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STATE-OF-THE-ART PAPER

Recommendations for Successful Training on Methods of Delivery of Biologics for Cardiac Regeneration

A Report of the International Society for Cardiovascular Translational Research

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The field of myocardial regeneration (angiogenesis and myogenesis) might prove to play an important role in the future management of cardiovascular disease. Stem cells are currently undergoing testing in Phase I and Phase II clinical trials. Methods of delivery will affect the outcome of such therapies, perhaps significantly. This document provides suggested guidance in 4 methods of delivery: endocardial, intracoronary, coronary sinus, and epicardial. (J Am Coll Cardiol Intv 2010;3:265–75) © 2010 by the American College of Cardiology Foundation

The mission of the International Society for Cardiovascular Translational Research (ISCTR) is to expedite scientific discovery into clinical application by providing an environment for collaboration and guidance among basic scientists, clinical scientists, sponsors, and regulatory authorities and to disseminate the science among the scientific community; promote research, development, and application of standards of care and guidelines; influence health care policy; educate the public; and improve the wellbeing of patients.

The report of the American Heart Association for the year 2005 documented that the incidence of myocardial infarction (MI) in the U.S. reached 850,000. Only one-third of these patients had a full recovery. Approximately 550,000 developed congestive heart failure, and 150,000 patients were diagnosed with refractory angina despite optimal medical care. The cost of coronary artery disease and congestive heart failure reached 170 billion dollars for that year (1).

The field of myocardial regeneration (angiogenesis and myogenesis) might prove to play an important role in the future management of such patients. Gene, protein, and stem cell therapies are currently being tested in both Phase I and Phase II clinical trials in the U.S. as well as Europe and Asia. Methods of delivery will likely affect the outcome of such therapies, perhaps significantly. This document aims to provide adequate guidance for delivery methods that fall into 4 categories: 1) endocardial; 2) intracoronary; 3) coronary sinus; and 3) epicardial.

Program Summary

The purpose of the task force recommendations is to provide suggested guidance for training of physicians on the delivery of stem cells for myocardial regeneration. The catheter training program will provide training to personnel, including physician investigators, cardiac catheterization laboratory staff, and coordinators involved in clinical trials

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using the devices. However, this document will emphasize the satisfactory training of physician investigators.

The complete training program for the catheter delivery system consists of 3 phases. Phase 1 should include a tutorial related to the variety of available percutaneous catheters and mapping technology. Phase 2 consists of instruction in pre-clinical (porcine) endocardial injections. Phase 3 involves investigational clinical cases proctored by the lead physician trainer.

Delivery of Biologics

The delivery methods identified use catheter systems to accurately deliver stem cells or other therapeutic substances to the myocardium. Biologics can be delivered via different routes: intracoronary (IC), intramyocardial (IM), and IV. There are advantages and disadvantages of each route and variable retention rate of cells in the myocardium. Hou et al. (2) demonstrated retention rates of 3% (IC), 11% (IM), and 3% (IV). High numbers of cells continue to travel to the lungs—47% (IC), 26% (IM), 43% (IV)—and lesser numbers travel to the liver, spleen, and kidneys. A limitation of cell therapy for heart disease is that stem cells injected directly into the myocardium are capable of entering the vasculature and migrating to remote organs. A variety of factors might play a role in the retention of stem cells in the myocardium, including adding collagen matrix to the cells (3–6).

Abbreviations and Acronyms

IC = intracoronary
IM = intramyocardial
ISCTR = International Society for Cardiovascular Translational Research
LAD = left anterior descending
LAO = left anterior oblique
LV = left ventricle/ventricular
MI = myocardial infarction
OTW = over-the-wire
PVC = premature ventricular contraction
RAO = right anterior oblique
2D = 2-dimensional
3D = 3-dimensional

Unresolved Issues

Several issues remain to be addressed. There is a need for a better understanding of the mechanisms underlying cell retention after coronary delivery in relation to the timing and extent of myocardial injury. This might better define the effective time window for coronary cell transfer after MI. In this regard, effects of ischemic pre-conditioning or similar approaches, such as ultrasound, should be explored as new approaches to enhance acute cell retention. Although the ideal end point for cell therapy is unknown at this point in time, many investigators have used objective findings such as size of infarct in patients with acute MI. Others have used quality-of-life assessments such as New York Heart Association functional classifications or Minnesota Living with Heart Failure questionnaires. Objective measurements for patients with congestive heart failure, such as left ventricular (LV) dimension, can be measured by echocardiography, and LV volume can be measured by nuclear single-photon emission computed tomography imaging. The exercise stress test and nuclear imaging have been used in patients with refractory angina. Overall, composite end points such as repeat hospital stay, revascularization, or mortality might be desirable.

Endocardial Delivery of Biologics

This delivery method uses catheter systems to accurately inject stem cells or other therapeutic substances into the endocardium of the LV.

Personnel. The training physician/proctor will provide all hands-on training. The trainer will be responsible for the evaluation of all investigator trainees, proctoring the initial 3 clinical cases; documentation of the training; and needs for additional training. The trainer should be board certified in Interventional Cardiology or equivalent if outside the U.S. and established as an expert in the field of catheter-based delivery and cell or gene therapy The trainer will have significant experience and demonstrate successful completion of 20 cell or gene transplant procedures in large animals and 20 cell or gene transplant procedures in humans. In addition, the trainer should be a knowledgeable and capable instructor or proctor.

