Optimized Delivery System Achieves Enhanced Endomyocardial Stem Cell Retention

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Background—Regenerative cell-based therapies are associated with limited myocardial retention of delivered stem cells. The objective of this study is to develop an endocardial delivery system for enhanced cell retention.

Methods and Results—Stem cell retention was simulated in silico using 1- and 3-dimensional models of tissue distortion and compliance associated with delivery. Needle designs, predicted to be optimal, were accordingly engineered using nitinol, a nickel and titanium alloy displaying shape memory and superelasticity. Biocompatibility was tested with human mesenchymal stem cells. Experimental validation was performed with species-matched cells directly delivered into Langendorff-perfused porcine hearts or administered percutaneously into the endocardium of infarcted pigs. Cell retention was quantified by flow cytometry and real-time quantitative polymerase chain reaction methodology. Models, computing optimal distribution of distortion calibrated to favor tissue compliance, predicted that a 75°-curved needle featuring small-to-large graded side holes would ensure the highest cell retention profile. In isolated hearts, the nitinol curved needle catheter (C-Cath) design ensured 3-fold superior stem cell retention compared with a standard needle. In the setting of chronic infarction, percutaneous delivery of stem cells with C-Cath yielded a 37.7±7.1% versus 10.0±2.8% retention achieved with a traditional needle without effect on biocompatibility or safety.


Key Words: catheters • myocardial infarction • regeneration • stem cells

Major advances in the understanding of stem cells as reparative biotherapeutics have been achieved during the past decade, yet regenerative procedures remain limited by the low retention rates after transplantation. To date, various routes of cell administration have been explored, with the average retention typically <10% of the injected dose.1-4

A surgical approach was originally used with delivery of stem cells via an epicardial route.5,6 A transition to percutaneous approaches, including intracoronary infusion with an over-the-wire balloon catheter, has enabled cell therapy at the time of myocardial infarction.7-9 Intracoronary administration of stem cells is thought to amplify endogenous repair processes at site of injury but requires efficient transendocardial migration of progenitors within the coronary bed.10-13 To circumvent the need for transendocardial homing, endomyocardial injection has more recently been used to deliver cells directly into the region of interest.1,11-13 Although endomyocardial injection has been suggested to improve delivery, overall retention rates remain limited, potentially compromising adequate stem cell dosing.1-3 Accordingly, optimized myocardial delivery of stem cells is a priority to advance regenerative procedures.

To date, straight and helical needle designs have been used for endomyocardial delivery in the clinical setting.14-18 The straight needle design has been extensively used in the delivery of myoblasts and mesenchymal stem cells (MSC), demonstrating an excellent safety profile.19-23 Yet, reported retention rates are typically in the single digits.4,20 Helical needles are thought to provide a better fixation during delivery and have also been associated with safe clinical outcome,11,24 although retention rate analysis has yet to be reported. Regardless of the design, the high pressure generated by the end-hole feature of all available needles has been suggested as a culprit in compromising reliable delivery of a desired viable and potent cell dose.1

The present bioengineering study is based on the hypothesis that uniform distribution of cells over the length of the...
WHAT IS KNOWN
• Percutaneous catheter-based methods are an established route for delivery of regenerative biologics to the heart.
• The current state-of-the-art devices and methodologies are associated with a significant loss of the delivered stem cell dose, creating a barrier to optimization of regenerative technologies.

WHAT THE STUDY ADDS
• Modeling provided an opportunity to test a variety of needle designs for optimization of cell retention.
• A curved needle design eliminated backflow of injectate and limited loss within contractile tissue.
• The use of a small-to-large graded side-hole design diminished interstitial pressure during delivery to improve retention.
• A curved needle design featuring small-to-large graded side holes resulted in a significant increase in cell retention in both healthy and infarcted hearts.

delivery needle, in tandem with alteration of the injection track, may provide a strategy to limit early cell loss. It was also hypothesized that larger exit diameters through the multiplication of holes would reduce shear stress on the cells, potentially yielding cells of better survival capabilities. A mathematical approach was initially used to model interstitial cellular retention. Modeling maintained injectate composition and tissue of interest as constants to test variable geometries and dynamics in endomyocardial delivery. Needle designs predicted to have the highest tissue retention rates by the Darcy law and 3-dimensional (3D) projections were then experimentally tested. This prototype development approach yielded a novel catheter design that achieves a superior cellular retention profile.

Methods
All animal protocols were reviewed and approved by the local Institutional Animal Care and Use Committee. Human bone marrow used in procurement of stem cells for biocompatibility testing was collected with written informed consent approved by the local institutional review board.

