pig	Permanent occlusion of LAD	Direct epicardial injection	Immediate	4 wk		Increased FS	Increased capillary and arteriole density	Decreased infarct size
UPy hydrogel	IGF-1 and HGF	Pig	Occlusion-reperfusion of LCx	Transendocardial injection	1 mo	1 mo	Decreased LVESV	Increased EF, FS	Increased capillary density (border zone)	Increased cardiac progenitor cell migration
HA	rTIMP-3	Pig	Permanent occlusion of LCx	Direct epicardial injection	Immediate	2 wk	Decreased LVEDd, LVEDV	Increased EF	Increased arteriole density	Decreased infarct size, increased wall thickness
Gelfoam	rPN	Pig	Embolization coil in LAD	Intrapericardial injection	2 days	1 or 12 wk			Increased capillary density (border zone)	CM proliferation in border zone
Fibrin-alginate composite		Pig	Permanent occlusion of LCx	Direct epicardial injection	7 days	7 days				Decreased infarct size, increased wall thickness
Alginate		Pig	Occlusion-reperfusion of LAD	Intracoronary infusion	4 days	60 days	Decreased LVEDA, LVESA			
HA		Sheep	Permanent occlusion of LAD	Direct epicardial injection	Immediate	2 and 8 wk				Decreased infarct size
Myocardial ECM hydrogel		Pig	Embolization coil in LAD	Transendocardial injection	2 wk	3 mo	Decreased LVEDV, LVESV	Increased EF, global wall motion index		Increased cardiac muscle at endocardium, decreased infarct fibrosis

Abbreviations: BM-MNCs, bone marrow-derived mononuclear cells; CM, cardiomyocyte; ECM, extracellular matrix; EF, ejection fraction; FS, fractional shortening; HA, hyaluronic acid; HGF, hepat factor; IGF-1, insulin-like growth factor-1; LAD, left anterior descending coronary artery; LCx, left circumflex artery; LV, left ventricular; LVEDA, LV end diastolic area; LVEDd, LV end diastolic diameter; L diastolic volume; LVESA, LV end systolic area; LVESV, LV end systolic volume; rPN, recombinant periostin peptide; rTIMP, recombinant tissue inhibitor of matrix metalloproteinase-3; UPy, ureidop VEGF, vascular endothelial growth factor.

Stem Cells Transl Med. 2014 Sep; 3(9): 1090–1099

Table 2: Clinical trials for injectable biomaterials in MI and PAD

Material	Product Name (Identifier #)	Trial Phase	MI/PAD	Study Design				Result
				Design	Control	Patient Population	Delivery	
Gelatin microspheres with bFGF	N/A	N/A	PAD	Nonrandomized	None	Patients with CLI, no option of medical or surgical treatment (7 total)	Single intramuscular injection (200 μg)	Significant improven 6-min wa distance, perfusion transcuta oxygen p and rest scale con with pre- treatmen
Alginate	Algisyl-LVR (NCT00847964)	I	MI	Nonrandomized	None	HF patients (9 total)	Intramyocardial injections during cardiac bypass surgery or valve replacement/ repair (9–15 injections, 0.25–0.35 ml each)	Improved LV and quali
	Algisyl-LVR (NCT01311791)	II (AUGMENT-HF)	MI	Randomized, single-blind	Standard medical therapy alone	HF patients, approximately one-half with previous MI (n = 78)	Intramyocardial injections via limited left thoracotomy (10–19 injections, 0.3 ml each)	Significant in in peak V and 6-mi test dista changes i end-diast diameter, end-syst diameter
Alginate	BL-1040 (NCT00557531)	I	MI	Nonrandomized	None	Experienced moderate to large MI, underwent successful primary PCI (n = 27)	Catheter-based intracoronary infusion (2 ml)	Preserved LV index, LV index, an
	IK-5001 (NCT01226563)	II (PRESERVATION I)	MI	Randomized, double-blind	Placebo (saline)	Experienced large MI, underwent successful primary PCI (n = 303)	Catheter-based intracoronary infusion (4 ml)	No differenc terms of index
Decellularized myocardial ECM hydrogel	VentriGel (NCT02305602)	I	MI	Nonrandomized	None	Experienced previous MI, 60 days to 3 years since event (18 patients projected)	Transendocardial delivery via MyoStar catheter	Ongoing