The preclinical staff, including the veterinarian, will have prior extensive training and experience in the oversight, care, and welfare of the animals. The clinical specialists are personnel who have extensive experience training customers and in providing technical support and education. The clinical trial research coordinator and cardiac catheterization laboratory staff will assist with the procedure and with case report documentation during clinical cases.

Catheter systems. There are varieties of catheters available for endoventricular delivery. For endoventricular delivery, investigators use either a fluoroscopic 2-dimensional (2D) guidance system (Biocardia helical infusion catheter; BioCardia, South San Francisco, California) or a 3-dimensional (3D) guidance system (Myostar Injection Catheter, Biosense Webster, Diamond Bar, California) (7,8). The advantages of 3D over 2D guidance are that the 3D system has the ability to identify the area intended for injection as well as the boundaries of the MI. Consequently, the investigator is able to perform the injections with increased accuracy. Furthermore, 3D guidance potentially allows for homogeneous distribution of the injections, decreasing the risk of local toxicity (adverse event related to cell concentration) and increasing efficacy. The disadvantage of 3D guidance is that the current mapping system requires significant training and skills and can be time consuming.

BioCardia's Helical Infusion Catheter (Fig. 1) is a catheter with a small, hollow, distal corkscrew needle, which

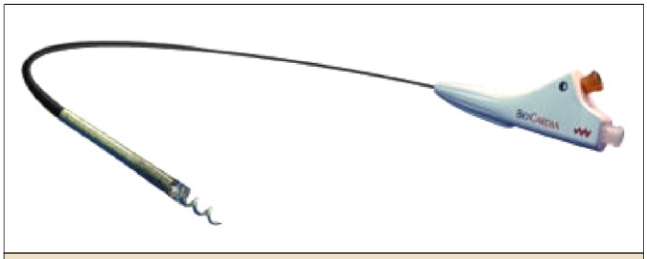

Figure 1. BioCardia Helical Infusion Catheter With Dual Fluid Ports

The BioCardia Helical Infusion Catheter has 2 fluid ports, 1 for therapeutic agent and 1 for contrast. Image reprinted with permission of Springer Science and Business Media (17).

might be rotated into tissue to provide active fixation during drug delivery similar to the active fixation electrodes used in cardiac pacing. This catheter is under investigational use in multiple ongoing clinical trials (9).

This system provides a means for fixation to the beating heart wall, uses simplified fluoroscopic imaging, crosses the aortic arch and valve safely over a guidewire with BioCardia's unique steerable guide, and allows 3 degrees of freedom to maximize operator control.

The Morph Deflectable Guide Catheter can be used to steer a guidewire across the aortic valve for transendocardial delivery from within the LV. For transendocardial delivery, the Morph Deflectable Guide Catheter is advanced over the wire, and the wire is removed to allow for advancement and navigation of the Helical Infusion Catheter (10).

The Myostar Injection Catheter (Biosense Webster) is a multi-electrode, percutaneous catheter with a deflectable tip and injection needle designed to inject soluble agents or cells transendocardially into the myocardium (Fig. 2). The tip of the injection catheter is equipped with a location sensor and a retractable, hollow, 27-gauge nitinol needle for fluid and/or cell delivery. Tip deflection is controlled at the proximal end by a tubular hand piece holding a piston and pull-wire mechanism. The high-torque shaft allows for controlled rotation of the curved tip to facilitate accurate positioning toward the desired site for agent injection (11,12). A second handle located at the proximal end of the catheter allows for controlled needle extension from the distal tip. The extended length of the needle is adjustable from 2 to 10 mm. The handle has a standard luer lock fitting for a syringe connection. The catheter interfaces with the NOGA (Biosense Webster) 3D electromagnetic cardiac mapping system for navigated local agent delivery into the myocardium (13). The NOGA map is a 3D reconstruction of the LV from points obtained by the mapping catheter. The NOGA map discriminates between areas of MI, the border zone, and normal myocardium (Fig. 3). With the combination of echocardiography and low voltage, one can identify the area of MI and thin wall (14).