Modeling
Computer simulations were run for 1D and 3D models to study the spread of injectate at the site of injection. The Darcy law provided modeling capacity for instantaneous discharge rate through a porous substance (permeability \( k \) [m²]), taking into account fluid viscosity and pressure alteration over a distance (\( P \)). One-dimensional flow models solved for transport and reaction of tissue in the static state. This approach was enriched with the addition of saturation modeling. Three-dimensional flow modeling was implemented to achieve head-to-head comparison among 4 scenarios, that is, catheters with (1) straight needle and end hole, (2) straight needle and side holes, (3) 45° needle and side holes, and (4) 75° needle and side holes. Complex 3D modeling was achieved with addition of fibers to mimic myocardial contractility (Figure 1).

Langendorff Perfusion and Microsphere Injection
Hearts were extracted from hybrid farm pigs after euthanasia with potassium chloride infusion (rapid intravenous KC1 bolus). Lateral thoracotomy or sternotomy was used for access to the myocardium, with careful resection of the heart to maintain integrity of the right and left atrium, proximal ascending aorta, and pulmonary artery. The right and left coronary vessels were canulated through the ascending aorta and perfused using a 95% O₂/5% CO₂-saturated perfusion solution (5-μm filtered NaCl 118.5, NaHCO₃ 25.0, KCl 4.7, KH₂PO₄ 1.2, glucose 11.0, and CaCl₂ 1.5 mmol/L) heated to 37°C to 37.5°C. Once the porcine myocardium displayed resumption of contractile activity, green fluorescent protein microbeads were injected epicardially into the left ventricular (LV) free wall using a traditional straight, altered straight, or curved needle.

Catheter Design and Biocompatibility
Based on model simulation, catheters were designed with a handle, an 8F shaft in tandem with an injection needle. A nickel titanium alloy (nitinol) was used to ensure capacity to retain a curved configuration with repeat use. Concomitantly, under good manufacturing practice, human MSCs were derived, thawed, and concentrated to between 65×10⁸ and 75×10⁸ cells/mL as previously described. To mimic clinical use, cells were next stored for 6 hours at 4°C. At final design, the curved needle catheter (C-Cath) was warmed to 37°C in the portion that would typically be within the patient, whereas the handle was maintained at room temperature. Biocompatibility was performed using human cardiopoietic cells to evaluate the viability of clinical-grade cardiopoietic cells (Figure 1 in the online-only Data Supplement). Cells were injected through the body temperature catheter 20× to mimic the clinical setting, with cell samples obtained on the first, 11th, and 20th injections. Exiting fractions were investigated for cell viability, survival rate, cell number, and myocyte enhancing factor 2C expression, a typical marker of human cardiopoietic MSCs. The cell number and viability were determined using the trypan blue exclusion assay. Viability was confirmed by cell-impermeant SYTOX green dye-assessed plasma membrane integrity, whereas reduction of C₅₋₇-resazurin to red-fluorescent C₅₋₇-resoruvin confirmed metabolic activity as analyzed by flow cytometry (FACS Canto II, BD Biosciences). Cell survival rate was defined as the ratio of the number of viable cells harvested 22±2 hours after seeding over the number of viable cells initially seeded.

Large Animal Healthy and Infarction Models
Male porcine MSCs were used in all studies assessing catheter safety and retention in pigs. Embryonic stem cells engineered with good manufacturing practice were cultured as previously described and delivered to 4 million cells per injection into healthy porcine hearts (n=3 straight needle catheters and n=4 curved needle catheters). Embryonic stem cell retention was quantified via a fluorescence-activating cell sorter-based approach by quantifying the number of green fluorescent protein-positive cells. MSCs were grown from iliac bone marrow aspirates obtained from 25 male Landrace-Yorkshire pigs. Bone marrow was cultured in α-modified Eagle’s medium (Invitrogen) with 20% fetal bovine serum (Wisent), l-glutamine (Invitrogen), and penicillin/streptomycin (Invitrogen) with isolated MSC cryopreserved. For injection, MSCs were thawed, washed, and concentrated to a density of 85 to 98 million cells/mL. Fluorescent labeling was performed for fluorescence-activated cell sorter-based quantification (PKH26, Sigma). Cell suspensions were loaded in 1 mL syringes and incubated at 4°C to preserve cell viability before administration. To identify injection site at necropsy, as part of the retention follow-up, Evans blue dye was added. In vivo catheter-based experiments were first conducted in 18 female Yorkshire crossbred swine in the absence of myocardial infarction (7 for straight and 11 for curved needle catheters). An additional cohort of 22 Yorkshire female swine were subjected to myocardial infarction and at an approximate age of 21 weeks and weighing 62.4±5.8 kg at the time of MSC injection, were included in the study for either safety or retention evaluation. After induction of anesthesia, percutaneous vascular access was obtained in the right
Percutaneous Cell Delivery

