Supplementary Information

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Supplementary Methods

GelMA Cryogel Preparation

Methacrylated gelatin cryogel (GelMA) was synthesized as previously described[1]. Pendant methacrylate groups were added primarily to free amines of gelatin by reaction with methacrylic anhydride Type A porcine skin gelatin (Sigma) at 10% (w/v) was dissolved in stirred Dulbeccos phosphate buffered saline (DPBS; GIBCO) at 50°C for 1 hour. Methacrylic anhydride (Sigma) was added dropwise to a final volume ratio of 1:4 methacrylic anhydride:gelatin solution. This resulted in GelMA with a degree of substitution of 79%. The solution was stirred at 50°C for 1hour, and then diluted 5x with DPBS. The resulting mixture was dialyzed in 12-14 kDa molecular weight cut-off tubing (Spectrum Labs) for 4 days against distilled water (dH2O) with frequent water replacement. The dialyzed solution was lyophilized, and the resulting GelMA was stored at -20ºC until use. The remainder of the preparation steps were conducted in aseptic conditions with sterile reagents. Free radical polymerization results in crosslinking of gelatin molecules, and freezing of GelMA in the presence of radical initiators allows polymerization to occur in the partially frozen state (cryopolymerization). Cylindrical acrylic molds (7.5mm diameter, 1.25mm thick for simplified Therepi) or (3mm diameter, 1.25mm thick for Therepi) were placed in a freezer at -20°, with direct shelf contact. The lyophilized GelMA was warmed to room temperature. A 137mg/ml ammonium persulfate solution (APS; Bio-Rad) was prepared, and protected from light. A 1% gelatin solution was prepared and placed on a hot plate at 40ºC, 500 rpm for 30 minutes, then stored in a 4ºC refrigerator for 20 minutes. 4ul tetramethylethylenediamine (TEMED; Bio-Rad) was added to the gelatin solution for every ml of solution, and stirred. The solution was placed in the fridge for three minutes, and then 36µl of APS was added to the gelatin for every ml of solution and stirred. The gelatin solution was pipetted into molds, filling to excess. An acrylic plate was placed over the mold, ensuring that all air was removed, and the assembly was clamped together with binder clips, and stored for seventeen hours at -20ºC. Ice crystals formed during the freezing and thawing after cryopolymerization resulted in the formation of a hydrogel with micron-scale pores. The gels were removed by displacing them from molds with a 7.5mm or a 4mm punch once semi-thawed (30 seconds-1 minute after removing from freezer), and they were stored in distilled sterile water until use.

Therepi manufacture

The fabrication of the *Therepi* device is described in Supplementary Figure S1 and the following text.

Formation of the polyurethane backbone

Thermoplastic polyurethane (TPU) was used as an impermeable membrane (HTM 8001-M polyether film, 0.003inch thick, American Polyfilm, Inc.). A sheet of TPU was mounted in a vacuum thermal former (Yescom Dental Vacuum Former, Generic). A 3-d printed hemispherical mold (Objet Connex 500) with a height of 3.9mm and a diameter of 3.5mm was used as the positive mold. The positive mold was placed on the vacuum platform and the sheet was heated until it was completely smooth. The heated TPU sheet was then lowered over the positive mold. Vacuum was simultaneously applied to the platform to ensure the sheet fully conformed to the shape of the mold. The formed TPU shape was then placed in a corresponding negative mold. A Teflon strip was inserted into grove of the negative mold to keep the channel open during the proceeding heat-sealing steps. A laser cut TPU window was placed on top and the assembly was heat-sealed with a heat transfer machine (Heat transfer machine QXAi, Powerpress) for 4 seconds at 330ºF (Figure 1.4E).

Impulse sealing of the semipermeable membrane

The formed TPU was taken out of the negative mold and aligned in an impulse heat sealer (H-2065 Deluxe Impulse Sealer, Uline). A circle of polycarbonate membrane (Cyclopore Track-Etched Membrane Filter, 13mm Diameter, 0.4 Micron, Whatman) was placed under the assembly and heat-sealed in place. This was repeated for two other sides of the device, leaving one side open for biomaterial insertion in a later step.

Thermo-sealing of the catheter

The laser cut teflon strip was removed from the part. Excess material was trimmed form the device with a fine scissors (Vannas Tübingen Spring Scissors, Harvard Apparatus) (Figure 1.4H). A 10cm, 3Fr, thermoplastic polyurethane catheter (Micro-Renathane® .040" x .023, Braintree scientific) was inserted into the formed channel. A PTFE coated mandrel (0.02196 inch, type 304 S.S/54076, Applied Plastics) was inserted into the catheter to retain patency during the proceeding heal sealing step. Heat shrink tubing (RNF-100-3/64-O-STK, TE Connectivity) was positioned at the unsealed interface between the catheter and the device. The part was inserted into an aluminum block containing a 2.5 mm diameter hole and the assembly was heated to 370ºF for 120 seconds with a heat press (Heat transfer machine QXAi, Powerpress). The part was removed from the aluminum block and the heat shrink tubing was cut away with a Vannas Tübingen Spring Scissors (Harvard Apparatus). After removal of the teflon coated mandrel, the device sent for ETO sterilization (low temperature ethylene-oxide cycle). The remaining steps were conducted in an aseptic environment in a class II laminar flow hood

Biomaterial insertion
A 2mm cylinder cut from a gel foam sponge (Gelfoam Hemostatic, Size 12-7mm, Pfizer Pharmaceutical) using a disposable biopsy punch (Integra Miltex). The final polycarbonate-TPU opening was sealed using an impulse heat sealer (H-2065 Deluxe Impulse Sealer, Uline).

In vitro studies

Cell transfection
For all studies using the IVIS Spectrum Xenogen 5000 (In Vivo Imaging System, Perkin Elmer), clonally derived mesenchymal stem cells from BALB/c mice (D1 ORL UVA [D1] (ATCC® CRL-12424™) were obtained from American Type Culture Collection (ATCC). ATCC cell lines are subjected to comprehensive and repeated authentication and contamination checks including short tandem repeat (STR) profiling, cell morphology, karyotyping and cytochrome C oxidase I (COI) testing. Testing was also completed to ensure that cell cultures were free of mycoplasma or other bacterial or fungal agents, conforming to the mycoplasma-testing stipulations recommended by the FDA "Points to Consider" protocol. To introduce firefly luciferase to these cells, lentiviral particles were produced containing a vector with mCherry-IRES-Firefly Luciferase driven by the CMV promoter by transfecting the plasmids in HEK-293T cells. The viral particles were then concentrated by centrifugation through an Amicon filter with a 3kDa cut-off. The viral titer was determined by evaluating mCherry signals after transducing MSCs with different concentrations of the particles. MSCs were then incubated with viral particles for 2 days. Cells expressing mCherry were then sorted via flow-activated cell sorting. Gaussia Luciferase expressing mouse MSC's were produced using the same approach.