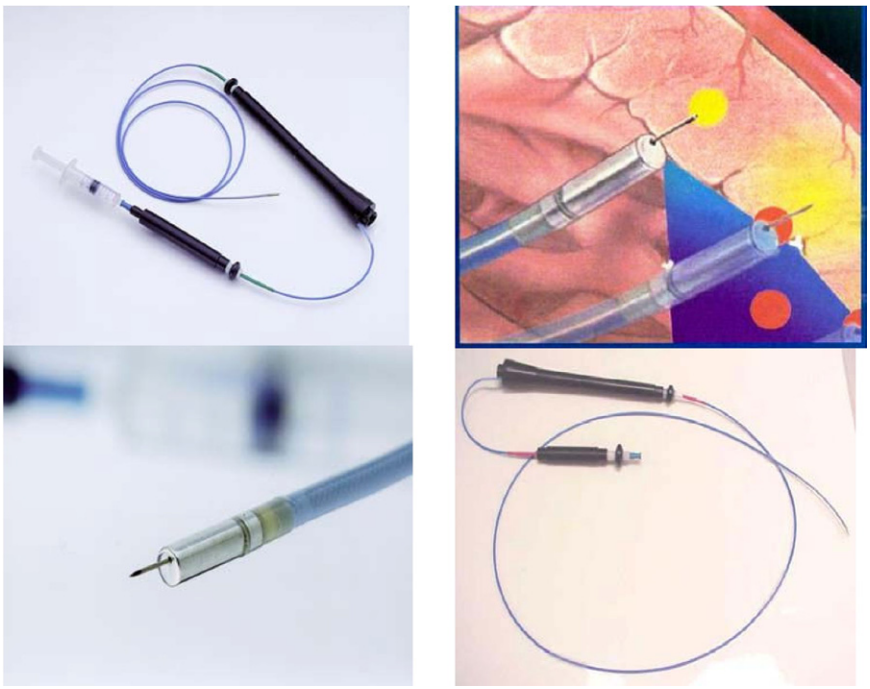

Figure 2. Myostar Cordis-Biosense Webster Needle Injection Catheter

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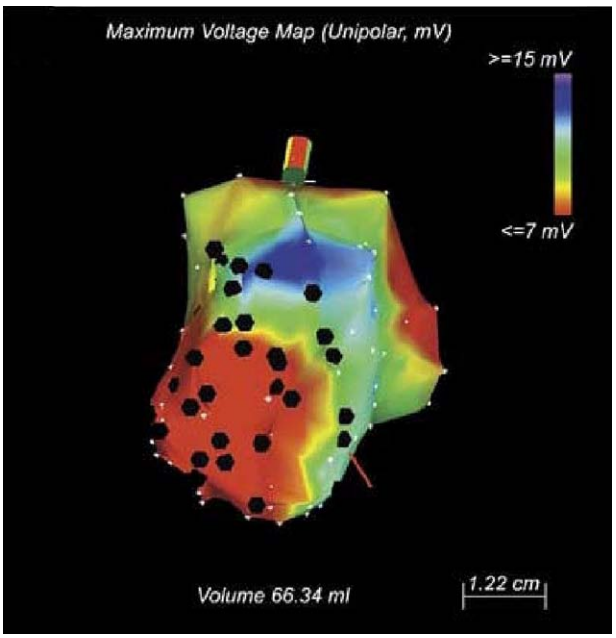

Figure 3. NOGA Mapping in Porcine Model

NOGA mapping in porcine model shows the location of injections in normal myocardium **(blue and green)**, chronic infarction **(red)**, and border zone of infarction **(yellow)**. Images have been reprinted with permission of Springer Science and Business Media (17).

Tutorial and injection catheter preparation instructions. A descriptive overview of the catheter system and instructive training can be provided by the manufacturer representative. A didactic review of all devices will include manufacturer directions for use, capacities, contraindications, and associated risks. Instruction should include: hands-on practice of catheter manipulation and injection procedure; review of the risks of the procedure and criteria for technical success; practice of injection catheter loading and delivery requirements; and a primer on how to determine catheter needle length and documentation of needle measurements before and after injection.

Pre-clinical training. The objective of the preclinical training program is to instruct and evaluate procedural performance of clinical investigators in the use of the catheter system in a porcine model as preparation for use in humans. The clinical indication for cell therapy might involve the treatment of acute MI, chronic MI, heart failure, and stable angina. Each clinical scenario will require different skills, proficiency, and training, but basic requirements are similar. The requisite instruction should be focused on targeting the therapy to the area of MI or ischemia while minimizing complications such as vascular injury and perforation. Myocardial tissue textures differ among normal musculature, ischemic tissue, peri-infarct zones, acute or chronic MI, and heart failure.

Animal model. For catheter-based cell transplantation studies, a large animal model is required to simulate conditions in humans (15). A swine model of MI produced by coil placement or balloon inflation in the left anterior descending (LAD) artery is a reproducible model and simulates MI in a human heart. de Prado et al. (16) demonstrated LAD balloon occlusion for 75 min caused a 20% compromise of LV and a mortality rate of 33%.

A coil model is preferred to surgical ligation, because mortality rates have been much lower with coils versus surgery (9% vs. 50%) (18). By placing coils distal to the diagonal, one can produce ejection fractions of 44% at 1 month and 41% at 3 months after MI (11). The post-MI swine remodeling process is similar to humans. The clinical investigator will be required to make an adjustment in the delivery technique by increasing pressure on the catheter during needle advancement, due to an increase in fibrotic tissue in the area of chronic MI, and must be aware that the incidence of premature ventricular contractions (PVCs) secondary to needle advancement is less likely. The Food and Drug Administration has accepted the described model to evaluate safety and efficacy of stem cell therapies.

Acute MI model. Myocardial infarction can be induced percutaneously in pigs by either balloon or coil occlusion in the coronary arteries. The MI can be achieved via balloon inflation within 60 to 90 min, whereas the coil method achieved total occlusion of blood flow in approximately 10 min. In approximately 4 to 10 days after MI injection, procedures can be done.

Chronic MI model. In the swine model, coil infarction forms scar tissue after 6 weeks (18). This resembles the chronic myocardial injury that occurs in humans. The procedure and training on the swine 6 weeks after MI will serve to qualify the trainee for cell transplantation in chronic MI and ischemic cardiomyopathy in humans.

In the chronic MI swine model, where contrast was injected in the myocardium under 3D electromechanical mapping guidance, only 30% of the injections within the scar were associated with PVCs. The standard notion that producing a PVC during needle penetration is indicative of successful injection might not apply for infarcted tissue. Sixty percent of the injections in the border zone were associated with PVCs. However, 90% of the injections in normal myocardium were associated with PVCs (17). This model will require more technical skills and training than the other models to guarantee successful injections. The challenges emerge from the following:

1. Lack of PVCs that provide assurance of needle penetration of the myocardium.
2. The scar tissue might make needle penetration more difficult.