On average, animal weight was 50±12 kg at the time of cell transplantation. Echocardiograms were conducted on all infarcted animals before and after cell administration and intracardiac echocardiography. Infarcted pigs were assigned to either the retention or safety arm of the study. Male porcine-derived MSCs were delivered via endomyocardial administration route 27 to 40 days postinfarction into female pigs. After anesthesia, percutaneous access to the right femoral artery and vein was obtained, and 9F sheaths (Cordis) were placed. A 0.035" guidewire (Cook Medical) was advanced into the LV retrograde across the aortic valve. A pigtail catheter (Cordis) was then inserted into the LV over the guidewire. Left ventriculography was performed in both the anteroposterior and lateral views to delineate regions of dysfunction. In a subset of pigs, an intracardiac echo catheter (8F; AcuNav, Biosense Webster) was introduced in the venous sheath for direct echo-based visualization of the myocardium. The injection catheter was flushed with prepared cell suspension, and needle length was adjusted to approximately half of the thickness of the myocardium with the help of an aortic arch simulator. The catheter was inserted through the sheath and advanced into the LV under fluoroscopy. The needle was deployed in the desired locations, avoiding the apex and scar, with cells administered by endomyocardial route into the free wall of the LV chamber guided by biplane fluoroscopy and in most cases intracardiac echo. Each injection was performed at a rate of ≈0.5 mL/min. The needle was held in place for ≈15 seconds postinjection before being retracted. Once all injections were delivered, the injection catheter system was withdrawn, and hemostasis was obtained as necessary. At the end of the ischemic period, the balloon was deflated and the ischemic area was allowed to reperfuse. Complete balloon deflation was confirmed via fluoroscopy. The angioplasty balloon, catheter, and sheaths were removed with hemostasis obtained by direct pressure on the vessels. Pigs were retained for 1 month, with ensuing scar confirmed on echocardiography (transthoracic echocardiography and intracardiac echocardiography). Infarcted pigs were assigned to either the retention or safety arm of the study.

Figure 1. Modeling based on the Darcy law provides putative needle design predicted to have a high degree of retention. A. Transport retention modeling predicts global retention within myocardial tissue over time in cylindrical coordinates. Two different needle lengths (blue and red) in spherical coordinate for a needle radius of the same size as in the cylindrical cases (green) and in cylindrical coordinates for a needle length of 6 mm but a shorter injection duration (cyan). B. Retention profiles are significantly upregulated with modulation of the absorption coefficient (λ). C. Altered needle design with injection over the length of the injection needle dramatically increases absorption. D. Three-dimentional modeling of tissue edema with end-hole versus side-hole needles. E. Tissue edema in the setting of contraction. F–H. Predicted retention rates for traditional straight needle with end hole, straight needle with side holes, and curved needle with side holes.

femoral artery and vein, and 9F sheaths were introduced. An angioplasty balloon was introduced by advancing it through the guide catheter to the left anterior descending coronary artery. The balloon was positioned to below the first major diagonal branch in the left anterior descending coronary artery and was inflated to sufficient pressure to occlude blood flow to the distal area of the left anterior descending coronary artery. Occlusion was confirmed via fluoroscopy. The balloon was left in place for 60 minutes. Animals were defibrillated as necessary. After anesthesia, percutaneous access to the right femoral artery and vein was obtained, and 9F sheaths (Cordis) were placed. A 0.035" guidewire (Cook Medical) was advanced into the LV retrograde across the aortic valve. A pigtail catheter (Cordis) was then inserted into the LV over the guidewire. Left ventriculography was performed in both the anteroposterior and lateral views to delineate regions of dysfunction. In a subset of pigs, an intracardiac echo catheter (8F; AcuNav, Biosense Webster) was introduced in the venous sheath for direct echo-based visualization of the myocardium. The injection catheter was flushed with prepared cell suspension, and needle length was adjusted to approximately half of the thickness of the myocardium with the help of an aortic arch simulator. The catheter was inserted through the sheath and advanced into the LV under fluoroscopy. The needle was deployed in the desired locations, avoiding the apex and scar, with cells administered by endomyocardial route into the free wall of the LV chamber guided by biplane fluoroscopy and in most cases intracardiac echo. Each injection was performed at a rate of ≈0.5 mL/min. The needle was held in place for ≈15 seconds postinjection before being retracted. Once all injections were delivered, the injection catheter system was withdrawn, and hemostasis was obtained as necessary. At the end of the ischemic period, the balloon was deflated and the ischemic area was allowed to reperfuse. Complete balloon deflation was confirmed via fluoroscopy. The angioplasty balloon, catheter, and sheaths were removed with hemostasis obtained by direct pressure on the vessels. Pigs were retained for 1 month, with ensuing scar confirmed on echocardiography (transthoracic echocardiography and intracardiac echocardiography). Infarcted pigs were assigned to either the retention or safety arm of the study.
described above. A total of 3 injections of 0.5 mL was used in the retention studies. For safety studies, 20 injections of 0.65 mL targeting separate areas within the myocardium were performed in each pig.