Cell preparation and biomaterial seeding
Mesenchymal stem cells were transfected with Firefly Luciferase or Gaussia Luciferase as described above. Luciferase-expressing mouse mesenchymal stem cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10% (v/v) bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco) at 37°C in a 5% CO_2 atmosphere. Cells were harvested using Trypsin (Sigma).

For GelMA seeding, cells were resuspended at ~10^6 cells/12.5µl in complete medium. GelMA discs were dehydrated by wicking with a Kimwipe, and rehydrated with 12.5µl of cell suspension (10^6 cells) in a 24-well plate. Well plates were incubated at 37°C for 60 minutes to allow for cell attachment then complete media was added and the gels were cultured overnight before implantation onto the heart as part of the simplified/direct delivery system or insertion into the *Therepi* reservoir.

Gelfoam® was inserted into *Therepi* devices as a dry cell free material and was subsequently seeded through the *Therepi* refill port. 1 million F-luc cells or 0.5 million G-luc+0.5 million F-luc cells were suspended in 30µl of media and injected through the refill line into the reservoir. 20µl of media was injected in order to ensure entirety of cell solution volume reached the reservoir, and refill line was free of cells. Seeded *Therepi* devices were cultured in 10 ml of media, which was changed at suitable intervals.

Bioluminescence measurements
To measure bioluminescence of F-luc cells *in vitro*, D-luciferin (XenoLight D-Luciferin Potassium Salt**,** Perkin Elmer) at 15mg/ml was added to each tissue culture flask. Flasks or animals were imaged in an IVIS Spectrum Xenogen 5000 (In Vivo Imaging System, Perkin Elmer). A sequence was acquired with the following settings; luminescent automatic exposure with a target count of 3,000 and a field-of-view set to C or D (automatic exposure preference range values were set to the following; exposure time: 0.5-170 seconds, binning: 1-8, F-stop:1). The sequence was ended once the signal had reached a plateau or was seen to drop. The highest value was used for the data. The automatic circular region of interest (ROI) tool from Living Image software was used to calculate the total flux of light in areas of interest (photons/sec).

In vitro G-luciferase bioluminescence was measured by transferring 100µl of media from the tissue culture flask to a black 96 well plate. 100 µl of imaging substrate coelenterazine (Rediject, PerkinElmer) at a concentration of 20 µg/ml was auto mixed with samples and imaged using a Spectramax L microplate reader. An integration time of 10 seconds was used. Each biological sample was measured in triplicate and readings were averaged. A cumulative concentration reading was recorded at days 1, 4, 7, 10, 14, 28 while accounting for a G-luc half-life of 6 days[2].

In vivo studies
Animal preparation and endotracheal intubation
Endotracheal intubation was required to mechanically ventilate the rat. This is described in Supplementary Figure S2 and the following text. A chamber was used for induction of anaesthesia and maintenance with a nose cone was used to maintain anaesthesia during preparation for surgery. The hair between the shoulder blades and on the left

side of the chest was removed with an electric razor and hair removal cream. Pre-operative analgesics were administered: buprenorphine (0.05mg/kg IP) and Lidocaine HLC 2% injection USP (100 microliter per injection site, SC). A tilting stand (Hallowell EMC, 000A3467) was used to position the animal for intubation. The arms were taped to the supports on the stand, and an elastic band with Velcro was placed around the two front teeth to hold the animal in place. The animal was tilted at a 45º angle and the glottis area was trans-illuminated with a spotlight placed close to the neck. The tongue was retracted to visualize the opening and closing of the larynx, just above the esophagus. Lidocaine (10µl of 1%) on tip of intubation tube was used to prevent laryngospasm. A forceps could be inserted into the mouth once the tongue was retracted to keep it open while a 16G, 2.5" catheter (BD angiocath) was inserted into the trachea, timed with the opening of the larynx. Intubation was verified with a dental mirror placed at the end of the catheter. Condensation from breathing was observed at the end of the catheter if correctly placed. The mechanical ventilator (SAR 830P, Harvard Apparatus) was then connected to the catheter via a luer connector on the end of an extension line on the ventilator output, and the chest of the animal was observed to ensure that the lungs were inflating and deflating in time with the ventilator. Settings were adjusted to volume control, flow rate of 200-500cc/min, with an inspiratory time of 0.55 seconds, a respiratory rate of 75 breaths per minute. Pressure was maintained between 12-14cm H_20 on inhalation.

Subcutaneous catheter tunneling procedure

Once the animal was intubated, the intubation catheter was tied in place using umbilical tape, and the animal was placed on a sterile drape over a heating pad for the surgery. An indwelling catheter was then tunnelled subcutaneously from a dorsal site between the shoulder blades of the animal to a ventral exit site close to the left fourth intercostal space. This is described in Supplementary Figure S2 and the following text. Briefly, both the dorsal and ventral surgical sites were washed with a 2-step iodine surgical scrub preparation. A small incision was made in the dorsal skin, in between the shoulder blades, and an incision was made at the ventral site, at the area of the fourth intercostal space (usually about the level of the third superior nipple). A forceps was used to tunnel subcutaneously from the dorsal to the ventral site. The forceps was directed through the incision, to grasp a 3Fr catheter (C30PU-RJV1303, Instech Laboratories) for the simplified *Therepi* procedure, or the fully formed *Therepi* device for the *Therepi* procedure, and retract it through the tunnel.

Thoracotomy and myocardial infarction

The animal was turned to its front, the chest was opened, pericardium was removed, and the left anterior descending artery was permanently ligated to induce a myocardial infarction. This is described in Supplementary figure S3 and the following text. A thoracotomy was performed by making a small incision parallel to the ribs at the 4th intercostal space, taking care not to cut the catheter at its ventral exit point. The intercostal fascia and muscle was opened using a blunt dissection technique, keeping the instruments parallel to the ribs and taking care to keep the ribs intact. A small chest retractor (Fine Science Tools, 17008-07) was used to spread the ribs and visualize the underlying lungs and heart. The left lung was retracted with a sterile cotton-tipped bud. The pericardium was removed with two fine curved forceps (Fine Science Tools 91197-00). A guide suture (6-0 prolene monofilament, Ethicon 8711) was then placed into the myocardium towards the apex using a spring-loaded needle driver (Castreviejo needle holder, Fine Science Tools 1265-14). Using the guide suture, the heart could be manipulated into position and held there using a hemostat (Fine Science Tools, 91308-12). For the hemodynamic study, rats underwent myocardial infarction as previously described [3]. The left anterior descending artery was permanently ligated with a suture (6-0 prolene, Ethicon 8711) approximately one third of the way from the apex to the base of the heart. Myocardial blanching was apparent after ligation of the LAD, confirming infarction.

Simplified Therepi (direct) placement

The simplified *Therepi* system, consisting of the catheter alone and a methacrylated gelatin cryogel was then attached to the heart. This is described in Supplementary Figure S3 and the following text. The catheter was positioned by retracting it at the dorsal opening until just enough of the length remained ventrally to be attached to the epicardium. A suture (6-0 prolene, Ethicon 8711) was brought through the tip of the catheter first, and then through the epicardium (on the left ventricle, close to the apex) before securing in place. Then the GelMA cryogel was placed on the heart by first placing a suture through the myocardium, just distal to the end of the catheter. The same suture was then placed through the GelMA and used as a guide to lower the GelMA cryogel on to the surface of heart so that it sat over the end of the catheter. The GelMA was then secured in place with a single suture loop and double knot, ensuring not to tear the GelMA. The guide suture was then removed, and the heart was gently guided back into the thoracic cavity.

