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A Novel Percutaneous Approach for Deployment of 3D Printed Coronary Stenosis Implants in Swine Models of Ischemic Heart Disease

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Corresponding Author:	Kim-Lien Nguyen, MD David Geffen School of Medicine at UCLA and VA Greater Los Angeles Healthcare System Los Angeles, CA UNITED STATES
Corresponding Author's Institution:	David Geffen School of Medicine at UCLA and VA Greater Los Angeles Healthcare System
Corresponding Author E-Mail:	klnguyen@ucla.edu
Order of Authors:	John Hollowed
	Caroline Colbert
	Jesse Currier
	Kim-Lien Nguyen
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Kim-Lien Nguyen, M.D.
David Geffen School of Medicine at UCLA
VA Greater Los Angeles Healthcare System
11301 Wilshire Blvd, MC111E
Los Angeles, CA 90073

August 29, 2019

Dear Members of the Editorial Board:

Enclosed, please find our manuscript entitled **"A Novel Percutaneous Approach for Deployment of 3D Printed Coronary Stenosis Implants in Swine Models of Ischemic Heart Disease"** by Hollowed et al. We are submitting the manuscript for exclusive consideration of publication as a ***Methods Article – JoVE Produced Video***. The contents have not been previously published. All authors agree to be accountable for all aspects of the work, have reviewed and agree with the contents of the manuscript. All authors have no relevant conflicts of interest to disclose. The co-authors have contributed significantly to the submitted work and their contribution are as follows:

Our work is of interest to a broad readership at *JoVE*, particularly readers with an interest in translational cardiovascular research using swine models of ischemic heart disease. We describe a novel technique for deploying 3D printed coronary stenosis implants to percutaneously create a swine model of myocardial ischemia. Our work has widespread implications for the development, testing, and validation of non-invasive diagnostic approaches in ischemic heart disease as well as for therapeutics aimed at reducing myocardial ischemic burden. Compared to open-chest approaches, the creation of our swine model is time-efficient, simple, and can be easily implemented.

Please address all correspondence concerning this manuscript to klnguyen@ucla.edu. Thank you in advance for consideration of our work. We look forward to hearing from you.

Sincerely,

Kim-Lien Nguyen, M.D.
Assistant Professor of Medicine and Radiology

TITLE:

Novel Percutaneous Approach for Deployment of 3D Printed Coronary Stenosis Implants in Swine Models of Ischemic Heart Disease

AUTHORS AND AFFILIATIONS:

John J. Hollowed[1], Caroline M. Colbert[2,3], Jesse W. Currier[1], Kim-Lien Nguyen[1,2,3]*

[1]Division of Cardiology, David Geffen School of Medicine and VA Greater Los Angeles Healthcare System, University of California Los Angeles, USA

[2]Physics and Biology in Medicine Graduate Program, University of California Los Angeles, USA

[3]Diagnostic Cardiovascular Imaging Laboratory, Department of Radiological Sciences, David Geffen School of Medicine, University of California Los Angeles, USA

Corresponding Author:

Kim-Lien Nguyen (klnguyen@ucla.edu)

Email Addresses of Co-authors:

John J. Hollowed (jhollowed@mednet.ucla.edu)

Caroline M. Colbert (colbertc@ucla.edu)

Jesse W. Currier (jesse.currier@va.gov)

KEYWORDS:

ischemia, swine, coronary artery, magnetic resonance imaging, coronary intervention, large animal model, ischemic heart disease

SUMMARY:

We describe a novel, cost-effective, and efficient technique for percutaneous delivery of three-dimensionally printed coronary implants to create closed-chest swine models of ischemic heart disease. The implants were fixed in place using a mother-and-child extension catheter with high success rate.

ABSTRACT

Minimally invasive methods for creating models of focal coronary narrowing in large animals are challenging. Rapid prototyping using three-dimensionally (3D) printed coronary implants can be employed to percutaneously create a focal coronary stenosis. However, reliable delivery of the implants can be difficult without the use of ancillary equipment. We describe the use of a mother-and-child coronary guide catheter for stabilization of the implant and for effective delivery of the 3D printed implant to any desired location along the length of the coronary vessel. The focal coronary narrowing was confirmed under coronary cineangiography and the functional significance of the coronary stenosis was assessed using gadolinium-enhanced first-pass cardiac perfusion MRI. We showed that reliable delivery of 3D printed coronary implants in swine models (n = 11) of ischemic heart disease can be achieved through repurposing mother-and-child coronary guide catheters. Our technique simplifies the percutaneous delivery of coronary implants to create closed-chest swine models of focal coronary artery stenosis and can be

performed expeditiously, with a low procedural failure rate.

INTRODUCTION:

Ischemic heart disease continues to be the number one cause of death in the United States[1]. Large animal models have been used experimentally to understand and characterize mechanisms driving coronary artery disease (CAD) and associated complications (including myocardial infarction, arrhythmic events, and heart failure), as well as for testing of new therapeutics or diagnostic modalities. Results from these studies have helped to broaden the understanding, diagnosis, and monitoring of ischemic heart disease and to advance clinical practice[2]. Several animal models including rabbits, dogs, and swine have been used. However, coronary stenoses, particularly discrete lesions, occur very rarely in these animals and are difficult to induce reproducibly[3]. Prior work described the creation of artificial coronary stenoses using ligation, occluders, or external clamps. Recently, we described how to use 3D printing technology to manufacture coronary implants that can be used to percutaneously create discrete artificial coronary narrowing[4]. Using computer-aided design software, we designed coronary artery implants as hollow tubes with varying inner and outer diameters as well as implant length and then fabricated them using commercially available additive materials. The implants are smooth, hollow, 3D printed tubes with rounded edges. We designed a library of implant sizes with a range of inner diameter, outer diameter, and length. The outer diameter of the implant is based on the size of the coronary guide catheter. The inner diameter is based on the size of a deflated coronary angioplasty balloon. We varied the length of the implant to tailor the desired severity of perfusion. However, safe percutaneous delivery of such devices can be challenging due to the lack of wires and catheters manufactured specifically for large animal use. In contrast, an extensive collection of catheters, wires, and supportive equipment are available for clinical use in human coronary arteries. In this work, we show how to repurpose a clinical grade mother-and-child coronary guide catheter for the delivery of the 3D printed coronary implants.

The GuideLiner catheter (**Figure 1A**) was developed for percutaneous coronary intervention (PCI) to allow for deep catheter seating and increased support for complex cases[5]. In our investigation, the GuideLiner catheter was chosen due to familiarity of use and availability, but similar catheters, where available, may also be considered. Considered a “mother-and-child” guide catheter (**Figure 1B**), the device fits inside a typical coronary guide catheter (“mother”) and is a coaxial flexible tube (“child”). This catheter can be inserted over a guidewire and effectively lengthens the reach of a typical coronary guide catheter by extending beyond the end of the coronary guide. The GuideLiner or a similar mother-and-child catheter can be used as added support for deployment of the 3D printed coronary implants. Because the implants are mounted over angioplasty balloons to be inserted as a unit over a coronary wire into the vessel (**Figure 1B**,**1C**), the catheter offers additional support to deliver the implant to the desired site. By positioning the mother-and-child catheter just proximal to the balloon, the implant remains at the desired location during balloon deflation and retraction. Despite having some firmness to its structure, the mother-and-child catheter’s unique ability to be advanced deep into coronary arteries over a guidewire and the radiopaque marker at the catheter tip were essential characteristics for implantation.

Our assembled delivery apparatus consisted of a typical coronary guide catheter, the mother-and-child catheter, and a 3D printed implant fixed onto a deflated coronary angioplasty balloon (**Figure 1B**). As a functional delivery unit, the mother-and-child catheter not only provided stable additional support for the delivery of the equipment but was also uniquely applied as a shearing device to keep the implants in place during deflation and removal of the balloon. The radiopaque marker at the catheter tip served as a positioning guide for the assembled apparatus and sits proximal to the angioplasty balloon. These characteristics allowed for precise deployment of the flow-limiting implants. The process was designed to be reproducible, efficient, and humane for the animal subjects.

In our application, the mother-and-child percutaneous delivery technique was used to create swine models with focal coronary stenosis for evaluation of contrast-enhanced stress cardiac perfusion magnetic resonance imaging (MRI). However, the technique may be employed in other investigations including vascular systems outside the coronary vessels.

PROTOCOL:

We conducted the experiments according to the guidelines by the Animal Welfare Act, the National Institutes of Health, and the American Heart Association on Research Animal Use. Our Institutional Animal Care and Use Committee approved the animal study protocol.