**Cell Retention**

Cell retention after endomyocardial delivery was measured 1 hour after injection of the last dose. Animals were euthanized by an intravenous overdose of sodium pentobarbital solution, and euthanasia was confirmed via auscultation. The heart was removed from each animal and was cut open along the posterior wall at the junction with the septum. The heart was laid flat to identify the injection sites by Evans blue dye staining. Each injection site was collected by cutting a 2.5×2.5-cm² section around the insertion point and analyzed using flow cytometry (fluorescence-activated cell sorter) analysis and real-time quantitative polymerase chain reaction methodology. The flow cytometry assay was performed after administration of prelabeled embryonic stem cells and male porcine MSCs. Here, heart samples were finely minced, and a cell suspension was obtained by digestion with a solution of 1 mg/mL collagenase/dispose and 200 μg/mL DNase in α-modified Eagle’s medium containing 5% fetal bovine serum, at 37°C, with constant agitation. After digestion, cells were washed, PKH26-labeled cells were quantified by flow cytometry (FACSCalibur, BD), and data were analyzed (Cellquest Pro Software, BD). Percentage of positive cells was measured, and the number of positive cells was normalized to total number of cells in the sample. To validate fluorescence-activated cell sorter–based findings, a quantitative polymerase chain reaction was developed to detect the Y chromosome of male stem cells injected into female swine. To this end, tissues were processed, nucleic acids were extracted, and genomic DNA concentrations and purities were measured by ultraviolet absorbance. The male porcine SRY gene sequence was detected in a background of female porcine genomic DNA using a Taqman PCR kit (Applied Biosystems). The primers used were 5’-CAAGTGGCTGGGATGCAAGT-3’ (forward) and 5’-CTCGAAGATGCGCTATT-3’ (reverse). Each quantitative polymerase chain reaction was conducted at 50 μL/well on a 96-well plate (7900HT Fast Real Time PCR, Applied Biosystem). Quantification was performed by the construction of a standard curve obtained by spiking a female porcine matrix prepared from female swine liver with DNA isolated from a known number of male porcine MSCs. The lower limit of quantification was established at 50 copies of male porcine MSC genomic DNA per reaction in the presence of 1 μg matrix DNA. Each sample was analyzed in triplicate. Separately, a canine model was used for independent documentation of retention using iodine-based contrast dye (Omnipaque, GE) visualized by a fluoroscopy approach (Artis zee, Siemens).

**Safety**

Safety was assessed by animal survival, adverse events, general well-being, electrocardiography, blood tests, and microscopic analysis of the heart. Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily. Observations were conducted pretest and once postdose. Body weights were measured and recorded pretest and once postdose. Physical examinations included skin, eyes, ears, nose, thorax, abdomen, and respiratory and circulatory systems. Electrocardiographic examinations were conducted pretest, before injection, and 1 hour postdose. Blood samples for clinical pathology evaluations were collected from all animals pretest, before injection, 1 hour postdose, and before necropsy (≥24 hours postdose). Blood samples for analysis of cardiac enzymes (creatine kinase MB, troponin I, and C-reactive proteins) were collected pretest, before injection, 1, 6, and 12 hours postdose, and before necropsy (24 hours postdose). At the end of the study, necropsy examination were performed, and tissue sections adjacent to injection sites were microscopically examined. Safety of the endomyocardial delivery of stem cells was assessed (n=10). A sham group (n=5) of swine were injected (needle insertion only) without cell delivery.

**Statistics**

Statistical differences were assessed using the nonparametric Wilcoxon rank-sum test and confirmed using the Kruskal–Wallis test. A 2-way ANOVA test was used for statistical assessment of troponin change over time after straight versus curved needle-based injections. Significance was assumed at P<0.05. Data are shown as mean±SEM for animal data and mean±SD for the biocompatibility assay.