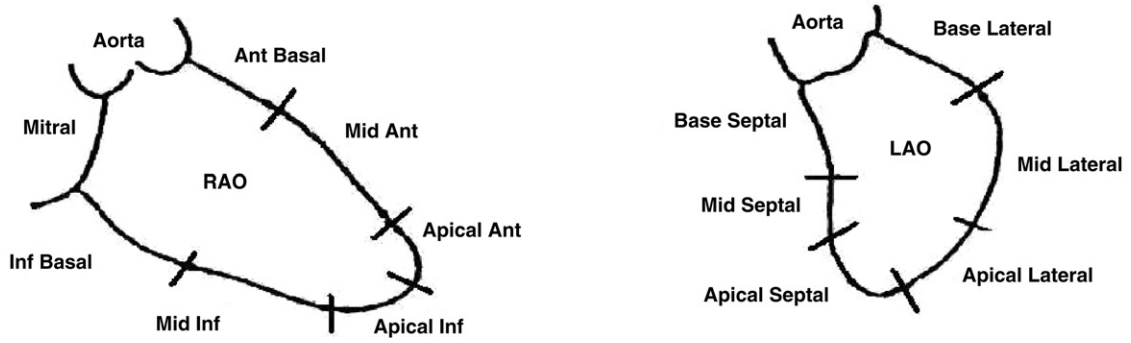

Figure 4. Segments of the RAO and LAO Projections

Ant = anterior; Inf = inferior; LAO = left anterior oblique; RAO = right anterior oblique.

3. The reduced thickness of the scarred myocardium might increase the risk of perforation and extravasations into pericardial space.

Consequently, operators need to develop tactile familiarity with the catheter and stability of the catheter during injections.

Procedure detail. LEFT HEART CATHETERIZATION. An 8-F sheath is introduced into the femoral artery after puncturing the femoral artery with the modified Seldinger technique or via a surgical cut down. After insertion of the arterial sheath, heparin is administered and supplemented as needed to maintain an activated clotting time of 200 to 250 s throughout the interventional portion of the procedure. A 6-F pigtail catheter will be used for left ventriculography performed in left anterior oblique (LAO) 7° to 15° and right anterior oblique 90 views (porcine model).

The right anterior oblique projection will identify the anterior basal, mid anterior, apical anterior, inferior, mid inferior, and inferior basal segments. The LAO projection will identify the basal lateral, mid lateral, apical lateral, apical septal, mid septal, and basal septal segments (Fig. 4).

INTRA-MYOCARDIAL INJECTION PROCEDURE. The catheter is placed via the femoral sheath and advanced to the aortic valve in a retrograde fashion. After full tip deflection in the descending aorta, gently prolapse the rounded distal tip across the aortic valve, then straighten the catheter tip toward the apex within the LV cavity.

The injection catheter is oriented to the treatment zone with fluoroscopic guidance. If the 3D guidance system is used, 3D imaging and mapping are also used. The injection needle is extended into the myocardium to a depth of approximately 4 to 6 mm, adjusted to wall thickness on the basis of prior echocardiographic assessment (approximately 50% of wall thickness). Four injections/LV wall (anterior, lateral, inferior, septal) are performed for a total of 16 injections. The operator must accurately make 3 of the 4 injections/wall. Each injection contains 0.2 ml of solution and is to be infused over a minimum of 20 s. A different color dye for each wall is used containing diluted mixed contrast tissue marking dye (10% dye + 40% contrast, 50% saline). The time (seconds) and volume (milliliters) for each injection are to be documented.

Accurate delivery will be evaluated by targeting well-defined areas on the NOGA map, provided 3D mapping system is used. In this case, injections should be separated by approximately 1 cm (Fig. 3). If a 2D catheter system is used, injection sites will be evaluated by a pathology exam. In either case, leakage of dye into the systemic circulation will be considered a failure of the procedure.

PATHOLOGY EVALUATION. The goal of the gross heart tissue evaluation is to accurately identify injection sites and evaluate any structural injuries. Because a different colored dye was used for each wall, the trainer should easily be able to identify the sites (Fig. 5).

The animal will be killed with pentobarbital per institutional standard operating procedure. The heart will be explanted, and gross dissection of the LV will be performed. The criteria for a successful procedure are as follows: the absence of LV perforation, pericardial effusion, and damage to cardiac structure (e.g., aortic and mitral valve structures, coronary and great vessels) due to the test device. Achieve an injection accuracy of 75% (3 of 4 injections/wall with the assigned dye).

Training goals. The trainer should visually assess trainee's technique, handling of equipment, and catheter use in conjunction with fluoroscopy. The following training goals should be verified by the physician trainer**:** the proper targeting of sites; animal stability including absence of sustained arrhythmias, ventricular tachycardia with/without cardioversion, severe hypotension (systolic blood pressure <60 mm Hg), and sustained tachycardia or bradycardia (change in heart rate >40% from baseline); and the ability to manipulate the catheter to achieve