Therepi placement

In lieu of attaching the cryogel and catheter separately to the ventricle, the encapsulated *Therepi* reservoir was secured to the ventricle of the heart at three suture points (6-0 prolene, Ethicon 8711) as shown in Supplementary Figure S3.

Thoracotomy closure
The thoracic cavity was closed, and negative pressure was re-established. This is described in Supplementary Figure S3 and the following text. The muscle layers were closed with interrupted sutures, leaving a small opening for the subsequent evacuation step (4-0 coated vicryl polyglactin 910 braided suture, Ethicon J310). A perforated tube connected to a 30cc syringe was placed into the thoracic cavity through a small opening in the muscle layers, and a vacuum was applied while the skin was sealed with fingertips to inflate the lungs and re-establish negative thoracic cavity pressure. A hemostat clamp was immediately placed on the skin, maintaining the airtight seal, and sutures (Ethicon J310) or wound clips (Reflex 9mm) were used to close the wound.

Subcutaneous port placement
Finally, the refill port was placed subcutaneously between the shoulder blades of the rat. This is described in Supplementary Figure S4 and the following text. The animal was carefully moved from the supine to a prone position. The catheter was then trimmed so that 2-3cm of catheter exited the dorsal site. Subcutaneous space sufficient for the vascular access button was then created using forceps. The vascular access button (VAB62BS/22, Instech Laboratories) was then attached and inserted into the subcutaneous pocket, ensuring that the catheter did not kink, but took gradual curves to allow slack for movement. The felt was secured to the underlying fascia using at least two interrupted sutures (Ethicon J310) taking care not to go through the catheter. The skin was then closed with four to five interrupted sutures (Ethicon J310). Finally, the isoflurane was turned off and the animal was ventilated with 100% oxygen on a heated pad until it started breathing on its own. The intubation catheter was then removed. 3ml of warm saline was administered subcutaneously and buprenorphine [0.05mg/kg in 50µl intraperitoneally (IP)] was given every twelve hours for three days post-operatively.

In vivo refills of cells and delivery of small and macromolecules
Cell refills were conducted through the port with 50µl of cell suspension at a concentration of $20x10^6$ cells/ml of complete media injected through the port of each animal with a 100µl microsyringe and a PNP-3M injector (Instech Laboratories). The line was then flushed with media to account for the dead volume for each system so that a total of 50µl of cell suspension reached the gel. Small molecules and macromolecules were delivered in the same manner ensuring 50µl of D-luciferin (at 15mg/ml concentration) or 100µl of BSA fluorescently labeled with Vivotag 800 reached the gel. For the IP groups either 150mg/kg of D-luciferin (at 15mg/ml concentration) or 100 µl of BSA/Vivotag 800 was injected IP. Fluorescently labeled BSA was prepared by incubating 3mg/ml of BSA with 100 microgram of Vivotag 800 (Perkin Elmer) for one hour. The non-reacted fluorophore was then separated using size exclusion chromatography (BioSpin P-6, Bio-Rad).

In vivo bioluminescence and fluorescence measurements
For the cell refill studies, animals were imaged on day of surgery (day 1), and at multiple days out to day fourteen for Therepi. On day four the refill group was replenished with cells, and imaged two hours before refill and four hours after refill. Animals were anesthetized with 3% isoflurane in oxygen. D-luciferin (XenoLight D-Luciferin Potassium Salt, Perkin Elmer) at 15mg/ml in complete media was injected through the port of each animal with a 200ul microsyringe and a PNP 3M injector accounting for dead volume so that 50ul of D-luciferin reaching the gel. An IVIS Spectrum Xenogen 5000 (In Vivo Imaging System, Perkin Elmer) was used for imaging. Animals were maintained under anesthesia with 3% isoflurane delivered through a nose cone during imaging. A sequence was acquired with the following settings; luminescent automatic exposure with a target count of 3,000 and a field-of-view set to D (automatic exposure preference range values were set to the following; exposure time: 0.5-170 seconds, binning: 1-8, F-stop:1, field-of-view D, no delay between images). The sequence was terminated once the signal was seen to drop. For the fluorescence images (macromolecule delivery) one image was taken for each timepoint at the following settings; automatic exposure, emission 820nm, excitation 745nm (exposure 0.5 seconds for direct injection or 120 seconds for IP injection, binning 8, field of view D, f stop 2). The automatic circular region of interest tool was used to calculate the total flux or radiance at the heart.

28-day pre-clinical study
Animals
Female Sprague Dawley rats (225-250g) were used for the study.

Transplanted cells preparation
For the 28-day study primary mesenchymal stem cells derived from Sprague Dawley rats were purchased from Cyagen (RASMX-01001). As per manufacturers data, they tested negative for bacteria, fungi, and mycoplasma. Cells at passage 5-7 were used in all groups for the study. Devices were seeded with $0.5x10^6$ cells (as described in Supplementary Movie S3), and cultured with AlphaMem/Glutamax (Gibco 32561037) supplemented with 10% (v/v) bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco) at 37°C in a 5% CO_2 atmosphere. for 3 days. Devices were then transferred to a serum free media, StemPro MSC SFM (Gibco), supplemented with Glutamax (Gibco), 24 hours prior to device implantation.

Intramyocardial Cell Injection
0.5 million cells were suspended in 50µl of StemPro MSC SFM (Gibco) 30 minutes prior to surgery, and stored in ice. 5 separate injection of 10µl were made at the border zone of the infarct. Cells were cultured in AlphaMem/Glutamax (Gibco 32561037) supplemented with 10% (v/v) bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco) at 37°C in a 5% CO_2 atmosphere for 7 days prior to injection.

Cell Seeding/ Cell Refills
Cell refills were performed on day 4,14 of the 28-day pre-clinical study. Negative pressure was applied to the *Therepi* system, using a syringe, to clear the line. 500,000 rat MSC's suspended in 30µl of serum free StemPro media was injected through the refill line into the reservoir. 20µl of StemPro media (Gibco) was injected to clear the refill line of cells

Functional hemodynamic measurements with Ultrasound
Hemodynamic measurements with echo ultrasound were performed as previously described using a Vevo 2100 Ultrasound machine and a MS200 transducer probe (9-18Mhz)[4]. B-mode measurements were taken using the parasternal long axis view of the heart. M-mode measurements were taken using the parasternal short axis view of the heart, at mid ventricle, with papillary muscles evident. Data was collected and analyzed using the Vevolab software (Visualsonics).