**Results**

**In Silico Prediction of Optimal Needle Design**

Because endothelium-disrupting techniques have previously been suggested to achieve the highest level of cellular retention in the preclinical setting, this mode of delivery was chosen for in silico analysis. Initial models were generated to allow prediction of retained cells within the myocardial interstitium after injection. To compute the flow of cells into the interstitial space during injection, the Darcy law was used (Figure IIA in the online-only Data Supplement). To further map the fate of the injectate over time, the model was then expanded to incorporate postinjection retention within the interstitial space, encompassing a retention and washout coefficient (κw/κr; Figure IIB in the online-only Data Supplement). Next, the influence of pressure induced by injection of cells within the interstitial space was introduced such that the interstitial pressure during injection would be equal to or greater than the pressure at the start or end of injection and that the interstitial pressure after the injection precipitously would diminish because of washout after the injection period (Figure IIC in the online-only Data Supplement). These parameters were integrated within a reaction retention model where influx during the injection phase is assumed to equal injectate retained within the interstitium plus washout (Figure IID in the online-only Data Supplement). Using the integrated model, 3D plots, incorporating pressure, retention, and washout, were made, demonstrating that optimization of needle length or timing of injection does not affect long-term retention (Figure 1A). However, it was noted that measures enhancing the absorption coefficient (termed λ) and diminishing tissue permeability (washout pressure: Dpwo) would dramatically affect tissue retention (Figure 1B). Thus, it was determined that use of the length of the needle for injectate delivery would eliminate interstitial pressure burden at the tip improving λ (Figure 1C). Intersitial edema occurs when the gradual influx of fluid within the tissue overcomes its lymphatic capacity without disrupting the cellular basement membrane. Furthermore, to eliminate the effect of Dpwo on retention, it was hypothesized that a curved needle design would more effectively trap the injectate within the interstitium, thereby mimicking processes seen with tissue edema (Figure 1D). Simulation of tissue edema was achieved through saturation (Figure IIIA in the online-only Data Supplement) and proelastic modeling (Figure IIIB in the online-only Data Supplement), allowing 3D modeling for traditional straight needle with end hole, straight needle with side holes, and curved needle with side holes (Figure 1D). Modeling of the inter-relationship between pressure and volume in the setting of tissue contraction provided a predictive index of cellular retention (Figure 1E). This approach allowed inclusion of varying pressure and volume tension at the site of injection to assess which needle design would provide the most ideal retention profile in the presence of contraction. In this setting, it was noted that compared with a straight needle with end hole or a straight needle with side hole...
holes, a curved needle with side holes would provide the highest degree of retention by allowing the longest period of injection without reaching tissue compliance limits for pressure and volume (Figure 1F–1H).

Endocardial Injection Catheter With Curved Needle Found Biocompatible

Using the predicted in silico needle designs, endomyocardial delivery catheters were manufactured encompassing 3 parts: a proximal handle, a catheter shaft, and an injection needle either straight or curved in the distal section (Figure 2A). Number of holes and their distribution were dictated by engineering limitations, whereas the ascending size of the holes was made to optimize flow dynamics. Needles were designed to be porous over the distal 4 mm with 6 incrementally sized side holes (0.007–0.017 inch) and an end hole (0.017 inch; Figure 2A, inset). The distal tip of the catheter shaft was made deflectable and controlled at the handle by a rotating wheel via an activation wire, internal to the shaft (Figure 2B and 2C). The catheter handle was designed to control shaft tip deflection via a thumb wheel and needle advancement or retraction via a rear-end push button. The catheter via a standard proximal luer lock connects to a syringe containing the therapeutic agent (Figure 2B and 2C). Biocompatibility assay using lineage-specified human-derived cardiopoietic MSC26,27 demonstrated no significant effect on stem cell viability or identity after passage through the catheter (n=5; Figure I in the online-only Data Supplement).

Curved Needle Catheter Recapitulates In Silico Models In Vivo

The optimized curved needle catheter featuring a curved needle with small-to-large graded side holes (C-Cath) was evaluated in both healthy and infarcted porcine models, in addition to healthy canine models. Area of infarction was documented by transthoracic echocardiography. After retrograde aortic passage of the catheter into the LV, biplane fluoroscopy was used to guide catheter positioning within the LV of all treated porcine models (Figure 3A). Of note, in a subset of animals, intracardiac echocardiography (Figure 4A) was used in tandem with biplane fluoroscopy to validate positioning within the LV (Figure 4). Serial injections of contrast dye visualized retention of injectate in the anteroseptal, inferolateral, and anterolateral distributions on fluoroscopy (Figures 3A and 4B). Close-up visualization of injection (Figure 3B) was noted to reproduce the predicted edema tissue distribution seen with in silico modeling of curved needle with side holes (Figure 1D, right). On gross inspection, porcine myocardium revealed areas of Evans blue staining suggestive of injectate retention (Figures 3C and 4C; Figure IV in the online-only Data Supplement).
Supplement). Histological assessment of Evans blue–stained areas revealed pockets of injected stem cells surrounded by a band of compressed myocardium (Figure 3D).

Curved Needle Results in Superior Myocardial Retention
To validate in silico prediction, the curved needle design was tested in a variety of models to exclude the biology of the transplanted cells as a contributing factor to retention (Figure 5). Initially, ex vivo evaluation of a variety of designs was made. Fluorescent microbeads were used to measure the quantity of injectate retained after epicardial delivery in a beating Langendorff-supported porcine heart. As predicted by the Darcy law–based modeling, needle curvature (75°), in combination with side holes graded with increasing size, produced the highest degree of retention (Figures 5 and 6A). Specifically, an average of 32,786±4,935 microspheres per injection site (n=5) was counted after delivery with an end-hole needle design versus 48,277±9,697 after addition of side holes (n=5; P=0.06). Retention was further enhanced with a 75°-curvature of the needle with side holes, improving retention of microbeads to 64,590±10,800 per injection site, a significant increase (n=6; P<0.05) versus the conventional straight needle with an end-hole specification (Figure 6A). This improvement was further corroborated in vivo with endocardial delivery of green fluorescent protein-positive embryonic stem cells (Figure 6B) or sex-mismatched porcine MSCs (Figure 6C), demonstrating a 2- to 3-fold improvement in retention. In normal hearts, cellular retention after delivery with a straight needle with end hole was measured at 10.0±2.8% (n=7) compared with 37.7±7.1% (n=11; P<0.02) with a 75°-curved needle with side holes (Figure 6C). Cell delivery was made in infarcted hearts to ensure safety after border-zone injection. In infarcted porcine hearts, retention after cellular implantation with a 75°-curved needle with side holes was noted to be 35.6±6.7% (n=15; P<0.05; Figure 6C). Transient troponin elevations induced by the curved needle (n=5) were comparable with those seen after straight needle with end-hole (n=5) delivery in the microbead study (Figure 6D). A good laboratory practice–grade safety study was performed with 20 injections in each porcine heart to mimic