1. Preprocedural preparation of 3D printed coronary stenosis implants

1.1. Using tweezers, dip-coat the printed implants in a 25% heparin solution to prevent thrombus formation and allow to air dry for 24 h.

2. Preprocedural preparation of animal subjects

2.1. Have male Yorkshire swine (SNS Farms, 30–45 kg) arrive at the institution 1 week prior to the experiment date and allow them to acclimate.

2.2. Keep the swine in a fasting state after midnight the day prior to the procedure.

3. Procedural anesthesia

3.1. Sedate the swine with intramuscular ketamine (10 mg/kg) and intravenous midazolam (1 mg/kg).

3.2. Ventilate the animals with an oxygen-isoflurane (1–2%) mixture.

3.3. Perform endotracheal intubation once the animal subject is sedated.

3.4. Infuse intravenous (IV) rocuronium (2.5 mg/kg/h) and give additional boluses (1–3 mg/kg IV every 20–30 min) when needed to achieve diaphragmatic immobilization.

3.5. Maintain a surgical plane of anesthesia throughout the procedure by checking for awakening, movements, wide fluctuation in vital signs, and other signs of distress or discomfort throughout the duration of the experiment. We monitored the swine for roughly 6 h under anesthesia.

4. Vascular access

4.1. Using the Seldinger technique, insert the arterial and venous sheaths into the bilateral femoral arteries and veins of the subjects.

4.2. Flush all catheter ports continuously with heparinized normal saline.

5. Preprocedural medication administration

5.1. Administer amiodarone intramuscularly (1.5 mg/kg), lidocaine intravenously (2 mg/kg), and esmolol intravenously (1 mg/kg) as needed for prophylaxis against arrhythmia. Give repeat dosages of amiodarone, lidocaine, and esmolol as needed throughout the course of the experiment to suppress ventricular rhythms and control heart rate response.

5.2. After vascular access is obtained, administer heparin (5,000–10,000 units) to keep an activated clotting time (ACT) >300 s. Check the ACT every hour during the course of the experiment and give additional intravenous heparin as needed to maintain the ACT goal.

6. Hemodynamic monitoring

6.1. Use a single lateral electrocardiography (ECG) chest lead for recording changes in ST segment, T-waves, and heart rate during the entire experimental period.

6.2. Use a pressure transducer to record continuous femoral arterial pressure throughout the procedure.

6.3. Attach a pulse oximeter to the animal's ear or lip for continuous pulse oximetry recordings.

7. Preparation of implant delivery equipment

7.1. Prior to performing coronary angiography, insert a deflated NC Trek over-the-wire coronary balloon through a mother-and-child catheter of the desired size such that the balloon tip extends beyond the tip of the catheter.

7.2. Mount the 3D printed implant onto the deflated angioplasty balloon such that the implant is positioned between the markers of the balloon and close to the proximal marker (**Figure 1B**).

7.3. Inflate the balloon with an insufflator to 2–3 atm in order to fix the implant onto the balloon.

Verify that the implant is positioned closer to the proximal half of the balloon so it will be closest to the mother-and-child catheter when ready for removal (**Figure 1B**).

8. Coronary angiography and deployment of coronary implant

8.1. Position the fluoroscopic C-arm in the anteroposterior (AP) projection.

8.2. Attach a control valve (see **Table of Materials**) to a left or right coronary guide catheter (see **Table of Materials**).

8.3. Introduce the guide catheter over a J-tipped wire through the right femoral artery sheath and, under fluoroscopic guidance, advance the catheter to the aortic root.

8.4. Selectively (or nonselectively) engage the catheter into the left main coronary artery (LMCA) and inject 5 mL of iodinated contrast under fluoroscopy to visualize the left coronary system.

8.5. Position the guide catheter towards the LMCA for the second angiogram (**Figure 2**). If coronary artery engagement proves difficult, due in part to the short aortic arch of the swine, consider performing non-selective angiograms as long as they provide adequate visualization of the vessels.

8.6. Once engaged within, or positioned near the LMCA, under fluoroscopy, advance a 0.014", 300 cm coronary wire (see **Table of Materials**) into the LMCA and further advance the wire to the distal left anterior descending artery (LAD) or left circumflex coronary artery (LCX) if desired (**Figure 3**).

8.7. Under fluoroscopic guidance, insert the previously assembled mother-and-child catheter with the inflated coronary angioplasty balloon and implant over the coronary wire and advance to the desired location along the coronary vessel. Inject 5 mL of iodinated contrast to visualize a discrete narrowing at the desired location where the coronary implant should be deployed (**Figure 4**).

8.8. Once the implant is in position, advance the mother-and-child catheter to the proximal marker of the inflated balloon.

8.9. Deflate the balloon and retract it through the mother-and-child catheter. This process allows the mother-and-child catheter to shear the implant off the balloon as it is retracted and fixes the position of the implant in the designated segment of the vessel.

8.10. Remove the balloon, mother-and-child catheter, and coronary wire.

8.11. Perform final angiograms to document the location of the new artificial stenosis within the vessel. When feasible, angiograms should be performed in two orthogonal views to acquire visual estimation of stenosis severity. A final angiography (**Figure 5**) can also be performed with

subselective positioning of the mother-and-child catheter in the proximal vessel, which provides excellent opacification with minimal contrast.

8.12. Immediately transfer the animal to the MR suite to undergo cardiac stress perfusion MRI using gadobutrol (0.1 mM/kg) injected at a rate of 2 mL/sec.

NOTE: The stress agent used was a 4 min infusion of adenosine at 300 µg/kg/min. The imaging protocol included 1) cine imaging (field of view [FOV] = 292 x 360 mm, matrix size = 102 x 126, repetition time [TR] = 5.22 ms, echo time [TE] = 2.48 ms, slice thickness = 6 mm, pixel bandwidth = 450 Hz, flip angle = 12˚); 2) first-pass perfusion at rest and at peak adenosine vasodilator stress using a spoiled gradient echo sequence (FOV = 320 x 320 mm, matrix size = 130 x 130, TR = 2.5 ms, TE = 1.1 ms, slice thickness = 10 mm, pixel bandwidth = 650 Hz, flip angle = 12˚; and 3) late gadolinium enhancement imaging using an ECG-gated, segmented, spoiled gradient-echo phase-sensitive-inversion-recovery sequence (FOV = 225 x 340 mm, matrix size = 131 x 175 mm, TR = 5.2 ms, TE = 1.96 ms, slice thickness = 8 mm, inversion time (TI) = optimized to null the myocardium, pixel bandwidth = 465 Hz, flip angle = 20˚). An illustrative first-pass perfusion image is shown in **Figure 6**.

8.13. After completion of the MRI protocol, euthanize the swine by an infusion of sodium pentobarbital (100 mg/kg).

8.14. Perform a lateral thoracotomy, excise the heart, and dissect the ex vivo heart to expose the coronary vessels. Note the location of the implant in relationship to either the diagonal branches (LAD territory) or obtuse marginal branches (LCX territory), and retrieve the implants.

8.15. Using blunted and curved Metzenbaum scissors, open the coronary vessel and inspect the vessel for gross injury (see **Figure 7**). Photograph the heart tissue for gross pathology and stain with triphenyltetrazolium chloride to exclude myocardial infarction (see **Figure 8**).

REPRESENTATIVE RESULTS:

After initial optimization of the procedure, the intervention component was completed within 30 min. The implants were successfully delivered in all 11 subjects (100%). The implant was retrieved at the autopsy in all 11 subjects (100%). Using the diagonal branches (along the LAD) or obtuse marginal branches (along the LCX) as positional markers, we found the position of the implant at fluoroscopic-guided deployment and at autopsy to be consistent in 10 of the 11 (91%) subjects where the implant was retrievable. In one subject, there was slight distal migration of the implant, which may be related to vasodilation induced by intracoronary nitroglycerin injection for coronary spasm. Of the 11 subjects studied, 9 survived for the entire catheterization and completed the MRI protocol, giving us an 82% procedural success rate. Two subjects died after the implants were deployed. The first subject developed ventricular fibrillation in the MRI suite well after deployment of the implant. The second died in the MRI scanner in the setting of hypotension midway through the experiment. At the time of dissection, we did not see thrombus within the implants or other signs of structural injury to the vessels. The high survival rate (2 deaths, 9 of 11 survived) highlights the importance an effective anti-arrhythmic prophylaxis

regimen. An illustrative example of stress cardiac perfusion MRI is provided in **Figure 6**. Detailed implant design and full results of the MRI validation will be reported separately.