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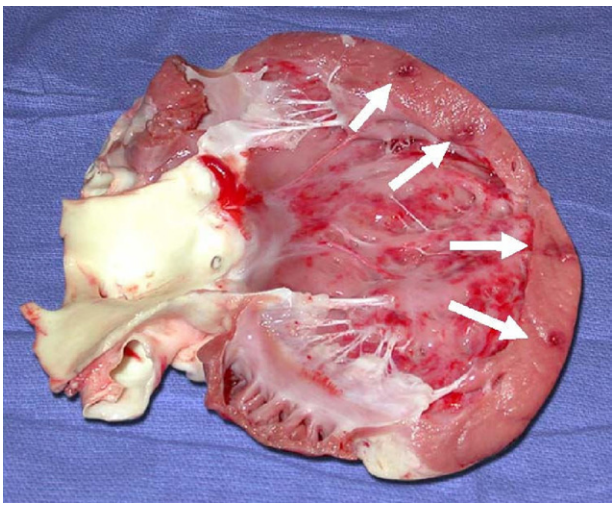

Figure 5. Methylene Blue Dye as Injected With the MyoStar Injection Catheter

Arrows indicate site of injection. Images have been reprinted with permission of Springer Science and Business Media (17).

a sufficiently stable endocardial position to perform the injection procedure.

Training should include techniques to avoid complications such as vascular injury of the iliac artery and aorta during the advancement of the catheter to the LV; catheter trauma to the coronaries due to inadvertent placement of the injection catheter into the coronary ostium, which might result in dissection, abrupt closure, perforation, or severe ischemia; trauma to the aortic valve causing hemodynamic compromise associated with acute aortic regurgitation; perforation or trauma to the mitral valve structure due to placement of the catheter or injector or due to needle puncture and injections; and LV perforation due to catheter placement or needle penetration into the pericardial space.

If the criteria described in the preceding text are met, the trainee should have successfully completed the preclinical training. If the goals are not met, the trainee should be required to repeat the training program. The investigator/trainee should also repeat the entire training protocol if there are any procedural adverse events during the clinical trial directly related to the injection (or mapping if the electromechanical mapping system is being used) or if more than 3 months of time have lapsed between completion of the training program and the first clinical case. Therefore it is recommended that the catheter training program not be completed until investigator site institutional review board approval and initial patient screening and consent have been completed.

Clinical training/case proctoring. After successful completion of the tutorial and preclinical training (i.e., all skills criteria have been met), the investigator trainee might begin scheduling clinical cases. The first 3 cases should include a representative of the catheter manufacturer and a physician trainer. Due to the possibility of reduced proficiency in this method over time, it is critical that there is no more than a 3-month lapse between completion of preclinical training and the first patient procedure and every following procedure.

After each mentored procedure, the investigator trainee's skills and knowledge of the catheter system will be documented and reviewed by a trainer physician proctor. The physician proctor might make further training recommendations if necessary. Any procedural event that occurs during the injection will be documented. The physician proctor has the option to increase the number of required mentored cases and can recommend remediation of any part of training.

Training program modification. This training program should be reviewed at least annually by the physician trainer and might be modified on the basis of trainee feedback, including content and passing requirements (i.e., preclinical evaluation, number of proctored cases) or any Food and Drug Administration recommendations or feedback. Modification to training might also be based on the progress of clinical trials and Data Safety Monitoring Board recommendations, new information on the device/interactions, the evaluation of procedure effectiveness, investigator qualifications, emerging issues and trends, any clinical safety results pertaining to use of the device, protocol-specific requirements, or updated source documents upon which training is based.

Suggested basic requirements should include:

1. Board certified in interventional cardiology or equivalent if outside of the U.S.
2. Attendance at 2 days of animal training.
3. Demonstrate acceptable mapping skills; identification of the apex, mitral valve, left bundle branch location, and LV outflow tract.
4. Perform a total of 16 contrast injections in the appropriate animal model, 4 in each wall (anterior, inferior, lateral, and septal).
5. Able to target 75% (12 of 16 injections) of total required injections and 75% (3 of 4 injections) in each wall identified by gross pathology (Fig. 5) for 2D catheter use and by NOGA mapping for 3D catheter use.
6. Absence of the following serious complications during the procedure: death, perforation, contrast leak into systemic circulation, left bundle branch block, mitral valve damage, aortic dissection, and coronary artery dissection.
7. Perform the 3 initial proctored clinical cases successfully.

Training records. All training sessions should be recorded and copied on appropriate (dated and signed) case report forms and filed with the sponsor and individual investiga-

tional sites. The ISCTR will issue certificates and retain copies of completed training records.

IC Delivery of Biologics

Coronary cell transfer is based on the combination of multiple factors that influence myocardial cell retention after myocardial injury. These factors include cell adhesion on the endothelial layer, transendothelial (vascular) passage and migration, and the presence of chemoattractive and adhesive forces in the myocardium. These factors lead ultimately to cell retention in the perivascular region and in the myocardium (19). If cells are given IC—mainly bone marrow mono nuclear cells—they might reside in any zone or location. Most of the current clinical trials are using a dose escalating model to determine the optimal number of cells delivered.

Setting and applications. Intracoronary infusion has been the method of choice for nearly all studies in patients with ST-segment elevation MI. Other applications include studies in chronic myocardial ischemia or ischemic LV dysfunction. Coronary transfer requires that target myocardium be subtended by an angiographically patent coronary artery or identifiable collateral vessel. The potential advantage of such coronary cell transfer is the homogenous distribution of the cells in the subtended target territory. Although distribution is important, it might not translate to survival. It is known that bone marrow-derived stem cells survive when transplanted into normal muscle or along the border zone of an MI, whereas myoblasts can be transplanted into an area with less blood flow, such as the scar area of an MI.