Figure 4. Use of fluoroscopy with intracardiac echo (ICE) provides optimal catheter positioning. A, ICE-based imaging of the left ventricle (LV) highlights catheter shaft and tip position in relation to the LV and right ventricles (RV). B, Anteroposterior (AP) fluoroscopic monitoring of catheter position performed in parallel with ICE highlights sites of injection (a, b, c) and injectate retention. C, Evans blue staining noted on gross evaluation of the myocardium after injection highlights sites of retained injectate (a, b, c) correlating with the AP fluoroscopic images.

Figure 3. In silico models recapitulated in vivo. A and B, Fluoroscopy-guided endomyocardial biologics delivery documents edema phenomenon with tissue retention of contrast dye in different regions of the myocardium. C, Evans blue–stained areas of injection document retention on gross inspection. D, Histological evaluation of Evans blue–stained areas documents pockets of mesenchymal stem cell (MSC) retention. CM indicates cardiomyocyte.
clinical cell delivery protocols. Cardiac enzyme increase after stem cell injection was noted to be transient (Figure V in the online-only Data Supplement). Pigs did not demonstrate any evidence for myocardial perforation (gross evaluation at necropsy) or periprocedural morbidity and mortality.

Discussion

Use of in silico modeling here yielded a novel catheter-based delivery system, demonstrating enhanced endomyocardial retention of stem cells. Through use of 1D and 3D modeling, a curved needle design with side holes was identified as providing the highest degree of retention. Accordingly, endovascular catheters designed for ex vivo and in vivo studies yielded an increase in retention by 3-fold compared with a straight needle with end-hole design. In tandem with retention, safety of the curved needle catheter (C-Cath) was documented in a porcine model of myocardial infarction, revealing no evidence of myocardial perforation or increased troponinemia versus the straight needle catheter. Fluoroscopic visualization in tandem with gross and histological tissue examination documented retention profiles matching those predicted by 3D modeling algorithms. C-Cath thus represents the first needle design shown to improve stem cell retention rates significantly within the myocardium.

Since the initiation of heart regenerative medicine protocols, myocardial delivery of stem cells has been a significant issue with consistent demonstration of low retention profiles of transplanted biologics. Two different endothelium-maintaining injection techniques have been used, namely intracoronary and intravenous administration. Both delivery techniques require robust and specific interaction between the coronary endothelium and transfused cells for successful transendothelial migration (diapedesis) into the injured myocardium.10,13,30 Despite similar transfer mechanisms, intracoronary...
delivery has been shown to provide a higher amount of homing compared with intravenous delivery because therapeutic concentration at the target site is significantly increased. However, retention achieved with direct endomyocardial delivery is thought to provide a greater degree of retention by disrupting myocardial interstitium in the deposition of the biotherapeutics.\textsuperscript{1,3,31}

Initial modeling assessment of the traditional injection needle revealed that endomyocardial delivery is limited by tissue compliance and pressures generated by the injectate at the tip of the injecting needle. Use of modeling informed development of an optimized needle design by allowing real-time estimation of tissue compliance and retention. Integration of saturation modeling in tandem with proelastic tissue distortion models was able to generate in 3 dimensions predictive algorithms for the dispersion of the injectate with respect to time. In this way, multiple needle shapes were efficiently evaluated to yield a prototype design predicted to have the highest degree of retention. Delivery needles expected to have the best retention profile were engineered through use of nickel titanium (nitinol), with shape memory and superelasticity ensuring maximal flexibility in catheter design.\textsuperscript{32}

The catheter was tested under a diversity of experimental conditions, with different cytotypes or microbeads, to ensure that cellular homing function was not a contributing factor to the augmented retentions noted (Figure 4). In vivo evaluation of putative high-efficiency catheters identified that a 75°-curvature with graded side holes had the capability to improve retention profiles versus the traditional straight needle with end hole by 3-fold. To ensure that this catheter could be safely used in patients with a prior history of myocardial infarction, good laboratory practice-grade evaluation of the final design of the catheter was made with 20 injections performed in infarcted pigs under fluoroscopic guidance corroborating an elevated cellular retention profile on gross, histological, and molecular evaluation, in tandem with high degree of safety.