Basic to translational science, vol.2, n. 2, 2017, 212-216

Currently, only one product, VentriGel, entered the Phase I clinical trial (NCT02305602) which is a biomaterial scaffold designed specifically for the repair of damaged myocardium. It is injected via catheter in a minimally-invasive procedure that does not require surgery or general anesthesia. The first-in-class, off-the-shelf, injectable biomaterial scaffold is built on technology licensed from the University of California, San Diego.

This could allow to think that AMCARE is in the forefront of biomaterials used for cardiac repairing treatment suggesting that up to day, there are not competitors in this specific sector, with the exception of Ventrix Inc (VentriGel).

STEM CELL THERAPY

The stem cell therapy represents an emerging novel alternative treatment approach for repairing the damaged myocardium. Indeed, stem cell therapy using several types of stem cells such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), cardiac stem cells (CSCs), and endothelial progenitor cells (EPCs) has been tested making remarkable advances in clinical and basic research. The Table 1 of the review article "Promising Therapeutic Strategies for Mesenchymal Stem Cell-Based Cardiovascular Regeneration: From Cell Priming to Tissue Engineering" (Stem Cells International Volume 2017, https://doi.org/10.1155/2017/3945403) reports a summary of the clinical trials completed and still ongoing performed with mesenchymal stem cells which are frequently used to treat the most common cardiovascular diseases.

It has to be underlined that the majority of completed human clinical trials are difficult to clarify and compare because the delivered cells are either mixed or enriched populations, and the number of implanted cells, delivery methods, and injection time intervals after myocardial infraction are different going from one trial to another one. This uncertainty is reflected in a few products having already entered the market. Between them, the only two products for cardiac repair are:

- Cellgram®-AMI: stem cell drug improving the ejection fraction in patients with acute myocardial infarction reperfused by coronary angioplasty within 72 hours after expression of chest pain. This product is commercialised in the Korean market only.
- CardioRel: autologous cardiomyocytes for myocardial infraction. This product is commercialised in the indian market only.

The corresponding companies with their total revenues at the end of 2016 are reported in Table 3.

Table 3: Stem-cell products for cardiac repair available on the market

Company	Product	Disease	Total revenues (USD)
Pharmicell - www.pharmicell.com	Cellgram®-AMI	Acute Myocardial Infarction (AMI)	23,4 million
Reliance Life Sciences - www.rellife.com	CardioRel	Acute Myocardial Infarction (AMI)	8 million

Nevertheless, there is significant corporate investment in cell therapy for ischemic heart disease, and multiple companies are currently conducting clinical trials. Table 4 resumes the potential competitors; it is noteworthy to emphasis that any of these companies is marketing a stem cell-based product for AMI treatment.

Table 4: Potential competitors of AMCARE

Company	Product	Phase	Cell type	Indicat
Mesoblast	Mesenchymal precursor cells	Phase III (n = 1,700)	Mesenchymal precursor cells (allogeneic)	Congestive failure
Caladrius Biosciences	NBS10	Phase II (n = 160)	Bone marrow-derived CD34+ cells (autologous)	Acute m infarction

Cytori	Adipose-derived regenerative cells	Phase II (n = 31)	Adipose-derived regenerative cells (autologous)	Chronic heart failure due to ischemic heart disease
Vericel	Ixmyelocel-T	Phase IIb (sample size undisclosed)	Bone marrow-derived mesenchymal stromal cells, CD14+ monocytes, and macrophage (autologous)	Ischemic dilated cardiomyopathy
Athersys	MultiStem	Phase II (n = 150)	Bone marrow-derived multicellular cell population (allogeneic)	Acute myocardial infarction
Osiris Therapeutics	Prochymal	Phase II	Adult mesenchymal stem cells	Acute myocardial infarction
TCA Cellular Therapy	BB-IND 11643 (MESENDO)	Phase I	Autologous adult mesenchymal stem cells	Acute myocardial infarction
Garnet BioTherapeutics	GBT009	Phase I	Allogeneic bone marrow-derived stem cells	Myocardial infarction
Baxter	CD34+ stem cells	Phase II	Allogeneic adult bone marrow stem cells	Ischemic stroke