Functional hemodynamic measurements with PV catheter
Hemodynamic measurements were carried out using the apical stick method as previously described[5], using the Powerlab 8/35 and LabChart Pro 8 software with the PV module. Pressure and volume were measured using a conductance catheter (SPR-838) in the left ventricle. An average of at least ten cardiac cycles was taken post-calibration for haemodynamic measurments in LabChart Pro software. Volume calibration was determined by inputting max diastolic and systolic volume obtained from ultrasound measurements, as per manufacturer's instructions (PV loop module, Labchart). LabChart Pro 8 (AD Instruments) was also used for PV data analysis.

Histology
Hearts were arrested in diastole by direct injection of 2-3ml of 1% KCl solution. Hearts were fixed in a 10% neutral buffered formalin solution for 24h. Tissues were then transferred to PBS and stored at 4°C until paraffin embedding. A transverse cut of the heart was made at the device/heart interface. Slices were stained for Hematoxylin & Eosin and analyzed by a practicing cardiac/medical device pathologist.

Epinephrine delivery and demonstration of functional effect by pressure monitoring
50 µL of epinephrine at a concentration of 1mg/ml (Pfizer) was injected either into the intraperitoneal space or directly into the device's delivery port and changes in blood pressure were monitored. Briefly, either healthy animals or animals with an MI and an implanted device (28 days prior to the experiment) were intubated and anesthetized. A terminal thoracotomy was performed and a PV catheter (Millar SPR-838) was inserted into the left ventricular space via apical stick. After verifying levels of systolic blood pressure were in a physiological range, epinephrine was injected into the intraperitoneal space of healthy animals (n=3) or directly into the device of the animals (n=3). Pressure changes were recorded for up to 2 minutes post-epinephrine injection using the PV catheter (SPR383), Powerlab data acquisition and LabChart 8 software (AD instruments).

Computed tomography imaging for distribution into tissue
An infarcted heart with an acellular *Therepi* device was explanted from a Sprague Dawley rat 26 days after myocardial infarction and *Therepi* implantation. Prior to scanning, a contrast agent phosphomolybdic acid (PMA) at a concentration of 12.5%w/w was used to simulate macromolecule diffusion from the *Therepi* into the *ex vivo* cardiac tissue through the surrounding fibrous capsule. 70µl of PMA was injected into the device and the entire assembly was placed in a humidity chamber. 12 h post PMA injection, a micro computed tomography (µCT) scan of the heart and the attached device was conducted using an XRA-002 X-Tek µCT system. 3D reconstructions were performed using CT-Pro (Nikon Metrology) and the surface renderings were generated using VGStudio Max.

Diffusion chamber for transport of macromolecules across a fibrous capsule
Heart tissue with attached *Therepi* was explanted at various times after placement. Real time measurement of diffusive transport of FITC-dextran through left ventricle tissue with attached polycarbonate membrane from explanted hearts was measured using a diffusion chamber consisting of two compartments, each with a volume of 2 mL. Heart tissue with attached polycarbonate membrane was clamped, using O rings, between the two compartments of the diffusion chamber. The total exposed tissue area for transport was 0.1257 cm^2. Each compartment was filled with 2 mL of Dulbecco's Phosphate Buffered Saline supplemented with protease inhibitors. At starting time t=0, FITC-dextran of molecular weight 40 kDa or 10 kDa (Sigma-Aldrich) was added to the 'upstream' compartment such that the final upstream concentration of the FITC-dextran was 50 M, allowing for diffusion from the upstream compartment through the tissue into the downstream compartment. The fluorescence at the downstream compartment was continuously measured using a custom fluorescence reader. The baths in both

compartments were magnetically stirred to minimize the effects of concentration variations within the compartments. The data from the real-time measurement of diffusive transport was fitted to the solution of the one-dimensional transient diffusion equation with constant concentration boundary conditions[1] using MATLAB and a diffusion time constant was determined. A partition coefficient of one was assumed.

Penetration of albumin across a fibrous capsule

An acellular *Therepi* device was placed in an MI model Sprague Dawley rat for 26 days. 50 microliters of 1mg/ml cyanine (Cy7) labelled bovine serum albumin was injected through *Therepi*, following heart explantation. Following 24 hours in a humidity chamber, the heart was imaged using epifluorescent microscopy, consisting of a Xenon lamp, Axio Zoom V16 microscope, and a Hamamatsu Flash 4.0 v3.

Statistical Analysis

A pilot study was performed to determine the mean and standard deviation in bioluminescent measurements for both conditions. This data was used to determine an adequate sample size (n=5) for a sufficiently powered study to determine significance of differences between the pre-refill and post-refill groups. The following exclusion criteria were pre-established; cells in the simplified *Therepi* and *Therepi* systems were assessed for viability *in vitro* prior to surgery by measuring their bioluminescence with the IVIS spectrum. Devices with bioluminescence less than 10^9 photons/second were excluded. Male rats and rats below 225g or above 250g were excluded. The following rats were also excluded; animals that did not survive the MI surgery, animals where the integrity of the device or the subcutaneous line or port was damaged, animals that did not display blanching of the left ventricle on LAD ligation, or animals that had an ejection fraction greater than 60% as assessed by echocardiography at day 0. For the PV loop data, measurement points that did not show a consistent PV loop due to catheter positioning or other reasons were excluded. No formal randomization was used but surgeries were carried out on groups alternately on each day of surgery. No blinding was used but all measurements were quantitative and non-subjective. Normal distribution was tested for each group using a histogram, and similar variances were checked for groups being compared. Graphpad Prism was used for statistical analysis. For normally distributed data a two-sided, unpaired t-test was used for comparing between two groups with an alpha level of 0.05. A one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-hoc test was used for studies with more than two groups, with an alpha level of 0.05.

