Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Methods Mol Biol*. Author manuscript; available in PMC 2015 March 26.

Published in final edited form as:

*Methods Mol Biol*. 2014 ; 1181: 109–120. doi:10.1007/978-1-4939-1047-2_10.

# Injectable ECM Scaffolds for Cardiac Repair

**Todd D. Johnson, M.S.**, **Rebecca L. Braden, M.S.**, and **Karen L. Christman, Ph.D.**

University of California San Diego in La Jolla, CA, Department of Bioengineering, Sanford Consortium for Regenerative Medicine

## Summary

Injectable biomaterials have been developed as potential minimally invasive therapies for treating myocardial infarction (MI) and heart failure. Christman et al. first showed that the injection of a biomaterial alone into rat myocardium can improve cardiac function after MI (1). More recently, hydrogel forms of decellularized extracellular matrix (ECM) materials have shown substantial promise. Here we present the methods for fabricating an injectable cardiac specific ECM biomaterial shown to already have positive outcomes in small and large animal models for cardiac repair (2–4). Also covered are the methods for the injection of a biomaterial into rat myocardium using a surgical approach through the diaphragm. Although the methods shown here are for injection of an acellular biomaterial, cells or other therapeutics could also be added to the injection for testing other regenerative medicine strategies.

### Keywords

Injectable; Extracellular Matrix; Hydrogel; Cardiac Repair; Rat; Decellularization

## 1. Introduction

A minimally invasive approach for cardiac repair has numerous positive benefits including decreasing local tissue trauma, surgery times, risk due to surgery, hospital stays, and recovery times. These positive attributes have lead to the investigation of injectable therapies for treating myocardial infarction (MI). Christman et al. in 2004 showed that injection of a biomaterial alone directly into the myocardium could lead to beneficial outcomes for cardiac repair post-MI (5). Since this initial study numerous naturally derived biomaterials including alginate, collagen, chitosan, decellularized tissues, fibrin, hyaluronic acid (HA), keratin, and Matrigel, along with several synthetic biomaterials composed of polyethylene glycol (PEG) or poly(N-isoproylacrylaminde) (PNIPAAm) have been investigated (6,7). Ideally these injectable biomaterials would be injected utilizing current catheter technology for quicker translation to the clinic. But this mode of delivery provides unique challenges and design parameters for the biomaterial such as incorporating the ability to pass through a 27G needle and the appropriate kinetics to not gel at body temperature for up to an hour due to the duration of these procedures (6). One material recently developed by Singelyn et al. in 2009 is a decellularized biomaterial derived from porcine myocardial

Corresponding Author: christman@eng.ucsd.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tissue, which provides a tissue specific material for cardiac repair (2). In brief, fresh porcine myocardium is decellularized by spinning the chopped tissue in detergents, and then the decellularized tissue is lyophilized and milled into a fine powder. This powder is then partially digested in acidic conditions by pepsin into a liquid form that once brought to physiological conditions (salt, pH, and temperature), gels with the appropriate kinetics for catheter delivery (3,4). This myocardial matrix hydrogel was initially tested by injection into rat myocardium post-MI and was shown to maintain cardiac function, increase the size of cardiomyocyte islands within the infarcted region, and even recruit cardiac stem cells into the region of repair (3). The matrix was also shown to be deliverable through numerous endocardial injections via catheter delivery in a porcine model (3). Later studies in a porcine MI-model, the myocardial matrix hydrogel lead to increasing cardiac function, decreased infarct fibrosis, and increased cardiac muscle at the endocardium (4). In this chapter, the methods for decellularization, material digestion and processing of the matrix into an injectable liquid form are presented. Also, detailed instructions for injecting a biomaterial into rat myocardium with a surgical approach through the diaphragm are included. Here the injection is occurring into a healthy rat heart but several methods for modeling myocardial infarction could be applied before the injection with either total coronary occlusion, coronary occlusion followed by reperfusion, or cryo-injury. Although the specific approach is for a biomaterial alone both growth factors and/or cells could be included in this procedure for further study options.

## 2. Materials

Use ultrapure water for all solutions and rinsing steps. All materials and supplies for the material processing should be sterile or as clean as possible to prevent contamination. Any surgical supplies or tools that come in contact with the animal during surgery should be autoclaved and/or sterilized.