FIGURE LEGENDS:

Figure 1: Catheter design and assembled apparatus with mounted coronary implant. (**A**) Diagram of the components of the mother-and-child catheter[6]. (**B**) Assembled apparatus showing the coronary balloon inflated with the 3D printed implant mounted and fixed at the leading head of the catheter, which protrudes through the guide catheter. (**C**) A magnified image of the 3D printed implant is shown mounted onto the angioplasty balloon.

Figure 2: Coronary angiogram in the anteroposterior projection shows selective contrast-enhancement of the left main coronary artery system.

Figure 3: Coronary angiogram in the anteroposterior projection shows the 0.014" 300 cm coronary wire in the left anterior descending artery.

Figure 4: Coronary angiogram in the anteroposterior projection. The image on the left shows the assembled mother-and-child catheter with the inflated coronary balloon and implant in the mid to distal segment of the left anterior descending artery. A higher magnification of the assembled apparatus within the coronary vessel is shown in the right panel.

Figure 5: Anteroposterior angiogram. The image on the left shows a focal stenosis in the distal left anterior descending artery after deployment of the implant. A higher magnification of the discrete coronary narrowing induced by the implant is shown in the right panel.

Figure 6: Stress cardiac perfusion magnetic resonance images of a coronary implant deployed in the proximal to mid left anterior descending artery. The images at rest (upper panel) and peak adenosine vasodilator stress (lower panel) show inducible perfusion defects in the segments subtended by the left anterior descending artery.

Figure 7: Autopsy images. (**A**) The implant at the distal left anterior, descending artery. (**B**) The absence of gross injury to the coronary vessel. (**C**) Implant without thrombus.

Figure 8: Histopathology of swine myocardial tissue. (**A**) Gross pathology and (**B**) triphenyltetrazolium chloride stains in one subject showed no evidence of myocardial tissue infarction.

DISCUSSION:

In this work, we focused on a novel percutaneous deployment strategy for coronary stenosis-inducing implants and showed that a mother-and-child catheter can be repurposed for effective percutaneous delivery of 3D printed coronary implants. Discrete artificial coronary stenoses of variable severity can be created quickly in swine models with a high success rate and in a minimally invasive manner using standard human percutaneous coronary interventional techniques and equipment. These implants were shown to be safe in the acute setting and were

also effective at creating severe angiographic stenoses, which correlated with stress-induced perfusion defects during vasodilator stress cardiac MRI. Compared to open-chest techniques, percutaneous delivery of stenosis-inducing implants is less invasive and more humane.

There are several other minimally invasive techniques currently available to create flow reduction in large animal models. The 3D printed coronary implants differ fundamentally from balloon occlusion and coil occlusion in that the stenoses induced by the 3D printed implants do not completely occlude the vessel. This is a major difference that allows for modelling of stress-induced ischemia rather than infarction[7,8]. Rissanen et al.[9] describe a percutaneous technique that creates flow limiting, non-obstructive stenoses in swine models using a coronary stent wrapped in a polytetrafluoroethylene tubing. The tubing could be shaped by employing needles and heat to create luminal narrowing of various degrees. It is clear that the implants we used differ in design and thorough description with full validation is beyond the scope of the current work, which is to describe the novel methodology used for delivery of 3D printed coronary implants. Utilizing the mother-and-child catheter allowed for precise deployment of the implants deep in the coronary arteries. It is difficult to compare procedural success between our studies as other investigators explored a chronic model and kept the swine alive for an extended period of time[9]. Bamberg et al. described a method using balloon catheters inflated within 3 mm stents to create stenoses of 50% and 75% in the left anterior descending artery. This latter method differs from our investigation in that the stenoses created required catheters to be left inside the animals. There is no way to create an artificial lesion and remove all equipment. While viable, the Bamberg method does not allow for investigation of ischemia beyond the acute setting and residual wires would cause image artifacts[10].

The role of mother-and-child catheters in coronary interventions has been well established, but their use to deliver implants into vascular beds has not been previously described[5,6]. The two most challenging aspects of percutaneous implant delivery include selective deployment into a precise coronary segment and prevention of retrograde migration. Attempting to deploy the device over angioplasty balloons was not effective because the implant could be pulled proximally in the vessel after balloon deflation. For several reasons, the mother-and-child catheter proved to be a valuable tool for fixing the implants in place during balloon withdrawal. The mother-and-child catheters fit easily in the coronary guide catheters and their size was ideal for our intervention. They were slightly larger than the deflated coronary balloon, allowing us to shear the implant off and to prevent retrograde migration of the implant as the balloon was withdrawn. The support provided by the mother-and-child catheter enabled the implants to be deeply seated in the coronary artery with strong apposition to the vessel lumen. Additionally, the radiopaque marker on the tip of the mother-and-child catheter helped position the catheter just proximal to the implant, as identified by the marker on the delivery balloon. Though the technique was mostly effective, in one subject there was slight distal migration after implant delivery. This may have been due to injection of intracoronary nitroglycerin for coronary vasospasm and resultant vasodilation leading to distal migration of the implant. The GuideLiner catheter was chosen due to familiarity of use, but there are a number of other similar devices which could potentially be used in its place. The Guidezilla Guide Extension Catheter (Boston Scientific, Marlborough, Massachusetts, USA) is also available in a 6F size and has a similar

structure to the GuideLiner. There is also a Guidion rapid exchange guide extension catheter (Interventional Medical Device Solutions, Roden, The Netherlands) which comes in sizes 5–8F and could also potentially be used in place of the GuideLiner catheter.

Our deployment technique can be performed efficiently and humanely in swine with a low procedural failure rate. In our preliminary study the procedural failure rate was 18%. There was a learning curve associated with the technique as we streamlined our interventions. However, despite the learning curve, all animal subjects survived the initial implant deployment intervention. The lesions created were focal and the narrowing ranged in severity, but they were not occlusive. These stenoses were angiographically significant and produced inducible perfusion defects during stress perfusion MRI. **Figure 6** is an example of a focal perfusion defect seen on MRI after successful implant deployment to the LAD. We aimed to create ischemia rather than infarction. **Figure 8** shows an example of histopathologic analysis of the myocardial tissue, which shows no evidence of infarction. The method relies on human coronary angioplasty equipment, and the similarity in swine coronary size to those of humans. The outer diameter of the 3D printed implant was based on the inner diameter of the guiding catheter and the inner diameter of the mother-and-child catheter. The minimal luminal diameter of the stenosis was based on the size of the deflated coronary balloon. The final flow-limiting severity of the discrete stenosis is based on the inner diameter and the length of the implant. Although resting angiographic flow was preserved, maximal coronary blood flow was reduced, as evidenced by the MRI perfusion scans. Future work will focus on replacing the balloon delivery wire with a pressure wire and measurement of fractional flow reserve or instantaneous flow reserve. Similarly, downstream microvascular injury can be produced by local injections of microspheres either through the delivery balloon or the mother-and-child catheter itself.

Our low procedural failure rate in a closed-chest swine model shows promise for future implementation. Because complete total occlusion was not performed, myocardial infarction was avoided, and may have contributed to the lower rate of malignant arrhythmias. In our study only 1 subject developed ventricular fibrillation. After an initial period of optimization, we cut down procedural time to roughly 30 min per case.

In summary, our results demonstrate a novel technique for deployment of 3D printed coronary implants and show the feasibility of creating a closed-chest swine model of discrete focal coronary stenosis. This minimally invasive technique can be used for testing and development of new diagnostic imaging techniques in ischemic heart disease. We used stress cardiac perfusion MRI, but other modalities may include nuclear imaging, ultrasound, and computed tomography. Although this model is immediately applicable to ischemic heart disease, with minor modifications, the technique can be employed for other occlusive vascular disease states.

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DISCLOSURES

The authors have nothing to disclose.