Supplementary References

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Supplementary Movies

Movie S1 Device manufacturing process

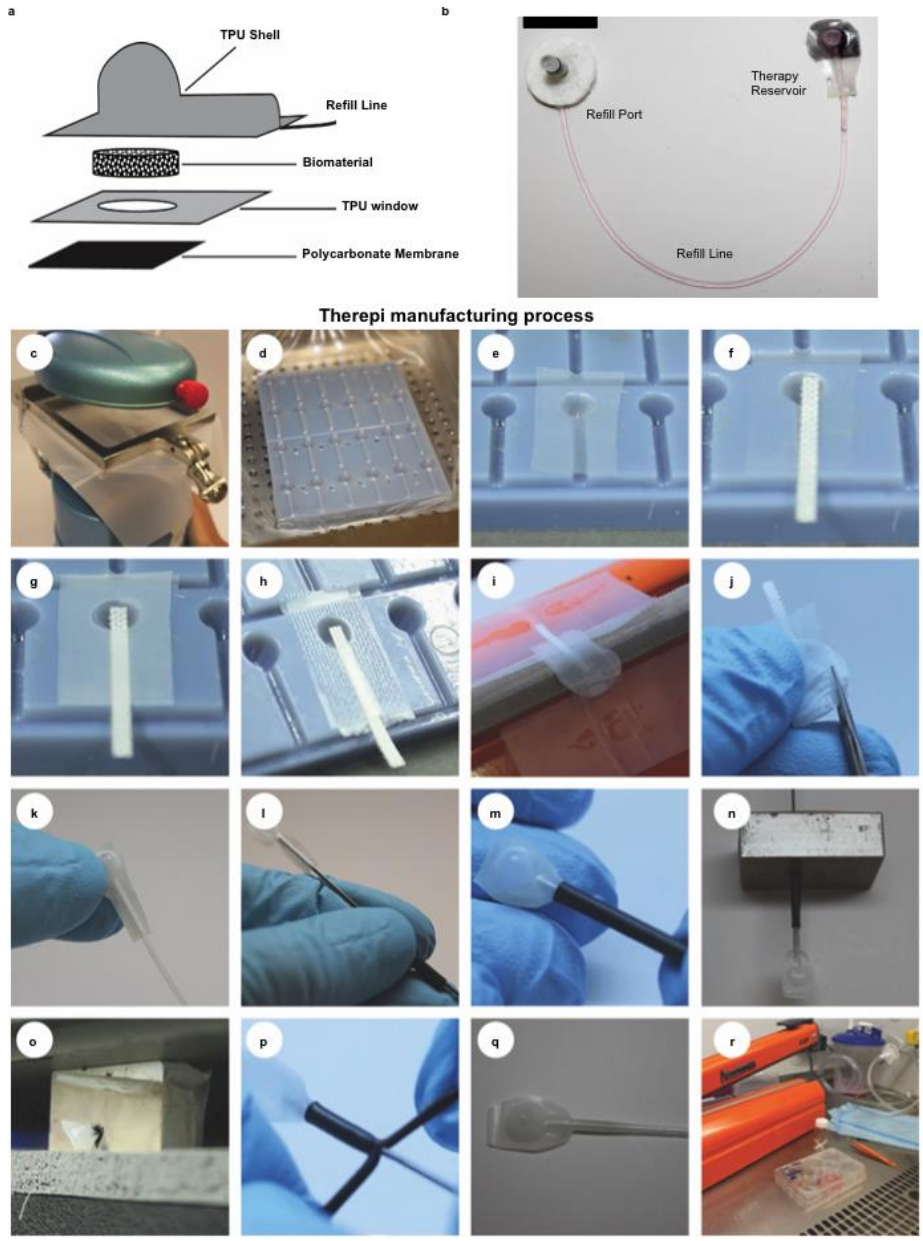
Movie S2 Minimally invasive delivery

Movie S3 Refill of therapy

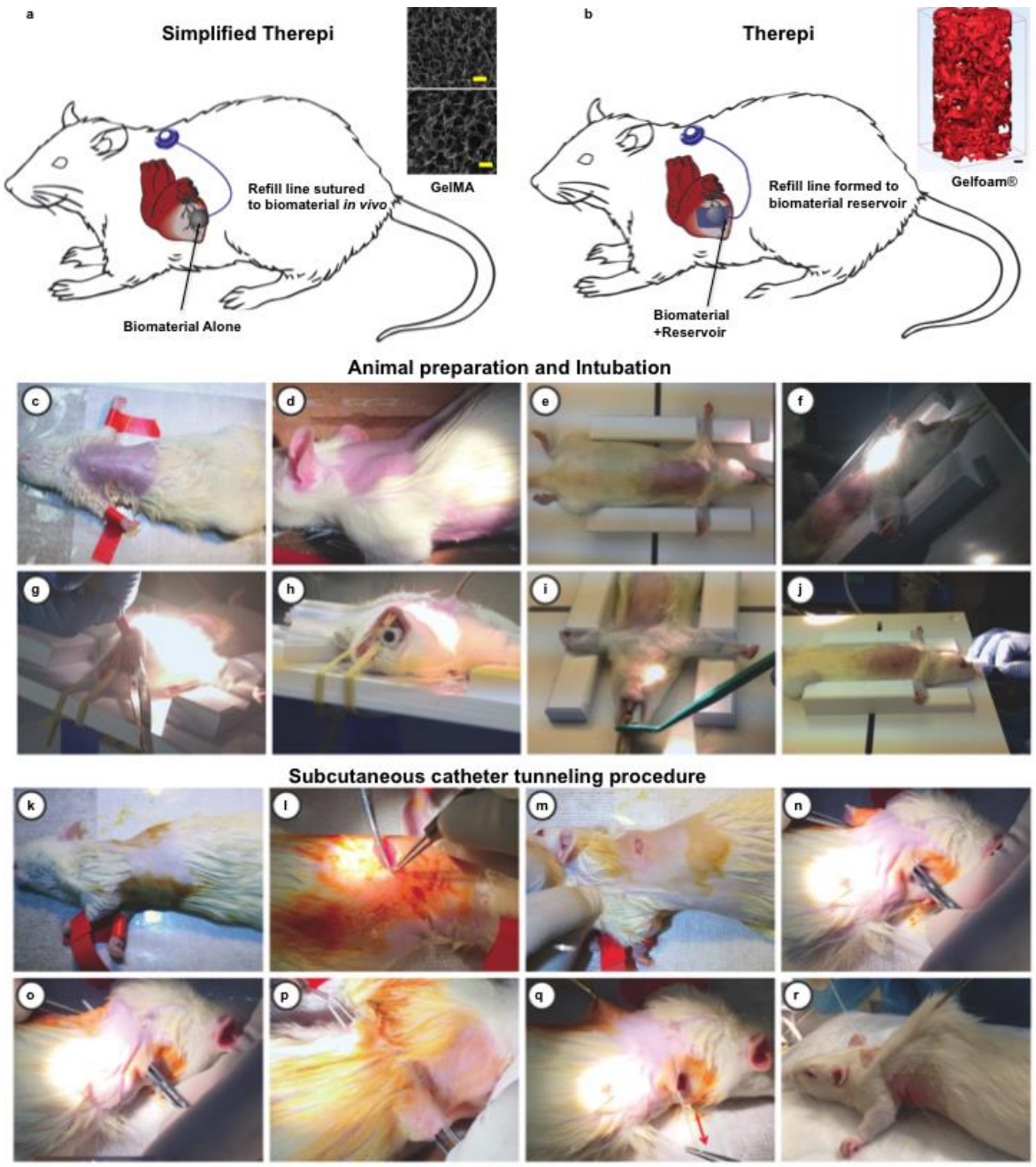
Movie S4 Overview of surgery.