Devices. Guide (5-F) and specialty catheters are preferred for selective cell infusion, given the caliber of their internal diameters. The technique typically uses commercially available devices, such as over-the-wire (OTW) angioplasty balloon catheters with short 8-mm balloons, to minimize potential vascular trauma (Fig. 6). New devices are being tested for the coronary perivascular (adventitial) delivery with the microneedle injection balloon catheter (Mercator, San Leandro, California).

Timing. The optimal time window for the coronary cell transfer seems to depend on the balance between multiple putative and detrimental factors governing the infarction healing. This seems to be most favorable between days 3 and 7 after the infarction. The kinetics and timing of coronary arterial cell delivery have received little attention in preclinical studies, and transition from isolated small animals to humans was rapid. Consequently, much of what is known regarding cell retention after IC administration has been learned from clinical studies. Clinical studies found a positive functional outcome if the cell injections were performed beyond day 3 (20). By contrast, efficacy of coronary cell transfer at the later stages or in the chronic

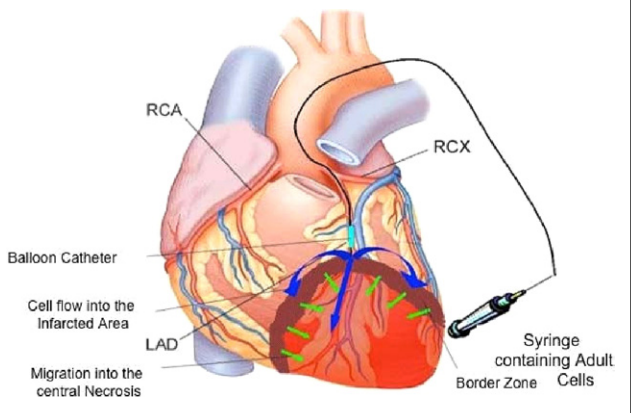

Figure 6. Intracoronary Delivery of Biologics

Images have been reprinted with permission of Springer Science and Business Media (17). LAD = left anterior descending coronary artery; RCA = right coronary artery; RCX = right circumflex artery.

setting is still a matter of ongoing research and requires better understanding of humoral mediated homing.

Methodology. The basic set-up for IC delivery of biologics is similar to balloon angioplasty with full heparinization (100 IU/kg) and hemodynamic and electrocardiographic monitoring. The most commonly applied technique of coronary cell transfer is anterograde delivery with selective coronary injections. Cells are injected through the central lumen of the delivery catheter at slow or high flow rates while maintaining coronary flow (nonocclusive) or interrupting it with balloon occlusion ("stop-flow" method). In both cases, care is taken that the delivery catheter is flushed with heparinized saline before use to prevent intraluminal clotting.

Currently, optimal timing for stem cell transplant is unknown. There are multiple clinical trials underway to investigate appropriate timing.

In the case of nonocclusive angioplasty method, a balloon catheter or specialty catheters are used for the sub-selective injection in the coronary vessel. The position of the catheter is determined by the extent of the target territory. The internal lumen is at the proximal end connected to the syringe containing the cell product. The cell product is infused as a continuous infusion or as a repetitive slow hand injection. In the case of hand injection, the cell product is loaded preferentially to the luer lock's small volume syringe, allowing smooth and controlled injections.

In the "stop-flow" method (Fig. 7), a balloon angioplasty (OTW) catheter is placed at the site of the stented segment in the vicinity of the culprit lesion in the infarct-related coronary artery. Care should be taken to avoid additional injury to the stented segment. Accordingly, balloon length should be smaller than the nominal length of the stent. The size should be comparable or, at most, 0.5 mm larger than

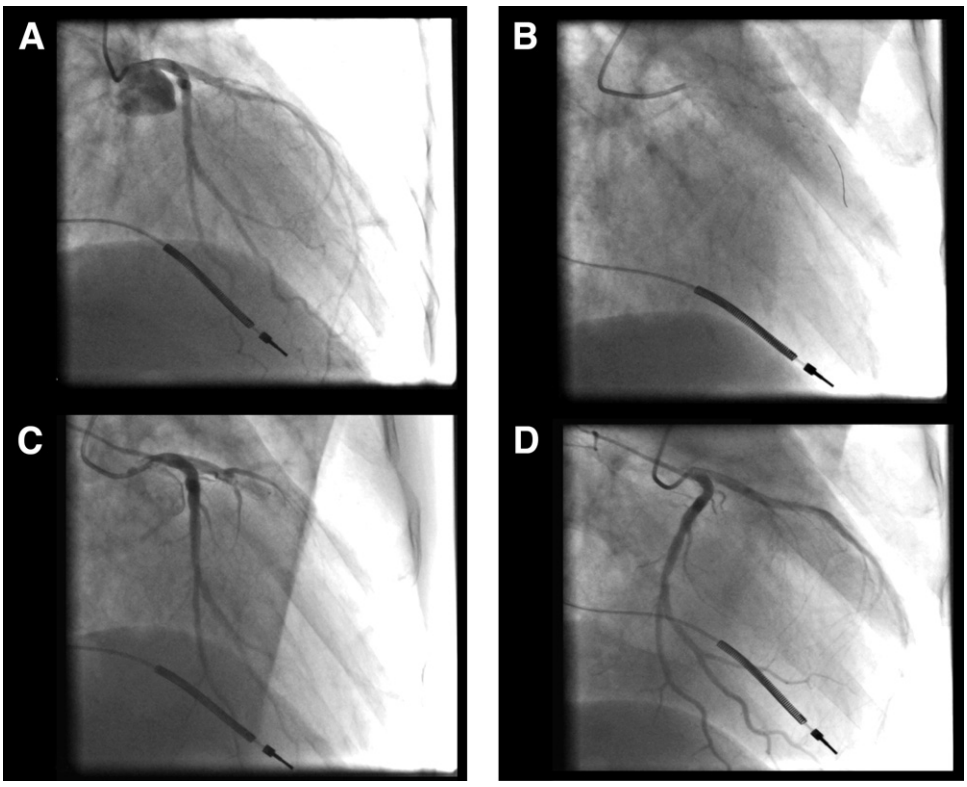

Figure 7. Procedure of Percutaneous Intracoronary Transplantation of Bone Marrow-Derived Stem Cells