This article highlights a cell retention after endomyocardial delivery with the curved needle catheter (C-Cath) at 30% to 40%, depending on the quantification method and large animal model (infarcted or healthy). Through a multiplicity of techniques used in the present study, data consistently demonstrated increased retention with the 75°-curved needle with side holes versus the straight with an end hole. These results are supported by the fact that retention rates obtained with the straight needle (10.0±2.8%) are in agreement with what has been previously reported with conventional designs (4%–10%).\textsuperscript{1,3,13,20} Safety assessment of the curved catheter (C-Cath) demonstrated that all animals survived cell transplantation via the catheter. No myocardial perforation or clinically relevant increases in the blood levels of cardiac enzymes were observed. However, use of the catheter in a model of infarction can only provide the basis for initial clinical use but cannot replace the experience gained with the use of the catheter in patients. Clinical assessment is, therefore, required before application in practice.

Optimization of myocardial retention of regenerative biologics is critical in the realization of genuine heart regeneration. Improving retention rates is a step toward this goal. Dose dependency can now be more readily assessed percutaneously with a significant reduction in the amount of initial cellular material needed, ultimately diminishing production and manufacturing costs. Although the curved needle catheter (C-Cath) was initially developed in the framework of regenerative cell therapy within the myocardium, any territory with large vascular access could benefit from the use of such a catheter for cell therapy in tandem with gene-, virus-, or protein-based biologics delivery.

**Study Limitation**

This study used modeling to identify optimized needle enhancing myocardial biologics retention. Specifically, this study evaluates acute myocardial retention of a variety of agents, including microbeads, embryonic stem cells, and MSCs within the healthy and infarcted myocardium. Here, the Darcy law was used for in silico approximation of myocardial tissue absorption and permeability. However, this approach can only aim to provide an approximation of these dynamic variables, which are varied across populations and in health and disease. Furthermore, additional variables such as injectate properties may have provided additional accuracy to the models used. Despite these limitations, this approach was proven valid because in vivo use of needle designs predicted advantageous improved retention. This study does not evaluate long-term retention of cells within the myocardium. Furthermore, it is unclear whether increase of retention within the myocardium will enhance regenerative or neovasculogenic influence of the biologics delivered. However, with enhanced acute retention, it is hypothesized that overall retention and beneficial influence would both be augmented if the cytotype delivered has a regenerative biological action.

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**Disclosures**

None.

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Supplemental Material

Supplemental Figures

Supplemental Figure 1. Multimodality-based assessment of human cardiopoietic stem cell viability following injection through C-Cath. (A-C) Assessment of viability and survival rate via trypan blue (A), 2-color fluorescent assay (B) and survival in culture (C). (D) Immunofluorescence-based confirmation of preservation of cellular identity through MEF2C staining.

Supplemental Figure 2. Use of Darcy’s Law to assess biologics-based retention within the myocardium.

Supplemental Figure 3. Addition of saturation and pro-elastic modeling to retention models allows for prediction of edema phenomenon.

Supplemental Figure 4. Evans blue staining documented on gross assessment of epicardial surface provides visual confirmation of injectate retention.

Supplemental Figure 5. Evaluation of cardiac enzyme change with delivery of saline versus MSC into procine hearts.
Supplemental Figure 1
A  Darcy System injection without washout

\[
\begin{align*}
&\begin{aligned}
    \{ & u + K_{is} \nabla p = 0 \\
    & \text{div } (u) = 0 \\
    & u \cdot n = \frac{V_{inj}}{T_{inj} A_{inj}} \\
    & u \cdot n = 0 \\
    & p = p_{is}^0
\end{aligned} \quad \text{in } \Omega \times (0, T_{inj}) \\
&\begin{aligned}
    & u \cdot n = \frac{V_{inj}}{T_{inj} A_{inj}} \\
    & u \cdot n = 0 \\
    & p = p_{is}^0
\end{aligned} \quad \text{on } \Gamma_{inj} \times (0, T_{inj}) \\
&\begin{aligned}
    & u \cdot n = 0 \\
    & p = p_{is}^0
\end{aligned} \quad \text{on } \Gamma_{epi/endo} \times (0, T_{inj}) \\
&\begin{aligned}
    & p = p_{is}^0
\end{aligned} \quad \text{on } \Gamma \setminus \{ \Gamma_{epi/endo} \cup \Gamma_{inj} \} \times (0, T_{inj})
\end{align*}
\]