Stem Cells Transl Med. 2015 Aug; 4(8): 863–867

Exploitation strategy

The highest exploitable value of the assets developed by AMCARE is achieved through their use in combination as a **regenerative therapy that treats damage caused by acute myocardial infarction**. This higher value is justified by the increasing relevance of regenerative therapies and their potential in the target market.

RCSI, Adjucor, BSC, Contipro, EXPLORA, and INNOVA are investing in further developing the assets in combination to bring them to a pre-commercialisation level. Although Celyad is not interested in pursuing its research on the CPC cells, they are in favor of licensing to a third-party.

In practice, the three components of the final therapy are:

a. Device (e.g. SPREDS, Viscath, C-cathGEL)
b. Carrier (e.g. HA-TA gel)
c. Therapeutic agent (e.g CPC gel)

A possible exploitation strategy would be for the interested partners to cooperate (e.g. under a follow-up call) to finalise pre-clinical studies, GMP production and obtain clinical authorisation for all three the components to arrive at First in Man. A notable exception would be the CPC-cells produced by Celyad, as those are already produce at GMP standards. The clinical studies could be carried out by a spin-out company managing the IP. Possible exit strategies via licensing to an external company (e.g. Medtronic, Edwards Lifesciences) could be envisioned before commercialisation, but likely after Clinical Phase I has been completed. That would require at least 4,5 millions € of additional investments that could be covered with another EU grant.