For intracoronary delivery of the cells, an over-the-wire angioplasty balloon catheter oversized by 0.5 mm is used **(A and B)**. After positioning at the site of the former infarct-related coronary occlusion where the stent had been implanted, the balloon is inflated at low pressure (2 to 4 atms) to block blood flow **(B)**. Then, the guidewire is removed, and the bone marrow-derived stem cells suspension is infused through the internal lumen of the balloon under stop-flow conditions **(C)**. Bone marrow-derived stem cells are infused with a pump at 1 to 2 ml/min during periods of 3 min of inflation and cell infusion alternating with 1 min of de-inflation and reperfusion until the total dose of cells is given to the patient. Integrity of the coronary artery used for transplantation and blood flow perfusion in the supplied myocardial area are checked after the procedure **(D)**. Images have been reprinted with permission of Springer Science and Business Media (17).

the nominal size of the implanted stent. Likewise, low-pressure balloon inflations are applied and typically do not exceed the nominal inflation pressure. Before cell injection, test inflation followed by test contrast can be performed to document the coronary artery occlusion at the desired segment. The cell product is loaded in the larger luer-lock syringe (10 or 20 ml), connected via a 3-way stop-cock to a small volume luer-lock syringe (3 ml). The system is connected to the proximal internal lumen of the OTW balloon catheter (Fig. 8). Cells are injected typically in boluses of 3 ml. After balloon inflation, cells are slowly injected over approximately 1-min, with a total occlusive time of 3 min. This timing is empirically chosen and is associated with prolonged contact between the infused cell product and the subtended endothelial surface of the macro- and microcirculation. After the 3-min occlusion period, reperfusion is allowed for 2 to 3 min. The balloon occlusion and cell delivery cycle is repeated, followed by reperfusion, until the process is complete. During balloon occlusion, the patient is monitored for signs of ischemia, arrhythmias, or hemodynamic instability. If severe arrhythmias, intolerable ischemia, or major hemodynamic instability are observed during balloon inflation, then the procedure should be halted and injections modified according to the patient's tolerance. If slow flow or signs of ischemia persist after balloon deflation (or completion of injection via special catheter), care should be taken to dilate the microvasculature that might be partially obstructed by injected cells (especially if large cells or a high concentration of cells is used). Intracoronary administration of arteriolar vasodilator (nitroprusside, adenosine) should be considered. At the end of the procedure, vessel patency and flow in the coronary artery are checked by control contrast injections. For IC cell delivery at high flow rates after MI, cells are delivered in a single 10-ml bolus over 30 s (20 ml/min) with a prototype balloon catheter with a large inner lumen allowing delivery at high flow rates. The catheter is placed in the mid LAD, and the balloon is inflated to a 1:1 ratio. A single infusion at 20 ml/min might be optimal from a safety standpoint without significantly decreasing cellular delivery (21).

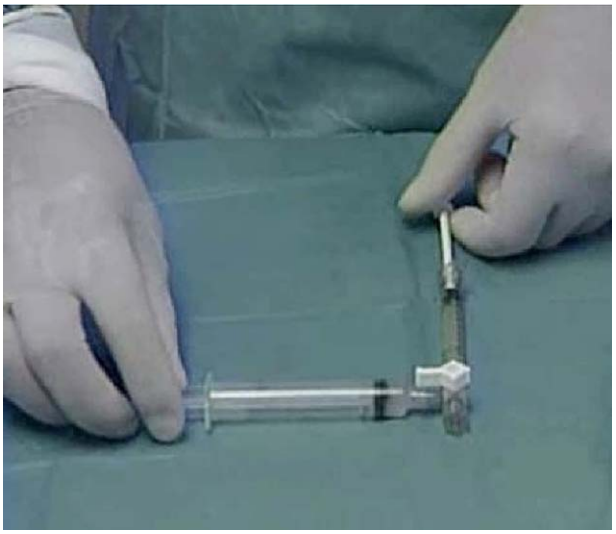

Figure 8. A 10-ml Luer Lock Syringe Connected Via 3-Way Stop-Cock to 3-ml Luer Lock Syringe

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Training requirements. Proctors should have experience in IC delivery of biologics (minimum of 10 cases) and be board certified in interventional cardiology or equivalent.

Trainees should observe at least 1 clinical case and successfully perform 2 proctored cases and be board certified in interventional cardiology (or equivalent).

Coronary Sinus Delivery of Biologics

Setting and applications. Percutaneous retrograde coronary sinus delivery is an alternative method for delivery of angiogenic proteins, genes, or stem cell therapy. The technique has been used for many years for delivery of cardioplegic solution in cardiovascular surgery and for protection against myocardial ischemia in patients undergoing high-risk percutaneous coronary interventions (22,23).