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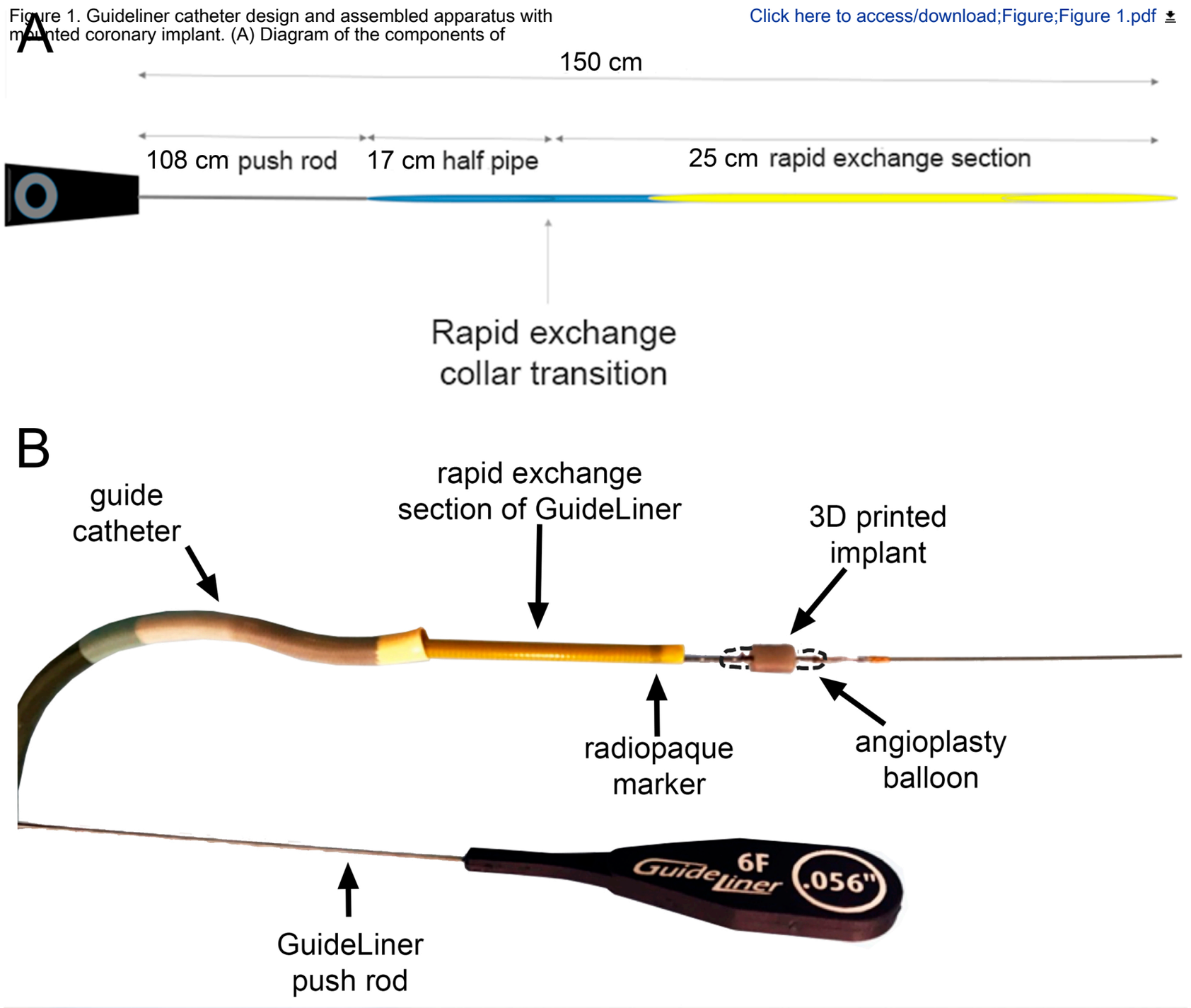
Figure 1. Guideliner catheter design and assembled apparatus with mounted coronary implant. (A) Diagram of the components of



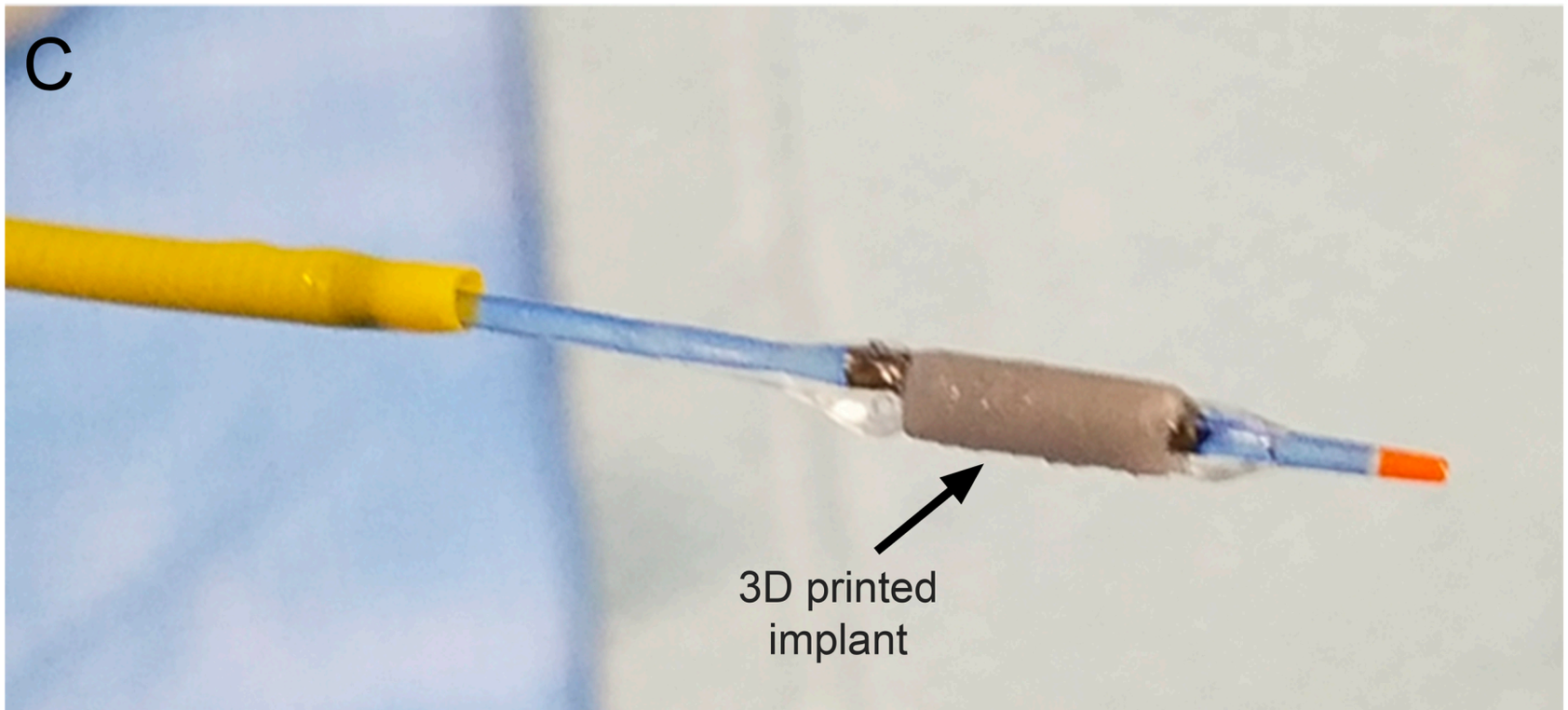


Figure 2

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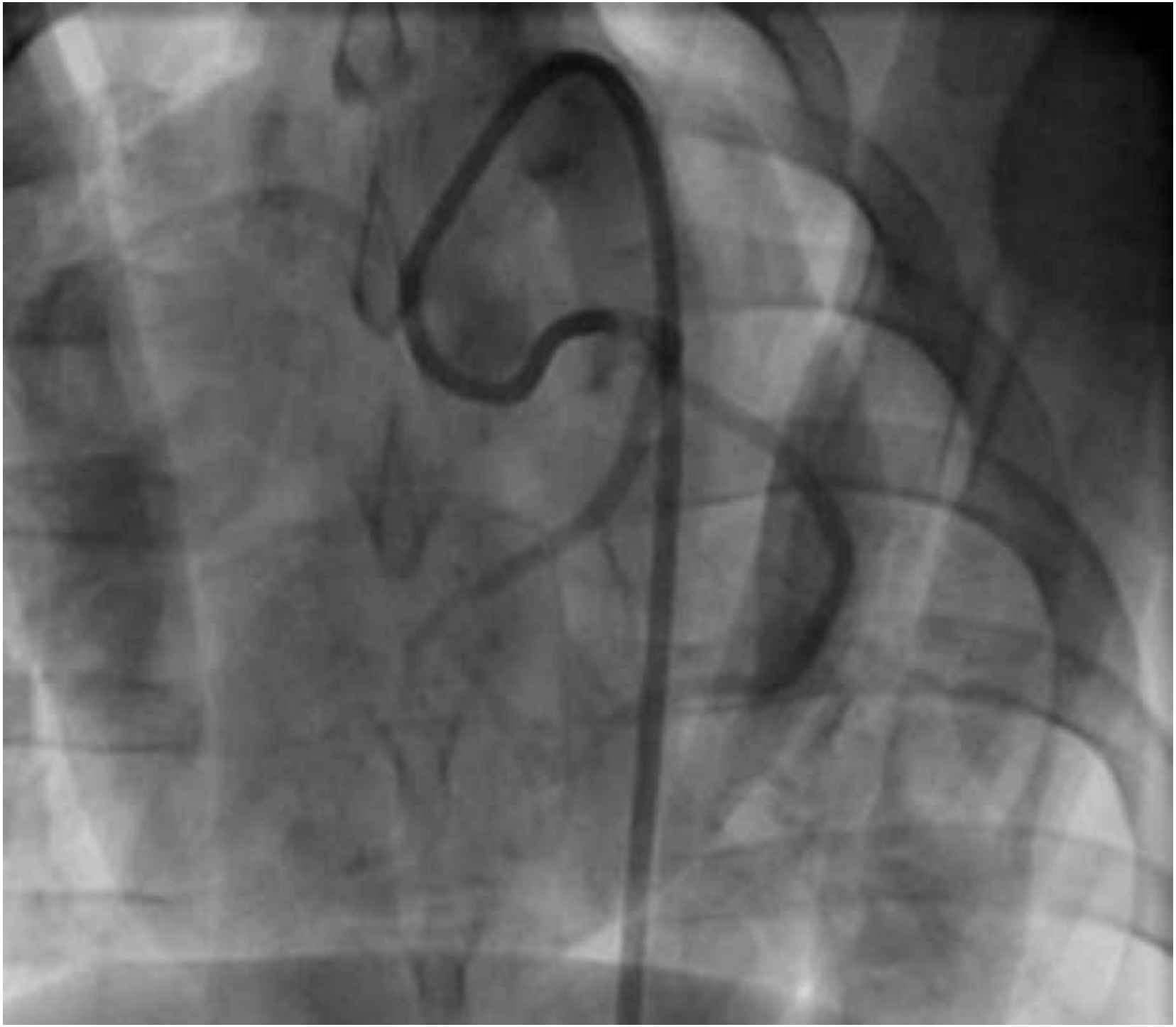