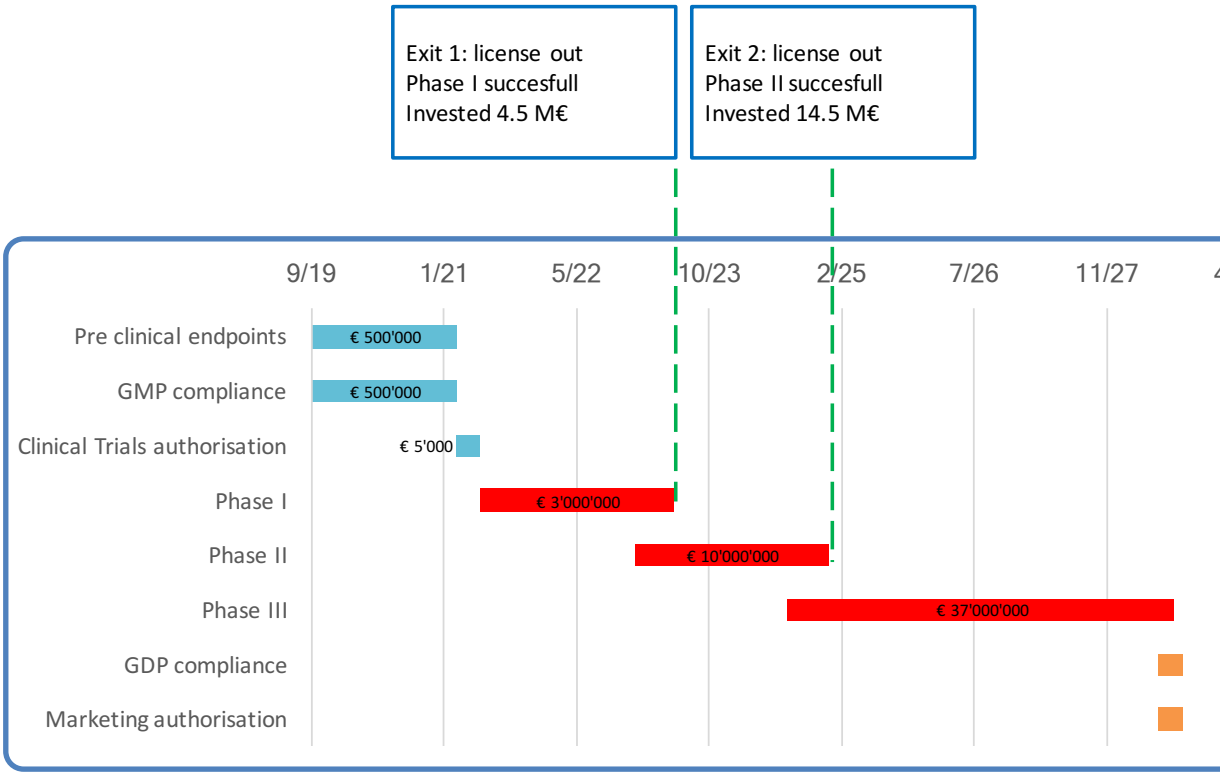


Figure 28: Pathway to commercialisation. Investment costs for clinical trials are based on: Sertkaya et. al Key cost drivers of pharmaceutical clinical trials in the United States", increased by factor 0.5 to adjust for ATMP

Dissemination activities

Several dissemination activities have been carried out by the Consortium:

1. A project website (www.amcare.eu) has been developed at the beginning of the project. It contains public information about the project, including news and events, and a password protected section for sharing data among the project partners.
2. dedicated pages were created by Innova in 2 social networks (Linkedin and Facebook) to reach a wide audience and achieve a widespread awareness of project outcomes and expected results
3. The results of the scientific research work have been submitted for publication to international, peer-reviewed high-level scientific journals
4. Partners in the consortium disseminated the project through their internal bulletins, secure intranet and by presentations at internal and external meetings.
5. Partners presented the results at several international conferences and workshops.
6. A leaflet has been designed and used in conferences and international events

Linking AMCARE to society

The use of stem cells and nanotechnology have evoked a public debate about their ethical dimension (Eberhardt-Metzger et al., 2003; Mieth, 2007, Nordgen, 1999; Rehmann-Sutter et al., 2003; Walters et al. 1999). In order to link AMCARE to society through science-society dialogues on chances, risks and ethical aspects of AMCARE, AMCARE held a **Patient Panel** for people suffering with cardiovascular disease in the **Croi** headquarters in Galway on the

19th October 2016. It was a very successful evening with a group of insightful and knowledgeable participants who gave great insight into the needs and desires of those who have experienced a heart attack.

Training

Training sessions and exchanges of researchers involved in AMCARE have taken place on several occasions throughout the 48 months. INNOVA delivered 4 training workshops about:

1. Technology transfer and innovation management
2. Effective scientific communication
3. “the main instruments of the Horizon 2020 programme” and “How to build a successful proposal for Horizon 2020”.
4. routes for a successful exploitation of AMCARE’s results.

Moreover, the AMCARE project in association with sister EU projects DRIVE and NEXT organised a Winter School training event: "Translational Reality of Advanced Therapy Medicinal Products (ATMPs)" in the Galway Bay Hotel from November 28th – December 1st 2016. All three projects are focused on the development of ATMPs (cell therapy products).

The training at the Winter School covered all the steps involved in the translation of ATMPs from the lab to the marketplace including: strategic engagement with industry, IP protection, GMP-compliant stem cell manufacture, preclinical validation of medical devices and clinical validation of cell therapy products, attracting venture capital and business planning and navigating the regulatory environment for ATMPs.

Invited speakers included experts from the AMCARE and DRIVE Consortium partners, collaborators from the National University of Ireland Galway (NUIG) and regulators from the Irish Health Products Regulatory Authority (HPRA). Attendees also visited the Centre for Cell Manufacturing Ireland (CCMI) at NUIG, Ireland's only GMP-compliant stem cell manufacturing facility, and Boston Scientific Ltd.'s ISO 14001-certified medical device research and manufacturing site in Ballybrit, Galway, the largest manufacturing facility in Boston Scientific's global network.