### 2.1. Decellularization Materials

1. Sharp knife and cutting board.
2. Decellularization Solution: 1% SDS, 1x PBS. Dissolve 80g of SDS powder (See Note 1) in 800mL of water to make a 10% stock solution of SDS. In an autoclaved 4L beaker combine 3400mL of water, 400mL of the 10% SDS stock, and 200mL of a 20x PBS stock solution. Stir until dissolved.
3. Plastic cryomolds and OCT compound.
4. Autoclaved 1L beakers with 3/8″ × 2 1/2″ stir bars.
5. Stir plate that can be set to 125 rpm and can hold a 1L beaker.
6. Penicillin/Streptomycin or PenStrep (PS): 10,000 Units/mL Penicillin and 10,000 μg/mL Streptomycin.

[1]The quality of SDS powder can vary greatly between brands and can even have variation from the same brand. The SDS powder should be white and very fine. When dissolved, the SDS solution should turn completely clear. Our lab has previously received batches of SDS that had gathered into large aggregates due to exposure to water before storage and a batch that dissolved into a yellow solution. Double check the quality of SDS since this could have significant impact on the decellularization of the tissue. We have had consistent results with ordering Fisher Scientific, #S529-500.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

7. Autoclaved fine mesh metal strainer.
8. Autoclaved 1L bottles.
9. Sterile 50mL plastic conicals.

### 2.2. Digestion and Injection Preparation Materials

1. Lyophilizer and Wiley® Mini-Mill.
2. Digestion Solution: 0.1 M HCl, 1 mg/mL pepsin from porcine gastric mucosa (2,500 units/mg protein). Fully dissolve the pepsin in acid by vortexing or shaking the solution. Then sterile filter through a 0.22um pore size filter.
3. Autoclaved 20mL scintillation vial and 5/16″ × 1/2″ stir bar.
4. Sterile filtered solutions for pH and salt adjustments: 1 M NaOH, 0.1 M HCl, and 10x PBS.

### 2.3. Cardiac Surgical Injection Materials

1. Isoflurane anesthesia system.
2. Ventilator with temperature probe.
3. Heating pump connected to warmed surgical table.
4. Electric razor and vacuum.
5. Sterile syringes: 1mL, 3mL, and 10mL.
6. Sterile needles: 25G, 27G, and 30G.
7. IV Catheter 14G × 2″.
8. Aspiration tube constructed from a 20G Intramedic tip with 10 cm of PE 100 tubing attached.
9. Autoclaved surgical tools: scalpel, scissors, fine forceps, standard forceps, needle holder, and towel clamp.
10. Topical: Betadine, Isopropyl Alcohol, Artificial Tears, Surgilube, and Triple Antibiotic Ointment.
11. Injectable: Lactated Ringers, 1% Lidocaine, and Buprenex.
12. Suture: Vicryl 4-0 FS-1, Vicryl 5-0 FS-2, and Prolene 5-0 RB-1.
13. Lab tape or masking tape.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## 3. Methods

### 3.1. Tissue Processing and Decellularization

1. Starting with a fresh unfrozen (see Note 2) porcine heart (Figure 1A), isolate only the left ventricle and septal wall by removing the thinner right ventricular free wall tissue, both atria, and the valves.
2. Remove any superficial fat, fascia, chordae tendineae, and papillary muscles just leaving behind only the red myocardial tissue (Figure 1B).
3. Cut the remaining tissue into small regular cubed pieces about 2 × 2 × 2mm (see Note 3) (Figure 2A).
4. Divide cut tissue into 1L autoclaved beakers with 20–35g of tissue per beaker (see Note 4).
5. Add 800mL of water and a 3/8″ × 2 1/2″ stir bar to each beaker.
6. Stir beakers at 125 rpm for 30–45 minutes. Cover beaker with parafilm (see Note 5).
7. Strain tissue through the autoclaved fine mesh metal strainer and rinse with water.
8. Place tissue back into beaker with stir bar. Add 800mL of decellularization solution and 4mL of PS.
9. Stir beakers at 125 rpm for 2 hours. Cover beaker with parafilm.
10. Repeat steps 7 and 8. Then stir beakers at 125 rpm for 24 hours.
11. Repeat step 10 for 2–4 times (for a total of 3–5 days in the decellularization solution) until the tissue has become completely decellularized or turned fully white in color (see Note 6) (Figure 2B).
12. Repeat step 7 and then place tissue back into beaker with stir bar and 800mL of water. Stir at 125 rpm for 24 hours.
13. Repeat step 12 but stir in water for only 30–45 minutes.
14. Repeat step 7 and then transfer decellularized tissue into 1L autoclaved bottles with 800mL of water. Vigorously shake for 1 minute.