Figure 3

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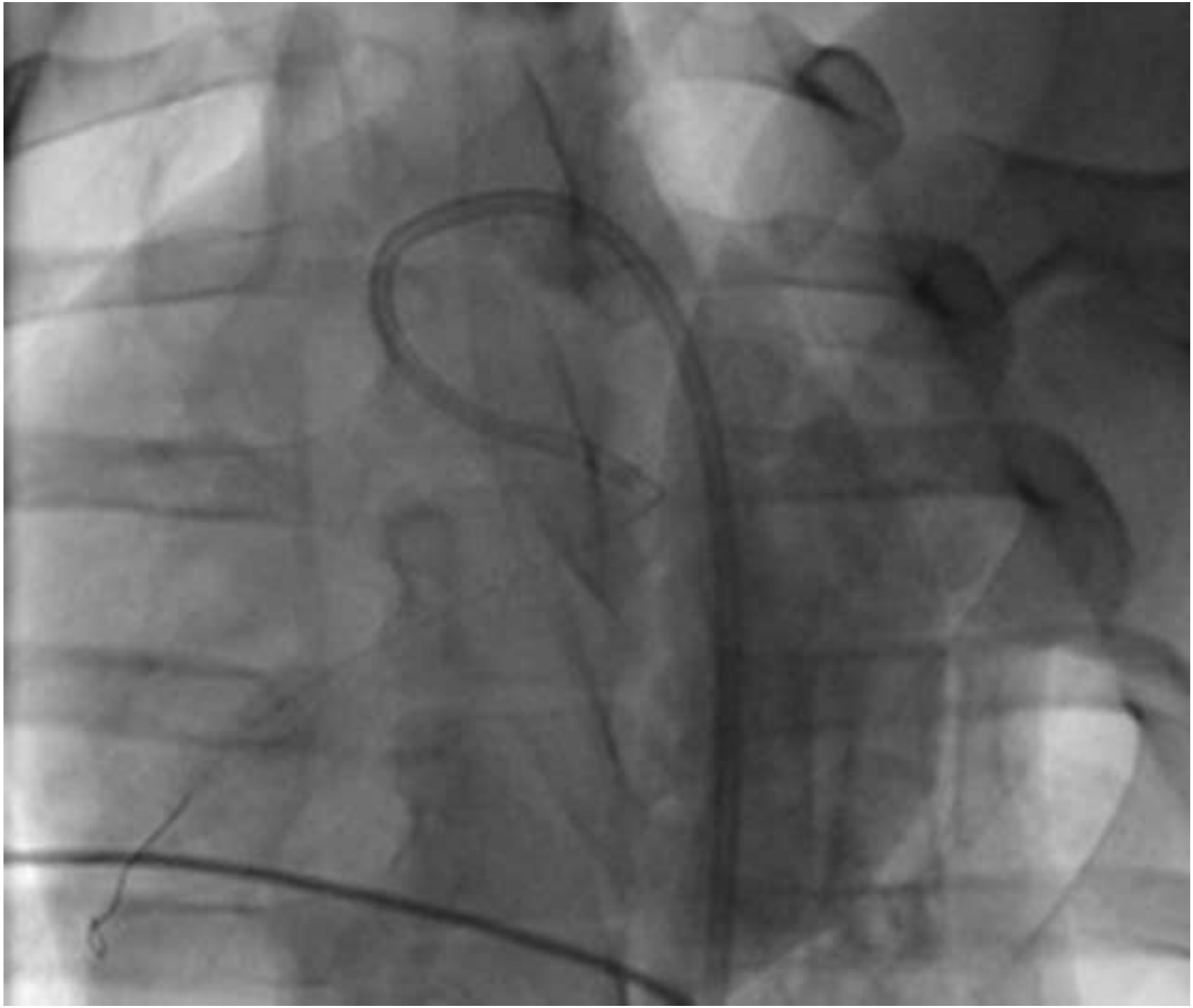

Figure 4

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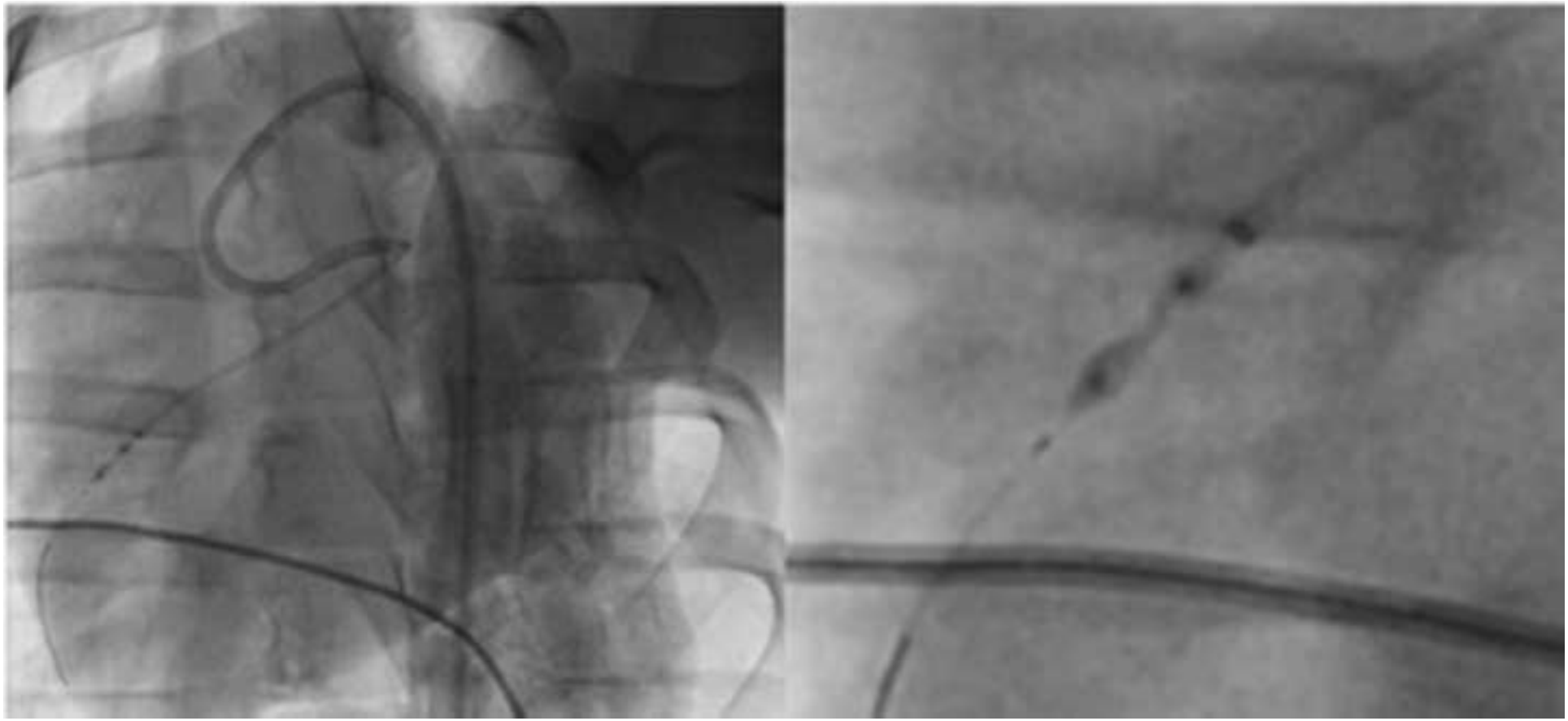