<table>
<thead>
<tr>
<th>value</th>
<th>def.</th>
<th>description</th>
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</thead>
<tbody>
<tr>
<td>c(x)</td>
<td>$\frac{N_{MS}}{V}$</td>
<td>free microspheres per volume interstitial space</td>
</tr>
<tr>
<td>$c_0$</td>
<td>$\frac{N_{MS}}{V_{inj}}$</td>
<td>MS-concentration in injection fluid</td>
</tr>
<tr>
<td>$\sigma_{is}(x)$</td>
<td>$\frac{N_{MS}}{V}$</td>
<td>retained microspheres per volume IS</td>
</tr>
<tr>
<td>$\sigma_b$</td>
<td>$\frac{N_{MS}}{V}$</td>
<td>lost microspheres per volume blood</td>
</tr>
<tr>
<td>$\phi$</td>
<td>$\frac{L^3}{V}$</td>
<td>porosity: volume of blood in total volume</td>
</tr>
</tbody>
</table>

B  Darcy System injection retention/washout coefficient added

\[
\begin{align*}
&\begin{aligned}
    \partial_t ((1 - \phi)c) + \text{div } ((1 - \phi)uc) = -(1 - \phi)k_{is}c - (1 - \phi)k_w c
\end{aligned} \quad \text{in } \Omega \times (0, T_{inj}) \\
&\begin{aligned}
    \partial_t ((1 - \phi)\sigma_{is}) = (1 - \phi)k_{is}c \\
    \partial_t (\phi \sigma_b) = (1 - \phi)k_w c + \phi c_0 \frac{V_{inj}^w}{T_{inj} V_b}
\end{aligned}
\end{align*}
\]

C  Darcy System with retention/washout and pressure coefficients

\[
\begin{align*}
&\begin{aligned}
    \text{div } (u) = -\beta^{osm}(p - p_{is}^0) \\
    u = -K_{is} \nabla p
\end{aligned} \quad \text{in } \Omega \times (0, T_{inj}) \\
&\begin{aligned}
    u \cdot n = u_{inj} = \frac{V_{inj}}{T_{inj} A_{inj}} \\
    p = p_{is}^0
\end{aligned} \quad \text{on } \Gamma_{inj} \\
&\begin{aligned}
    p \geq p_{is}^0 \quad \text{to} \quad p \leq p_{is}^0
\end{aligned} \quad \text{on } \Gamma \setminus \Gamma_{inj}
\end{align*}
\]

D  Integration of a parameters in a reaction retention model

\[
\begin{align*}
V_{inj} = V_{absorbed} + V_w \\
= \int_0^{T_{inj}} \int_{\Omega} \beta^{osm}(p - p_{is}^0) d\Omega dt + \int_{T_{inj}}^t \int_{\Gamma_{inj}} u \cdot nd\Gamma dt
\end{align*}
\]

Supplemental Figure 2
A  Injected versus retained cell relationship assessed in saturation model

\[
\frac{k_{is} + k_w}{k_{is}} \int_{\Omega} \phi \sigma_{is} \, d\Omega + \int_{\Omega} \phi c \, d\Omega = \frac{c_0 V_{inj}^i t}{T_{inj}}
\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
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<tbody>
<tr>
<td>(k_{is})</td>
<td>s(^{-1})</td>
<td>0.1</td>
</tr>
<tr>
<td>(k_w)</td>
<td>s(^{-1})</td>
<td>0.0</td>
</tr>
<tr>
<td>(\beta)</td>
<td>cm(^2) s(^{-1}) dyn(^{-1})</td>
<td>(0 - 1 \times 10^{-4})</td>
</tr>
<tr>
<td>(\sigma_{max})</td>
<td>-</td>
<td>0.01</td>
</tr>
</tbody>
</table>

B  Pro-elastic modeling incorporated tissue distortion

\[
\begin{align*}
(\rho_0 + m) \ddot{y} &= \nabla \cdot \left( \frac{E}{\Sigma} \right) \\
\nabla \cdot w + \frac{1}{JM \int dt} \left( \frac{f'}{M f^2} (p - p_0) \nabla \cdot u^s \right) &= -b \nabla \cdot u^s - \beta p \\
w &= -K \cdot \nabla p \\
p - p_0 &= M f(J) \left( b(1 - J) + \frac{m}{\rho_0 f} \right) \\
\partial_t (\phi c) + \nabla \cdot (\phi u^f c) &= -k_{is} \phi c \left( 1 - \frac{\sigma_{is}}{\sigma_{max}} \right) - k_w \phi c \\
\partial_t (\phi \sigma_{is}) &= k_{is} \phi c \left( 1 - \frac{\sigma_{is}}{\sigma_{max}} \right) \\
\partial_t \sigma_b &= k_w \int_{\Omega} \phi c \, d\Omega \frac{V_{inj}}{V_b} + c_0 \frac{V_{inj}}{T_{inj} V_b}
\end{align*}
\]

\text{in } \Omega \times (0, T_{inj})

Supplemental Figure 3
Supplemental Figure 4
** p-value < 0.01 treated versus control at a given time point (Wilcoxon)

* p-value < 0.05 treated versus control at a given time point (Wilcoxon)

# p-value < 0.01 treated 24h versus treated 12h (Wilcoxon)

Supplemental Figure 5