---

[2]After the heart is harvested it should be kept on ice to minimize degradation of the ECM, which is an issue due to the immediate release of matrix metalloproteinases (MMPs) from cell death.

[3]The size of tissue pieces for this process has been optimized for the type of tissue being decellularized. If the pieces are too large, the core of the tissue will not decellularize properly and can even begin to decompose. If the tissue pieces are too small, the tissue pieces will shred and fall apart in the decellularization solution. Denser tissues decellularize slower and require smaller pieces. Weaker tissues, or those with little ECM, can decellularize more quickly and should be cut into larger pieces.

[4]It is helpful to remove a couple pieces of fresh tissue to freeze for analysis against the decellularized tissue. Some of the fresh tissue should also be frozen in OCT compound in a cryomold for cryo-sectioning and histological staining.

[5]The tissue is first spun in water to not only rinse off the blood but it is also a hypotonic solution, which can actually begin the decellularization process by rupturing the exposed cells.

[6]The length of time needed to decellularize the tissue varies and is dependent on the tissue type and size of the cut tissue pieces. Larger pieces take longer than smaller ones and thus, after 24 hours in the decellularization solutions the larger pieces can be cut in half if needed. While spinning in the decellularization solution the tissue will release the cellular content, as seen by a cloudy haze that accumulates over time in the beaker. Eventually the tissue will turn completely white, leaving behind only the ECM scaffold of the tissue.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

15. Repeat step 14 and then check for bubble formation after shaking. Lack of bubbles indicates removal of SDS solution. If bubbles remain then keep rinsing and shaking in the 1L bottle with water to remove SDS from tissue.
16. Strain tissue with fine mesh metal strainer and transfer each into a 50mL conical and freeze at −80°C (see Note 7).

### 3.2. Digestion and Injection Preparation

1. Using a lyophilizer, freeze dry the decellularized tissue.
2. Once fully dry, with a Wiley® Mini Mill process the material through a #40 mesh filter (pore size of 0.422mm) into an autoclaved 20mL vial (Figure 2D). Before using the mill, make sure to fully clean all surfaces of the mill and mesh with 70% ethanol to minimize contamination of the decellularized tissue.
3. In a sterile environment (such as a tissue culture hood), mass out 15–25mg of milled ECM into an autoclaved 20mL vial with a 5/16″ × 1/2″ stir bar.
4. Still in a sterile environment, add an appropriate amount of sterile filtered digestion solution to the 20mL vial until the ECM is at 10 mg/mL. Stir at 60–85 rpm for 48–56 hours (see Note 8).
5. Once digested (Figure 2E), the ECM in liquid form is brought to physiological conditions on ice with ice-chilled solutions. Add 1M NaOH and thoroughly mix until at pH 7.4 as confirmed with a pH strip.
6. Calculate the new total volume of liquid in the vial and add 1/9 this volume of 10x PBS to bring the solutions up to 1x PBS salt concentration.
7. Dilute the solution to the desired ECM concentration of 6 mg/mL for the injection with 1x PBS.
8. The liquid form of the ECM can either be kept on ice for immediate injection preparation or frozen and re-lyophilized for long-term storage at −80°C. If stored in a lyophilized state, the material can simply be resuspended with the appropriate amount of sterile water back to 6 mg/mL ECM.
9. Liquid form of the ECM is then loaded into a sterile syringe and kept on ice until injected into the myocardium. Upon injection into the animal the material will gel or self-assemble into a nanofibrous structure (Figure 2F).