Figure 5

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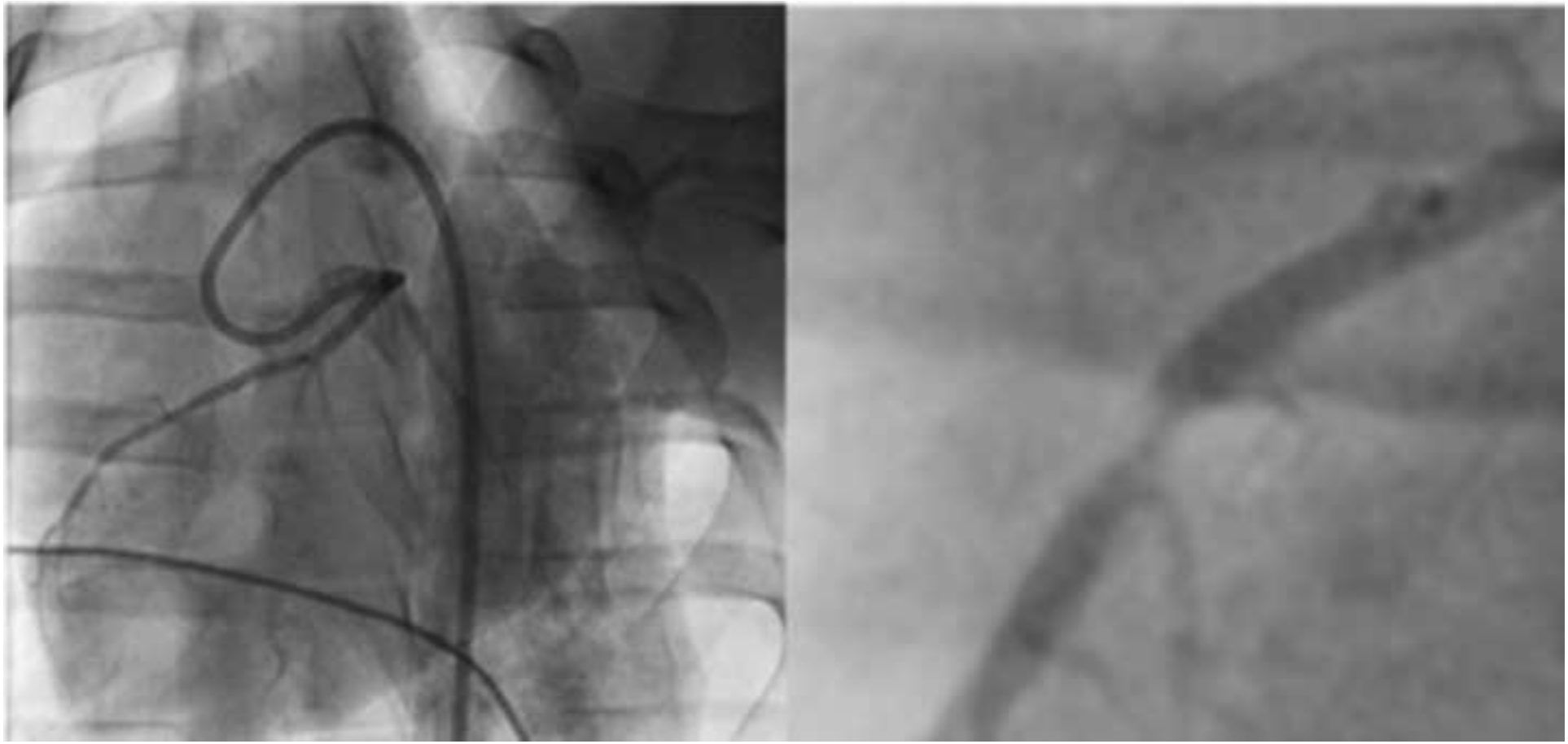