Movie S5 Overview of the terminal PV surgery

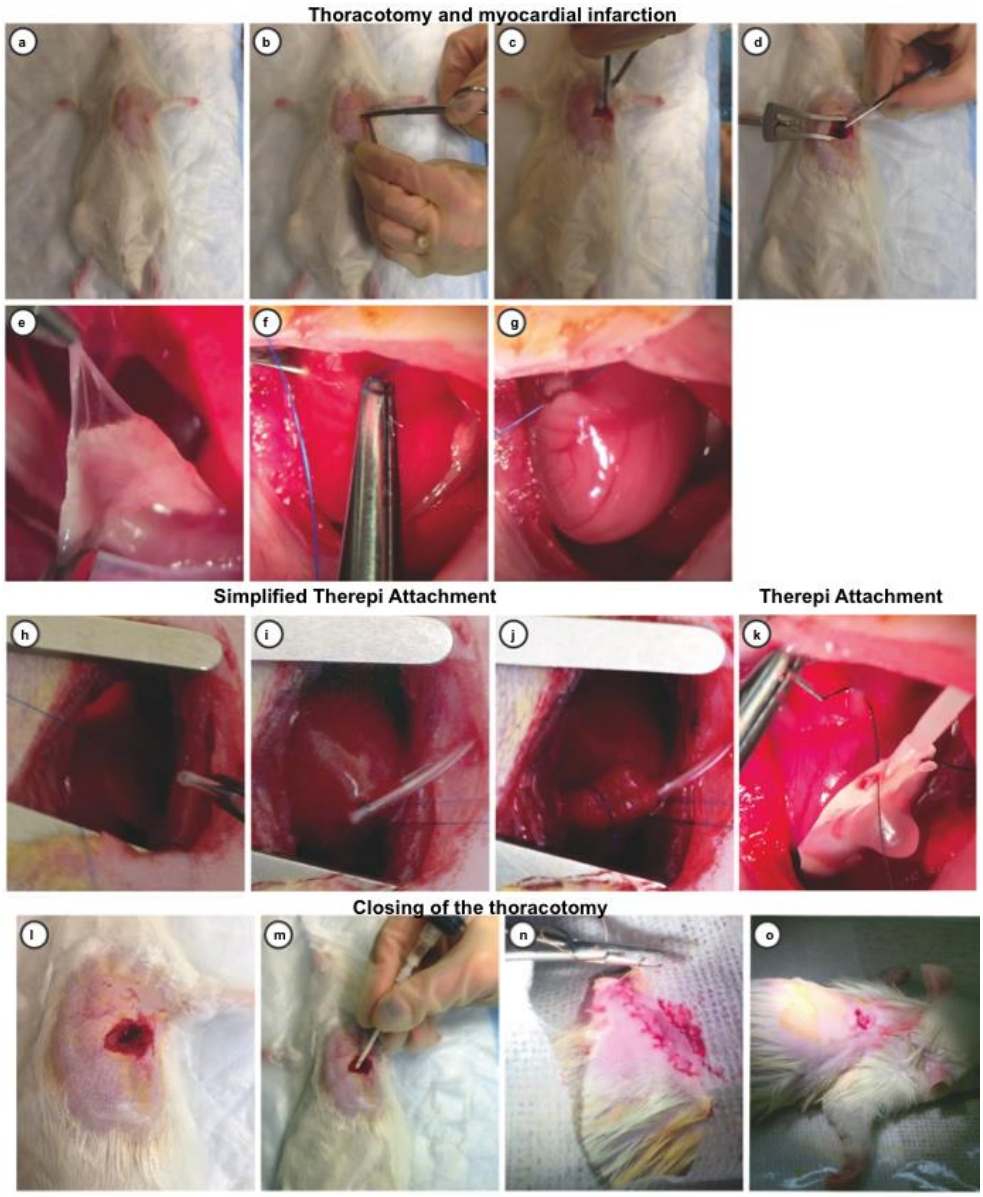
Supplementary Figures



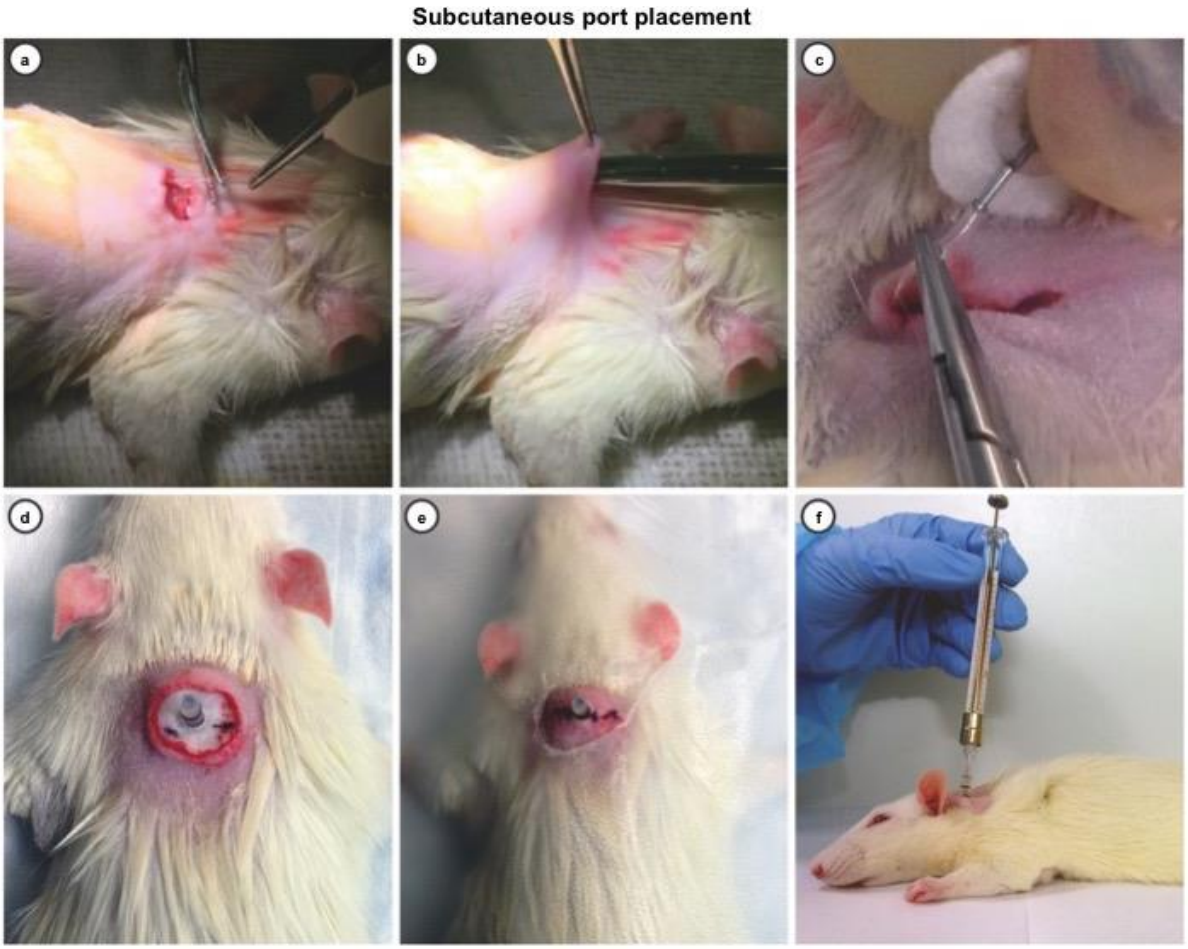
Supplementary Figure S1: Therepi components and fabrication. a) An exploded view of the layers of the *Therepi* reservoir b) The entire *Therepi* system showing refill line, refill port and reservoir c) A thermo-plastic urethane sheet is mounted in a thermal former with a vacuum platform and a positive 3-D printed mould was placed on the platform d) The thermoplastic urethane sheet is heated until it sags in the centre. The platform is lowered and vacuum is applied so the thermoplastic sheet is formed over the positive mould. e) The formed TPU is placed in a negative mould. f) A Teflon strip was inserted into grove of the negative mould to keep the channel open during the proceeding heat-sealing steps. g-h) A laser cut TPU window was placed on top and the assembly was heat-sealed with a heat transfer machine i) The assembly was aligned in an impulse sealer over a layer of polycarbonate and sealed at three sides, leaving one side open for biomaterial insertion j) Excess material was trimmed from the device with a fine scissors. k) A thermoplastic polyurethane catheter was inserted into the formed channel l) A PTFE coated mandrel was inserted into the catheter to retain patency during the catheter healing process m) Heat shrink tubing was positioned at the unsealed interface between the catheter and the device. (n-o) The parts were bonded in a heated aluminium block, with the heat provided conducted by the block and pressure provided by the contraction of the heat shrink tubing (p) The heat shrink tubing was snipped at the bottom at opposite sides and peeled away q) The PTFE coated mandrel was removed. At this stage the device was ETO sterilised r) A gel foam cylinder was aseptically inserted into the device. The device was aseptically sealed with an impulse sealer. Cells were injected through the catheter and seeded onto the gel foam when required for surgery.