### 3.3. Cardiac Surgical Injection

All animal work should be done under an approved animal protocol as governed by your institution. For our studies, all experiments were performed in accordance with the

[7]Similar to the fresh tissue, some of the decellularized tissue should be kept for analysis and frozen in OCT compound in a cryomold for cryo-sectioning and histological staining to confirm complete decellularization of the tissue (Figure 2C).
[8]The digest should be checked regularly throughout 48–56 hours. The ECM material can creep up the walls of the glass vial and out of the digestion solution due to the motion of the stir bar. If this happens, the material should be scrapped down back into the solution using a small autoclaved metal spatula. Once digested, the material should have an increased viscosity and be homogeneous but it will not be transparent.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

guidelines established by the Committee on Animal Research at the University of California, San Diego, and the American Association for Accreditation of Laboratory Animal Care.

1. Anesthetize a female Sprague Dawley rat (225–250g) for 3–5 minutes with 5% Isoflurane.
2. Intubate with a 14G × 2″ IV catheter. Secure the rat in a supine position on the surgical table and connect to the ventilator where anesthesia is maintained with 2.5% Isoflurane.
3. Apply ophthalmic ointment (Artificial Tears) to the eyes.
4. Insert the temperature probe coated in Surgilube.
5. Administer two 1.5mL subcutaneous injections of Lactated Ringers through a 25G needle away from the incision region.
6. With the electric razor, shave and vacuum the abdomen and chest region free of hair. Disinfect the incision region with Betadine and wipe clean with 70% Isopropyl alcohol swabs.
7. Use a 25G needle to inject 3–4 beads of 1% Lidocaine subcutaneously along the length of the initial incision region.
8. Use the scalpel to make a 3–4cm cutaneous incision beginning posterior to the xiphoid process and continuing lateral left approximately 1cm caudal to the ribcage (Figure 3).
9. Carefully cut through the muscle to expose the xiphoid process without damaging the liver. Once the xiphoid process is exposed, cut through the muscle along the length of the cutaneous incision.
10. Expose the diaphragm by lifting and anchoring the xiphoid process. This can be accomplished by running half the length of an appropriate suture (Vicryl 4-0 FS-1) through the xiphoid process and taping the free ends of the suture to a nearby high point (see Note 9).
11. Use fine forceps to hold the diaphragm dorsal to the heart apex and slightly pull the diaphragm caudal in order to prevent damaging lung or heart tissue. Cut a small opening in the diaphragm at a point dorsal to the heart apex. From this opening make a ventral incision that extends 1–1.5cm being careful not to cut the lungs (Figure 4).
12. Tear the pericardium at the apex without damaging the lungs to expose the left ventricle free wall (see Note 10).
13. Hold the heart lightly enough to provide stability but not interfere with its regular beating. Inject 75μL of the biomaterial through a 27G or 30G needle at a steady

[9]To increase visibility, a second suture can be used in the same manner but placed along the ribcage edge approximately 1cm lateral left from the xiphoid process. With the diaphragm exposed, the location of the heart apex will be visible through the diaphragm between the lungs.

[10]Once the heart apex is free from the pericardium, visibility can be aided by anchoring a sterile cotton swab with a towel clamp to keep the incision open and the right lung from blocking visibility.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

rate with the bevel or bore hole oriented toward the left ventricle lumen. The needle tip should enter the left ventricle free wall almost parallel to the epicardial surface to ensure the injection does not enter the lumen (see Note 11) (Figure 5).

14. Close the diaphragm with a taper needle suture (Prolene 5-0 RB-1). Anchor the suture using a double knot at the dorsal edge of the incision and close 3/4 of the incision with a running stitch (see Note 12).

15. When 3/4 of the opening is closed, insert an aspiration tube into the cavity along the incision and continue the running stitch around the tube until the incision is completely closed (see Note 13).

16. Place one last stitch around the tube but leave the loop large enough to use in a final double knot (Figure 6).

17. Attach a 10mL syringe to the aspiration tube.

18. With one hand, hold the running stitch tight while leaving the last stitch loose (Figure 6). Use the other hand to suction the air from the thoracic cavity while simultaneously pulling the tubing free (see Note 14).

19. Continue to hold the suture and watch the diaphragm once the aspiration tubing is free. If the diaphragm remains tight and concave the sutures can be closed with a double knot (see Note 15) (Figure 7).

20. Reduce the Isoflurane level to 1% after closing the diaphragm.

21. Release and remove the anchored suture used to make the diaphragm visible.

22. Suture the muscle layer closed with an appropriate suture (Vicryl 4-0 FS-1) using close-spaced intermittent stitches.