Figure 6

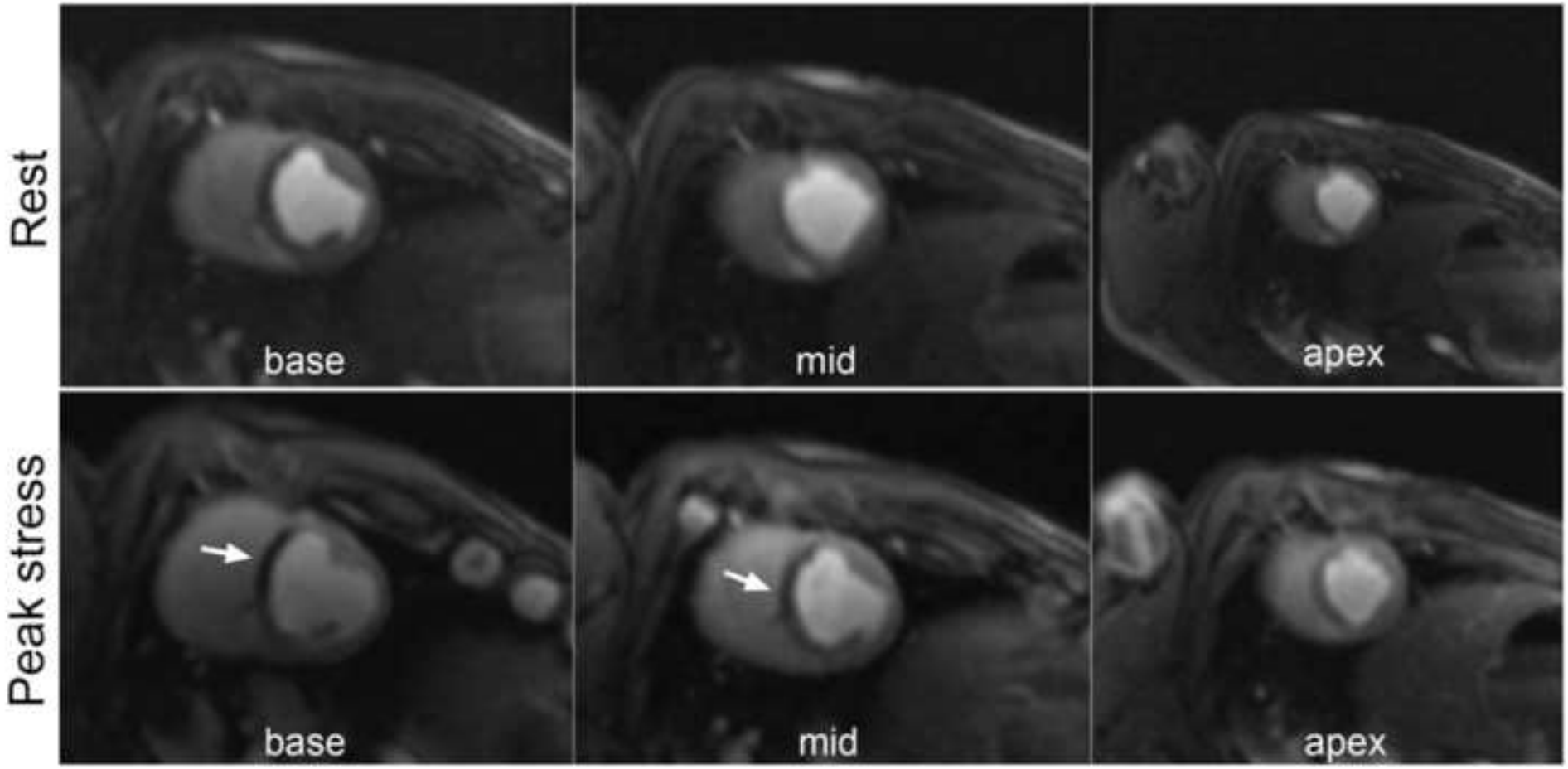

Figure 7

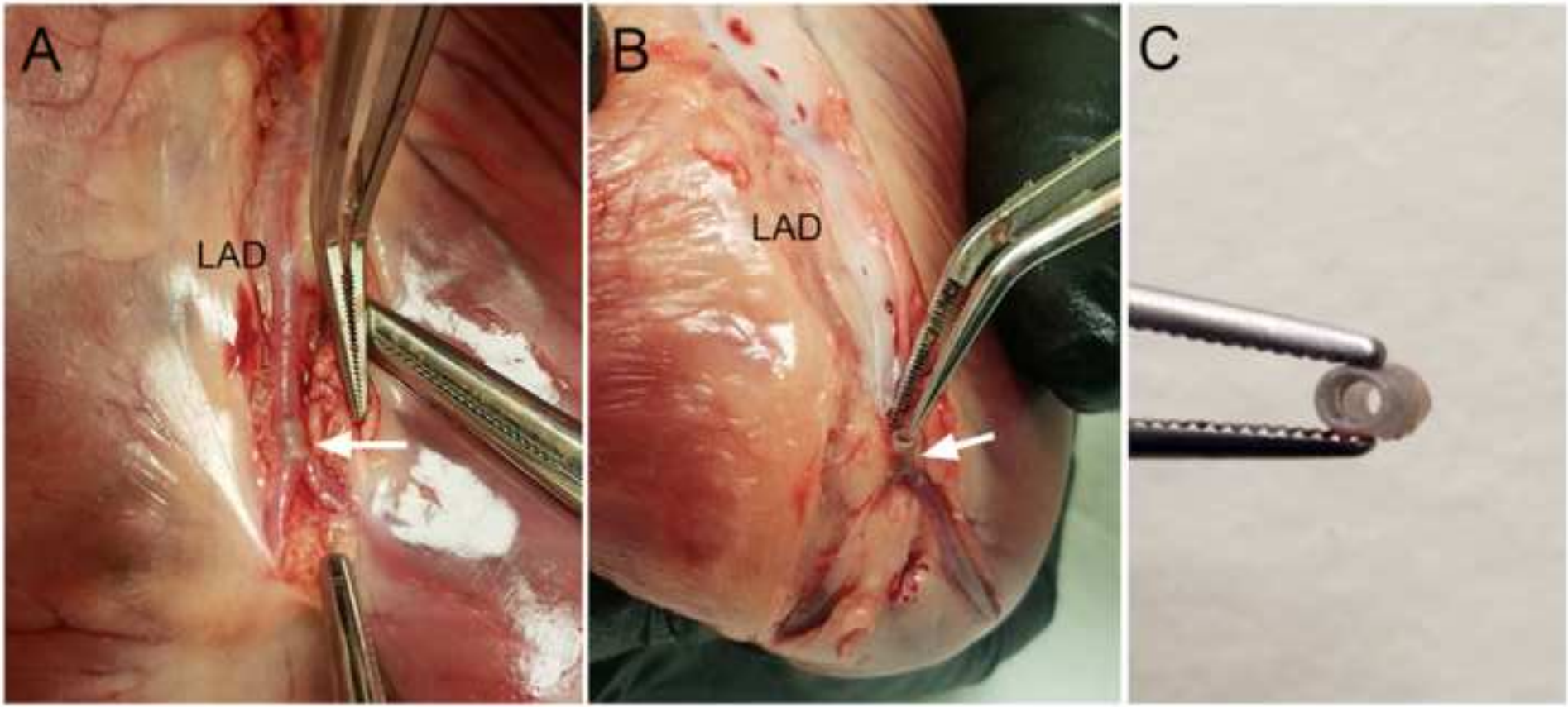

Figure 8. Histopathology of swine myocardial tissue. Gross pathology (A) and

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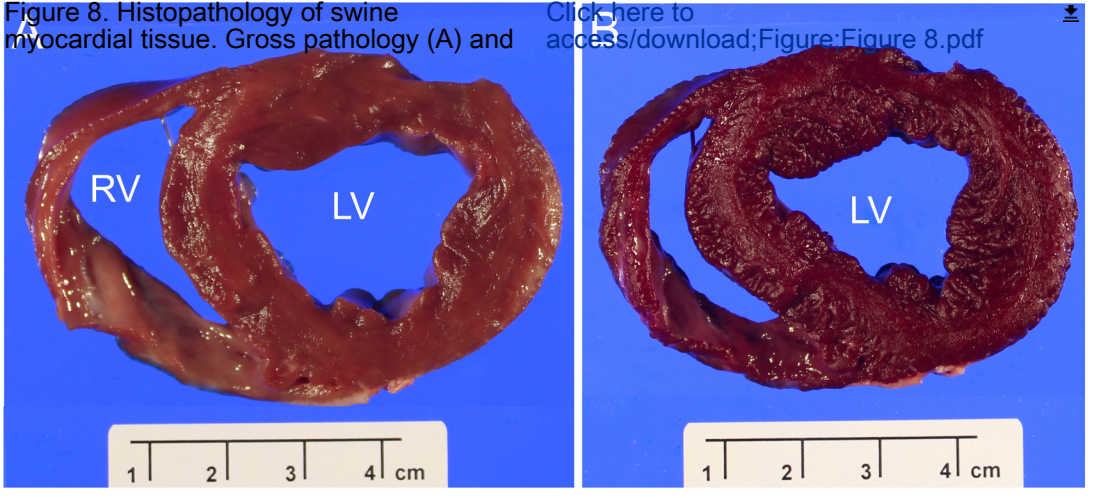

Name of Material/Equipment
3D-Printed coronary implants
Amiodarone IV solution
Amplatz Left-2 (AL-2) guide catheter (8F)
Balance Middleweight coronary wire (0.014” 300cm)
COPILOT Bleedback Control valve
Esmolol IV solution (1 mg/kg)
Formlabs Form 2 3D-printer with a minimum XY feature size of 150 µm
Formlabs Grey Resin (implant material)
Gadobutrol 0.1 mmol/kg
GuideLiner catheter (6F)
Heparin IV solution
Ketamine IM solution (10 mg/kg)
Lidocaine IV solution
Male Yorkshire swine (30-45 kg)
Midazolam IV solution
NC Trek over-the-wire coronary balloon
Oxygen-isoflurane 1-2% inhaled mixture
Rocuronium IV solution
Sodium Pentobarbital IV solution (100mg/kg)
Triphenyltetrazolium chloride stain

Company
Study Site Manufactured
Study Site Pharmacy
Boston Scientific, Marlborough, Massachusetts, USA
Abbott Laboratories, Abbott Park, Illinois, USA
Abbott Laboratories, Abbott Park, Illinois, USA
Study Site Pharmacy
Formlabs Inc., Somerville, Massachusetts, USA
Formlabs Inc., Somerville, Massachusetts, USA
Gadvist, Bayer Pharmaceuticals, Wayne, NJ
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Study Site Pharmacy
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Institution Pathology Lab

Re: JoVE-60729

Dear Editors:

Thank you for the opportunity to resubmit our manuscript. We appreciate your insightful comments and suggestions. Below, please find a point-by-point response to the Editorial and reviewers' comments.

EDITORIAL COMMENTS

General:

E1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
We have carefully reviewed the manuscript for spelling and grammar issues.

E2. Please include email addresses for all authors in the manuscript itself.
We have provided the email addresses for the co-authors.

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We removed all trademarks and registered symbols.

Protocol:

E4. For each protocol step, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.
We have reviewed the protocol and addressed the "how" in each step.

Specific Protocol steps:

E5. 1.1: How long to air-dry?
The heparin-coated implant was allowed to air dry for 24 hours. We have added the duration to the revised manuscript.

E6. 2. 3: Please mention how proper anesthesia is confirmed.
In the revised manuscript, we described that adequate anesthesia was ensured by checking for arousal, signs of distress, and wide fluctuation in vital signs.

E7. 8.13: Please give the euthanasia method.
In the revised manuscript, we clarified that euthanasia was achieved using sodium pentobarbital 100mg/kg IV.

Representative Results:
E8. This is slightly confusing-were there 12 or 13 subjects?
We apologize for the lack of clarity. There were 12 subjects. However, in the initial version of the manuscript, deployment of the implant did not involve the use of the Guideliner. We have corrected the number of subjects to 11.

Figures:

E9. Figure 1: Please include a space between numbers and their corresponding units (e.g., '108 cm').
Figure 1 has been edited in the revised manuscript.

E10. Figure 6: There is no legend for panel C.
We have added a legend for panel C in Figure 6.

References:

E11. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.
We have made the modifications in the revised manuscript.

E12. Please do not abbreviate journal titles.
Journal titles are no longer abbreviated.