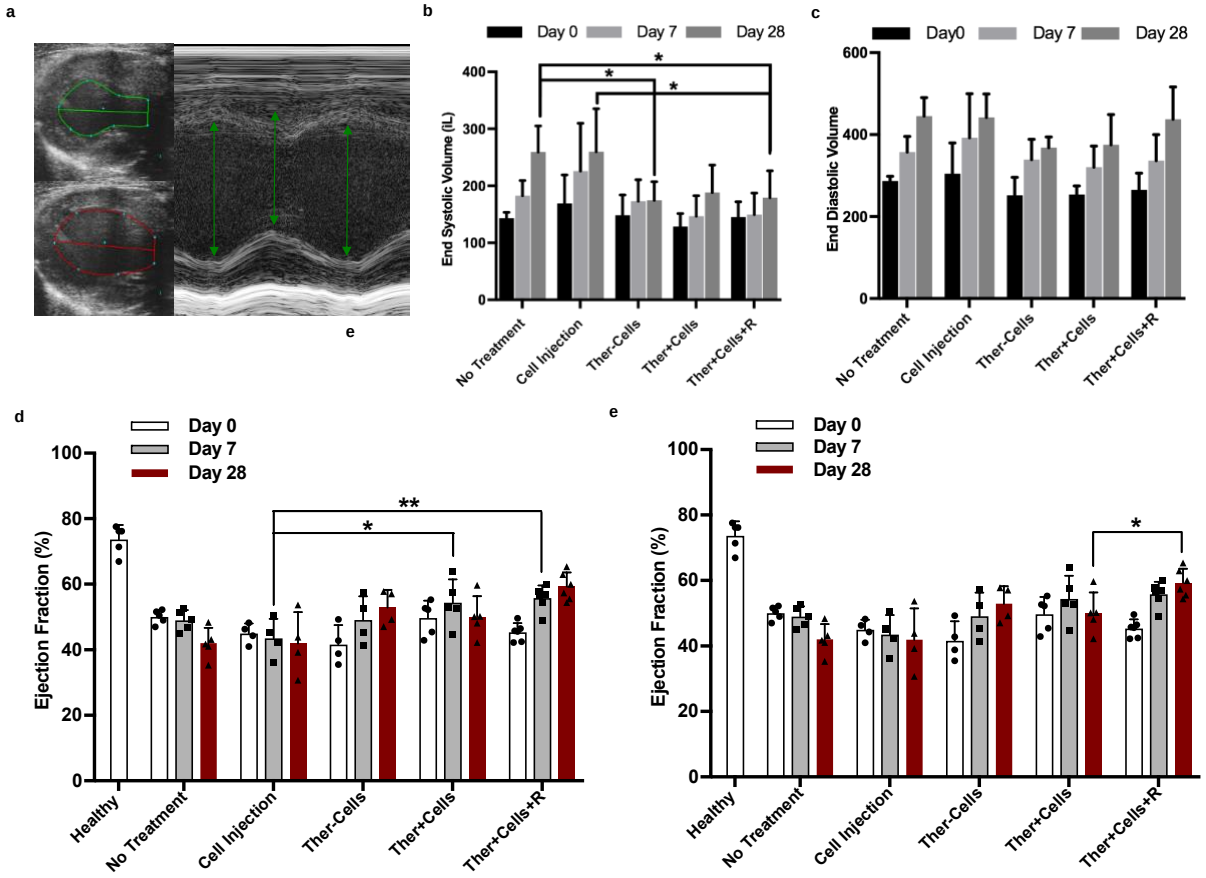
Supplementary Figure S2: Overview of Therepi concept, initial surgical steps and subcutaneous tunneling: a) Simplified *Therepi* model: schematic showing a rat model with a biomaterial alone on the heart, and an implanted catheter leading to the biomaterial that can be refilled with bioagents. Image above tail show surface and cross-sectional view of the GelMA biomaterial, (yellow scale bar is 100µm) b) *Therepi* model: Schematic showing a rat model with a *Therepi* attached to the heart. Image above tail shows 3-dimensional reconstruction of cylindrical sample of Gelfoam® from a micro-computed tomography scan. Black scale bar is 100µm. Animal **Preparation and Intubation (c-j):** c-d) Hair is removed at the dorsal and ventral site. e) The limbs are taped to a tilting stand and an elastic band around the incisors is attached to the stand. f) The stand is raised and the glottis is transilluminated with a light source. (g) The stand is positioned so that the operator has a direct view of the larynx, the tongue is retracted and pulled up so that the vocal cords can be visualized. (h) A 16G catheter is inserted into the trachea, timed with the opening of the vocal cords. (i) Correct insertion is verified by visualizing a small area of condensation on exhalation when a dental mirror is placed at the end of the catheter. (j) The ventilator is connected to the intubation catheter with an extension line. The lungs are observed to be moving with the ventilator and settings are adjusted as appropriate. **Subcutaneous catheter tunnelling procedure (k-r):** k) Iodine surgical scrub preperation is applied. l-m) An incisison is created at the dorsal and ventral sites n-o) Using a blunt tweezers, a subcutaneous tunnel is created between the dorsal and ventral site p) The forceps are placed through the tunnel so that they exit at the ventro-lateral incision and one end of the catheter is grasped with the forceps q) The catheter is pulled back through the dorsal site r) The catheter can be seen at the tunnel entry and exit points.