23. Close the skin with an appropriate suture (Vicryl 5-0 FS-2) using intermittent stitches and seal with tissue adhesive (Vetbond).

24. Apply triple antibiotic ointment over the wound.

25. Reduce the Isoflurane level to 0% and monitor breathing until the rat can breathe independent of the ventilator.

26. Administer an appropriate dose of post-operative analgesic such as Buprenex. Check with your animal care facility to determine which analgesic, administrative route and dosage is recommended for your facility (see Note 16).

---

[11]Blanching of the left ventricular free wall during the injection is a visible indication of injection success, but may not always be visible.

[12]The stitch placement should be close set to make an airtight closure to allow the lungs to properly inflate. If air is present in the thoracic cavity the animal will not be able to breathe. Mouth breathing or gasping post surgery may be an indication of air in the thoracic cavity.

[13]The aspiration tubing can be taped to a light in order to keep the aspiration tube positioned next to the sutures. The placement of the tube along the diaphragm closure is necessary to limit the chances of aspirating lung tissue or pericardium.

[14]Between 1–3mL of total volume may be removed from the cavity. If resistance is felt, the suction may be blocked by pericardium or lung tissue. If this occurs, stop suctioning and adjust the aspiration tubing. Keep the aspiration tubing next to the suture line to reduce the chances of aspirating any lung tissue.

[15]If there is a leak in the sutures the diaphragm will not remain tight and will balloon. The sutures can be loosened to reinsert the aspiration tube and repeat the process.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## References


1. Christman KL, Fok HH, Sievers RE, et al. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. Tissue Eng. 2004; 10
2. Singelyn, JM.; DeQuach, JA.; Seif-Naraghi, SB., et al. Biomaterials. Vol. 30. Elsevier Ltd; 2009. Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering.
3. Singelyn JM, Sundaramurthy P, Johnson TD, et al. Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction. Journal of the American College of Cardiology. 2012; 59
4. Seif-Naraghi SB, Singelyn JM, Salvatore MA, et al. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. Sci Transl Med. 2013; 5
5. Christman K, Vardanian A, Fang Q, et al. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. Journal of the American College of Cardiology. 2004; 44
6. Johnson TD, Christman KL. Injectable hydrogel therapies and their delivery strategies for treating myocardial infarction. Expert Opin Drug Deliv. 2012
7. Rane AA, Christman KL. Biomaterials for the treatment of myocardial infarction: a 5-year update. Journal of the American College of Cardiology. 2011; 58
8. Yang CH, Lee BB, Jung HS, et al. Effect of electroacupuncture on response to immobilization stress. Pharmacol Biochem Behav. 2002; 72


[16]Buprenorphine is a controlled substance commonly sold under the brand name Buprenex and may require special licensing to purchase. When a 0.05 mg/kg dose is subcutaneously administered using a 27G needle the animal will be mobile, sternal, and alert during their immediate recovery. Within an hour the animal's behavior and coat appearance should be normal. There should be no signs of distress such as hunched back, ruffled fur or mouth breathing.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

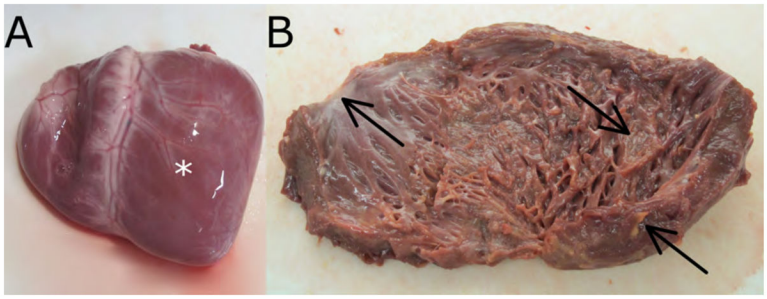


**Figure 1.**

A fresh harvested porcine heart before processing. Labeled with a (*) is the left ventricular myocardium used in the protocol (A). The fresh porcine heart is trimmed down such that only the myocardial tissue of the left ventricle remains. As indicated with arrows, the valves, superficial fat, fascia, chordae tendineae, and papillary muscles were all removed leaving behind the red myocardial tissue (B).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