Retrograde coronary sinus delivery has been compared with both IC and IM delivery in preclinical models. Gene therapy studies using retrograde coronary sinus delivery demonstrated more homogenous delivery and increased gene expression compared with IC delivery and IM delivery: either surgical epicardial approach or percutaneous endocardial approach (24). Another study using retrograde coronary sinus delivery of fibroblast growth factor-2 demonstrated improved myocardial function and regional myocardial blood flow compared with IC delivery. Coronary sinus delivery resulted in a 2-fold increase in tissue binding of fibroblast growth factor-2 (25).

The technique seems to be very safe and has potential advantages for more homogenous delivery across the myocardium. Theoretically, it might be lower risk than IM delivery, with more uniform and higher rates of delivery. The coronary sinus approach would also be available for patients excluded from trials using percutaneous endomyocardial delivery, such as patients with severe aortic valve disease. Compared with IC delivery, it has the theoretical advantage of more homogeneous delivery, in particular, in patients with severe subtotal stenosis (26).

Methodology. The technique involves placement of a catheter into the coronary sinus via either the internal jugular or femoral vein, with the infusion catheter placed over a wire. A single or double balloon is inflated, followed by infusion of the angiogenic agent for 5 to 30 min at a pressure approximately 20 ml higher than the coronary sinus pressure. The balloon inclusion time and infusion pressure might vary depending on the indication.

The TransAccess MicroLume Intramyocardial Injection System (Medtronic, Inc., Santa Rosa, California) might be an appropriate system to use for this delivery method. This system can be used for angiogenesis, and myogenesis through its venous approach with fluoroscopic guidance and accurate, stable targeting is achieved. It allows epicardial, tangential access to the myocardium, with a reduced risk of embolization.

Training requirements. Proctors should have experience in IC delivery of biologics (minimum of 10 cases) and be board certified in interventional cardiology or equivalent.

Trainees should observe at least 1 clinical case and successfully perform 2 proctored cases and be board certified in interventional cardiology (or equivalent).

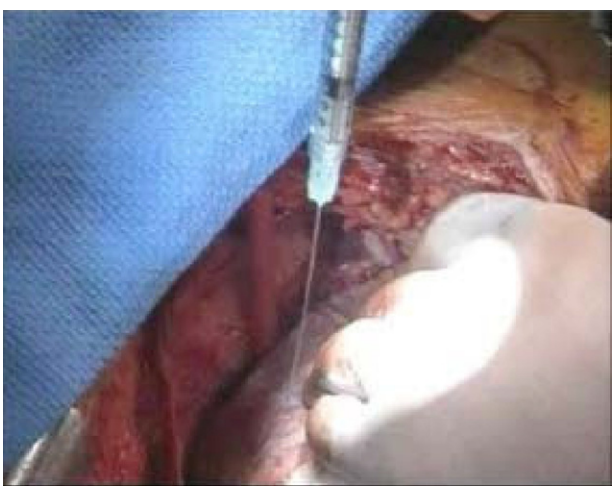

Figure 9. Epicardial Delivery of Biologics by Direct Exposure and Injection

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Epicardial Delivery of Biologics

Settings and applications. Epicardial delivery for cell therapy is considered the most reliable method. Cells are injected into the epicardium, which is highly accessible, due to exposure by surgical incision (Fig. 9). Avoidance of injection into epicardial arteries is achieved easily by direct visualization. The concentration of the number of cell injections will depend on the dose intended for the patient (27).

The areas of injection are identified before surgery by echocardiography and nuclear imaging and during surgery by direct visualization. Transplanted cells are targeted to ischemic areas and/or scar tissue. Injections can be made into a beating or arrested heart and are made with either a 27-gauge customized pre-bent needle (for multiple perpendicular injections) or a straight needle, depending on the technique used.

The injection procedure can be performed by either of the following methods. For the first method, introduce the needle deep into the muscle in an oblique fashion. To verify that the needle is not inside the ventricle, apply suction to the syringe, no aspiration of blood should occur, then inject intended dose. Next, pull the catheter back 0.5 to 1 cm and inject the next intended dose. Repeat this process for the specific location. Use of this injection procedure will result in fewer puncture sites. The second method is to select separate injection sites for each intended dose, thus increasing the number of needle penetrations. The disadvantage of this approach is that it increases the likelihood of leakage, thereby decreasing the actual amount of injected material that can remain trapped in the myocardium. This issue can be addressed by closing the puncture sites by finger pressure or with a drop of surgical glue. The real efficacy of these procedures remains elusive.

It is currently unclear whether corticosteroids are needed to reduce inflammatory responses possibly generated by the injections. As such, IV corticosteroids might or might not be given at the time of injection and 24 h after injection.

In the future, improvements could come from either more elaborate computer-driven injection devices (as used for cell injections for patients with Parkinson's disease) that allow a tighter control over pressure, duration, and location of injections or through the replacement of injection by epicardial application of cell-seeded biocompatible patches or even scaffold-free cell sheets that are simply overlaid onto the target area and maintained by glue or a couple of stitches.

Training requirements. Proctors should have experience in IC delivery of biologics (minimum of 5 cases) and be board certified in interventional cardiology (or equivalent).

Trainees should observe at least 1 clinical case and successfully perform 2 proctored cases and be board certified in interventional cardiology (or equivalent).

All training recommendations are from the ISCTR to provide a framework for others who are interested in engaging in this new endeavor.

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Key Words: myocardial regeneration ■ stem cells ■ training ■ injection ■ catheter.