Supplementary Figure S3 Procedural details. Thoracotomy, myocardial infarction and *Therepi* attachment. Thoracotomy and myocardial infarction (a-g) a) The rat is carefully turned supine, and draped with a clear sterile plastic drape, taking care not to dislodge the intubation catheter or the ventilator connection. b) A small incision is made at the 4th left intercostal space. c) The tissue is dissected bluntly between the ribs to access the thoracic cavity. d) A chest retractor is inserted underneath the ribs each side of the incision and opened to visualize the underlying heart. e)The lung is gently moved aside with a sterile cotton bud and the the pericardium was removed with two fine curved forceps f) The left anterior descending artery is permanently ligated with a suture g) Myocardial blanching is apparent after ligation of the LAD, confirming infarction. **Simplified *Therepi* Attachment (h-j)** h) The catheter position is adjusted from the dorsal end so that the implanted catheter terminates at the heart. i) The catheter is sutured to the ventricle of the heart, ensuring not to block the catheter. j) The biomaterial is placed on the heart using a pre-placed suture and "parachuting" the biomaterial onto the ventricle then securing it with a single knot so that it is placed at the end of the catheter. ***Therepi* Attachment (k)** In lieu of attaching the cryogel and catheter separately to the ventricle, the encapsulated *Therepi* reservoir was secured to the ventricle of the heart at three suture points **(c) Closing of the thoracotomy (l-o):** l) The muscle layer is closed with sutures, leaving a small opening for the next evacuation step. m) A perforated tube connected to a 30cc syringe is placed into the thoracic cavity through a small opening in the muscle layers, and a vacuum was applied while the skin was sealed with fingertips to inflate the lungs and re-establish negative thoracic cavity pressure. A hemostat clamp is immediately placed on the skin, maintaining the airtight seal. o) The rat is turned prone, ensuring not to dislodge the intubation catheter or ventilator attachment, animal is re-draped in this position.



Supplementary Figure S4: Subcutaneous port placement. a) The catheter is trimmed so that 2-3cm of catheter exits the dorsal site, ensuring there is no kinking in the line but enough slack for movements b) Subcutaneous space sufficient for the vascular access button was then created using a forceps c) The subcutaneous port was then attached and inserted into the subcutaneous pocket. d) The felt is secured to the underlying fascia using at least two interrupted sutures, taking care not to go through the catheter f) The skin is closed with four to five interrupted sutures and the animal is recovered g) Repeated, minimally invasive delivery is enabled through the port



Supplementary Figure S5: Echocardiography measurements. a) Representative echocardiographic measurements of an infarcted heart for calculating ejection fraction (left) and fractional shortening (right) b) End systolic volume for all groups assessed by echocardiography. c) End diastolic volume for all groups assessed by echocardiography. *p<0.05 , see Table S2 for exact p values. Data are mean ± SD (n=5); as analyzed by a two-way analysis of variance (ANOVA), with Tukey's multiple comparisons post-test. Ther=*Therepi*, R=refill. d) Ejection fraction data from echocardiographic data with statistics for day 7 *p=0.0276, **p=0.0057. e) Ejection fraction data from echocardiographic data with additional statistics for day 28 *p=0.0494.

Supplementary Table 1. Diffusion coefficient following formation of fibrous capsule in vivo *(Fig. 4f,g)*

Days Post-Implantation	**0**	**20**	**20**
Molecular Weight (kDa)	40	40	10
Tissue Thickness (cm)	0.167	0.225	0.216
Lag time (s)	8909	28750	8172
Diffusion Coefficient (cm^2/s)	**$3.13*10^{-6}$**	**$1.76 * 10^{-6}$**	**$5.71 *10^{-6}$**

Supplementary Table 2. Multiple comparisons testing for ejection fraction (%) measured by echo (Fig. 6d)

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**Summary**	**Adjusted P Value**
Day 0					
No Treatment vs. Cell Injection	5.114	-4.84 to 15.07	No	ns	0.6003
No Treatment vs. *Therepi* - cells	8.473	-1.48 to 18.43	No	ns	0.1307
No Treatment vs. *Therepi* + cells	0.3553	-9.029 to 9.74	No	ns	>0.9999
No Treatment vs. *Therepi* + cells + refill	4.696	-4.289 to 13.68	No	ns	0.5843
Cell Injection vs. *Therepi* - cells	3.359	-7.133 to 13.85	No	ns	0.895
Cell Injection vs. *Therepi* + cells	-4.759	-14.71 to 5.195	No	ns	0.6635
Cell Injection vs. *Therepi* + cells + refill	-0.4176	-9.996 to 9.161	No	ns	>0.9999
Therepi - cells vs. *Therepi* + cells	-8.118	-18.07 to 1.836	No	ns	0.1607
Therepi - cells vs. *Therepi* + cells + refill	-3.777	-13.36 to 5.801	No	ns	0.8002
Therepi + cells vs. *Therepi* + cells + refill	4.341	-4.644 to 13.33	No	ns	0.6546
Day 7					
No Treatment vs. Cell Injection	5.449	-4.505 to 15.4	No	ns	0.5401
No Treatment vs. *Therepi* - cells	-0.1212	-10.07 to 9.833	No	ns	>0.9999
No Treatment vs. *Therepi* + cells	-5.326	-14.71 to 4.058	No	ns	0.5043
No Treatment vs. *Therepi* + cells + refill	-6.817	-15.8 to 2.168	No	ns	0.2188
Cell Injection vs. *Therepi* - cells	-5.57	-16.06 to 4.923	No	ns	0.5697
Cell Injection vs. *Therepi* + cells	-10.77	-20.73 to -0.8209	Yes	*	0.0276
Cell Injection vs. *Therepi* + cells + refill	-12.27	-21.84 to -2.688	Yes	**	0.0057
Therepi - cells vs. *Therepi* + cells	-5.205	-15.16 to 4.749	No	ns	0.5839
Therepi - cells vs. *Therepi* + cells + refill	-6.696	-16.27 to 2.882	No	ns	0.294
Therepi + cells vs. *Therepi* + cells + refill	-1.491	-10.48 to 7.494	No	ns	0.9899
Day 28					
No Treatment vs. Cell Injection	0	-9.954 to 9.954	No	ns	>0.9999
No Treatment vs. *Therepi* - cells	-11	-20.95 to -1.046	Yes	*	0.0233
No Treatment vs. *Therepi* + cells	-9	-18.38 to 0.3845	No	ns	0.0661
No Treatment vs. *Therepi* + cells + refill	-18	-26.99 to -9.015	Yes	****	<0.0001
Cell Injection vs. *Therepi* - cells	-11	-21.49 to -0.5078	Yes	*	0.0354
Cell Injection vs. *Therepi* + cells	-9	-18.95 to 0.9538	No	ns	0.0945
Cell Injection vs. *Therepi* + cells + refill	-18	-27.58 to -8.422	Yes	****	<0.0001
Therepi - cells vs. *Therepi* + cells	2	-7.954 to 11.95	No	ns	0.9794
Therepi - cells vs. *Therepi* + cells + refill	-7	-16.58 to 2.578	No	ns	0.2522
Therepi + cells vs. *Therepi* + cells + refill	-9	-17.99 to -0.01498	Yes	*	0.0494