Table of Materials:
E13. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

REVIEWERS' COMMENTS

REVIEWER #1

R1.1. What about implant design and preparation? Only reference is not a publication but an

ISMRM abstract. Need a publication as reference to describe how the implant was designed and manufactured (material, design, adherence to the vessel wall, etc.). Could the authors provide implant design details in this manuscript? Without this description, manuscript seems to be incomplete since the implant is an integral part of the procedure. The interventional procedure tested appears to be tuned to the delivery of this particular implant that the authors prepared in their lab and published an abstract on.
A separate manuscript focused on the implant design, manufacturing, and validation is currently under review. Extensive detail about the implant design and manufacturing is beyond the scope of the current manuscript, which focuses on the method of delivery for such a device. In the revised manuscript, we provided additional detail about the implant, but did not want to detract from the focus of the manuscript, which is on the method of delivery. The technique we describe is applicable to the delivery of a number of different investigative tools and as such was the focus of this paper.

R1.2. What about determination of coronary vessel diameter and accordingly the outer diameter of the implant? It seems that the implant simply plugs into the vessel, please clarify this. Vessels will have slight difference between proximal and distal diameters (tapering effect). Specify if and how this is taken into account when designing the implant. In this case length of implant will also matter. Line 297 in the Discussion says that the implant dimensions are limited by the size of the guiding catheter and GuideLiner. How does this translate to where in the vessel the implant can be placed? - which cannot be consistent across subjects as it would depend on vessel diameter and/or custom implant design. The abstract states that the implant can be placed anywhere along the vessel. Can the authors comment?
Please refer to the response above for R1.1. The focus of our current manuscript is on the method of delivery. The technique we describe is applicable to the delivery of a number of different investigative tools and as such was the focus of this paper. We have a separate manuscript focused on the implant design, manufacturing, and validation currently under review. In the revised manuscript, we provided additional details about the implant in the context of varying vessel geometry and size. Briefly, we created a library of varying sizes and used angiographic data. The outer diameter of the implants was based on the coronary guide catheter. The inner diameter was based on the size of a deflated coronary angioplasty balloon. Coronary guide catheters and the Guideliner are available in several sizes and a larger size could be used if a more proximal segment is desired. Coronary CT imaging could also be performed prior to the intervention to create a more custom print to improve the fit of the implant.

R1.3. Considering all the above, one needs to know coronary vessel geometry and dimensions in order to design the implant in the first place so that implants can be deployed in specific locations along the vessel - for example beyond first diagonal or second diagonal of LAD. Were all implants of the same physical dimension across pigs? If so, then one would end up occluding at varying levels along the vessel (no control)? This is an important consideration since it will determine the ischemic risk zone, which often needs to be controlled. Please clarify.

Please refer to responses in R1.1-R1.2. We created a library of implants with different sizes and selected the appropriate size in the cath lab. Our specific targets in these swine were mid to distal LAD and distal the second diagonal branch. For even greater precision or pig-specific implants, we could perform a coronary CT, then print the implant, and deploy the implant using the same technique.

R1.4. Can the authors comment on the design of the inner diameter of the implant? This will determine the degree of flow past the implant to create ischemia of variable severity. Line 263 in the discussion mentions this but no details are provided as to designing such implants for catheter deployment. What was the inner diameter of the implants in all the animals and did the authors determine what flow reduction these would create? This has been described in the introduction section.
Please refer to responses in R1.1. The inner diameter was based on the size of the deflated angioplasty balloon; the length of the implants was also varied to achieve the desired severity of ischemia. In the revised manuscript, we added clarification in the discussion. Because our paper is focused on the method of deployment, extensive detail about the implant design is available in another manuscript currently under review. In this early proof-of-concept work, we did not quantify the degree of flow reduction using invasive methods such as fractional flow reserve or instantaneous flow reserve. However, this is an area of ongoing evaluation in our lab. Angiographically all implants deployed created a moderate to severe stenosis which was determined visually based on two orthogonal views.

R1.5. What was the stress agent used during the MRI? Provide agent, dose, timing etc.
We used adenosine, 300 μg/kg/min, 4 min infusion. **We clarified the stress agent in the methods section of the revised manuscript.**

R1.6. Specify the contrast agent and the sequence (with parameters) used in the MRI exam.
We provided the contrast agent and the pulse sequence parameters in the revised manuscript.

R1.7. Line 211: Please provide protocol for animal euthanization.
In the revised manuscript, we clarified the euthanasia protocol in the methods section.

R1.8. Line 218: At autopsy, the implant was retrieved in 11 of 12 animals. What happened to the implant in 1 remaining animal?
In the very first swine and before implementation of our Guideliner technique, we could not find the implant anywhere along the length of the coronary vessel. This pig served as the control for subsequent experiments where with the Guideliner technique, we were able to retrieve all the implants. In the revised manuscript, we removed the first subject because it is not relevant to the technique described in our paper. With this in mind the implant was retrieved in all subjects who underwent the study with the GuideLiner catheter (11 of 11).

R1.9. Line 219: 10 of 12 animals completed the study. Please provide details of the 2 unsuccessful animals - how did they succumb, at what stage of the process (during

intervention, MRI, stress?) did they succumb. Were any precautions possible to help their survival?
We apologize for the lack of clarity. In the revised manuscript, we provided additional details regarding the deaths of the animals and commented on our anti-arrhythmic regimen.

R1.10. Line 268: In the discussion, the authors state that their method is less invasive and faster compared to existing techniques. However, there are other techniques that can offer minimally-invasive stenoses, the authors should acknowledge other methods described previously (two mentioned below). How does the proposed method compare with these in terms of advantages and disadvantages?
We thank you for this suggestion. However, our paper is focused on the deployment of such stenosis-inducing implants rather than the implants themselves. In a separate manuscript currently under review and which describes our implants in detail, we compared and contrasted our implants with other techniques that have been published. In the revised manuscript, we briefly touched upon alternative implant designs and focused on the technique of implant delivery. We compared our delivery technique with those used by other investigators.

R1.11a. Line 274: It is stated that the GuideLiner mother-and-child catheter helped fix the implant in place during balloon withdrawal. After the catheter is withdrawn and procedure completed, what is to prevent the implant from dislodging from its position (implant design, blood flow)?
We apologize for the confusion and have clarified this point in the revised manuscript. The support provided by the Guideliner enabled the implant to be deeply deployed in the coronary artery and allowed for strong apposition to the vessel lumen and secure seating. As a result, the risk of retrograde migration after implantation was low.

R1.11b. Did the authors validate the positioning of the implant in the vessel during intervention with that actually found post-sacrifice?
We used the diagonal branches and obtuse marginals as position markers. When retrieved, in general, we did noted that they were where we expected them to be In one pig, there was slight migration distal to where we expected the implant to be. This may have been due to injection of intracoronary nitroglycerin for coronary vasospasm and resultant vasodilation leading to distal migration of the implant.

MINOR COMMENTS
R1.12. Line 45: Suggest rephrase to "We showed that reliable delivery of 3D printed coronary implants in swine models (N=12) of ischemic heart disease can be achieved…"
We made the modifications as suggested.

R1.13. Line 75: Sentence construction does not seem correct - "…inserted over a guidewire and through and extended beyond…". Consider rewording this part.
We made the revised the wording as suggested.

R1.14. Line 77: Should probably be - "The Guideliner or a similar catheter can be used as added support for the deployment…"

R1.15. Line 174: Should be "…advance the catheter TO the aortic root."
We made the modifications as suggested.

R1.16. Line 306: There should be a period after "…reserve (FFR) wire.".
We made the modifications as suggested.

REVIEWER #2

R2.1 Please describe how long you monitored the pigs under anesthesia after the 3D printed implant implantation.
Roughly six hours. The length of the experiment. This has been included in the methods section.

R2.2 Compare in a more detailed manner the advantages and disadvantages of this method compared to balloon occlusion and coil occlusion.
We have included these comparisons in the discussion section.

R2.3 Do you have any data about histopathologic analysis of ischemic heart tissue in this study? Please show the histopathologic results.
In the revised manuscript, we provided histopathologic results, which show normal myocardial tissue and confirmed the absence of myocardial infarction. We aimed to create hypoperfusion and stress-induced ischemia, which would not result in injury of the myocardial tissue.

1 Alewife Center #200
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Author(s):	John J. Hollowed MD, Caroline M. Colbert BS, Kim-Lien Nguyen MD, Jesse W. Currier MD

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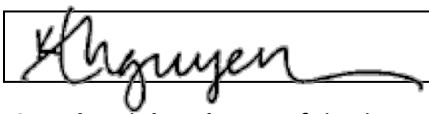
CORRESPONDING AUTHOR

Name:	Kim-Lien Nguyen, MD
Department:	Dvision of Cardiology and Department of Radiology
Institution:	David Geffen School of Medicine at UCLA and VA Greater Los Angeles Healthcare System
Title:	Assistant Professor of Medicine
Signature:	
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