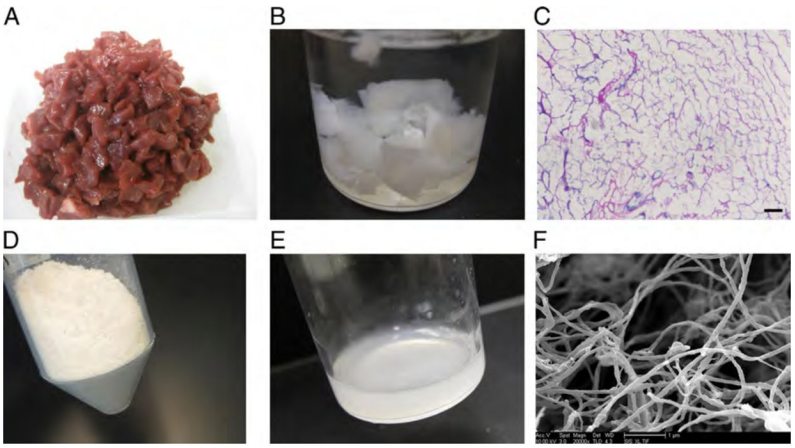


**Figure 2.**

Fresh myocardial tissue chopped into fine pieces (A). Fully decellularized, white myocardial tissue (B). An H&E stained section of fully decellularized myocardial tissue showing no remaining nuclei (C). Lyophilized and milled porcine myocardial matrix (D). Once partially digested in acid by pepsin, the material is now in a liquid form (E). When brought to physiological conditions including salt, temperature, and pH the material forms a nanofibrous gel as viewed by scanning electron microscopy (SEM) (F). Modified and reprinted from (3) with permission from Elsevier.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

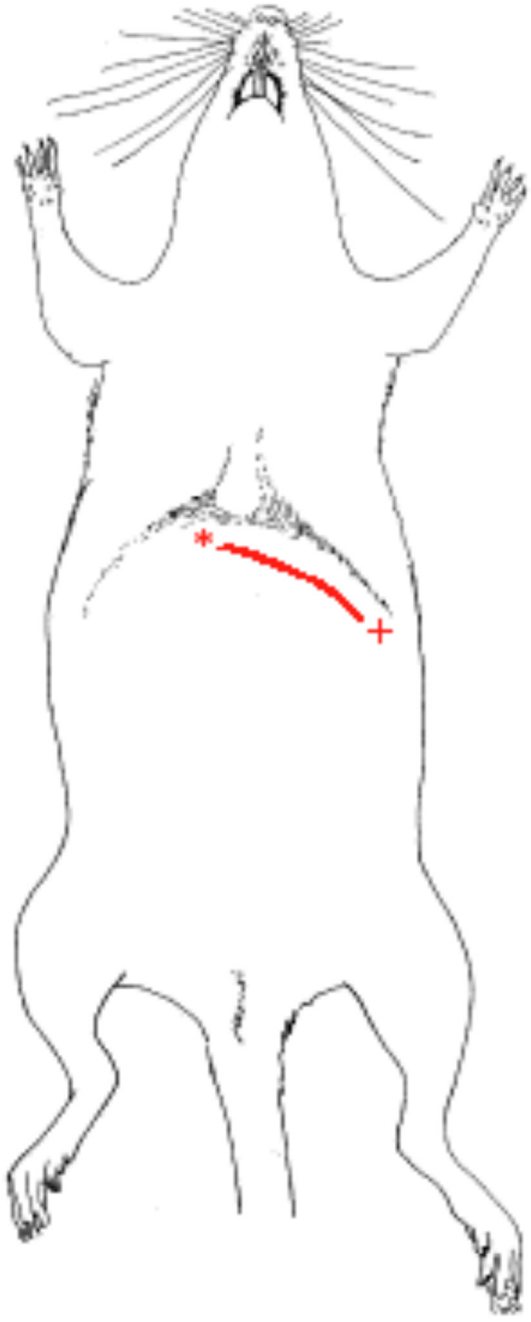

**Figure 3.**
The initial cutaneous incision beginning posterior to the xiphoid process (*) and continuing lateral left approximately 1 cm caudal to the ribcage (+). Modified and reprinted from (8) with permission from Elsevier.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

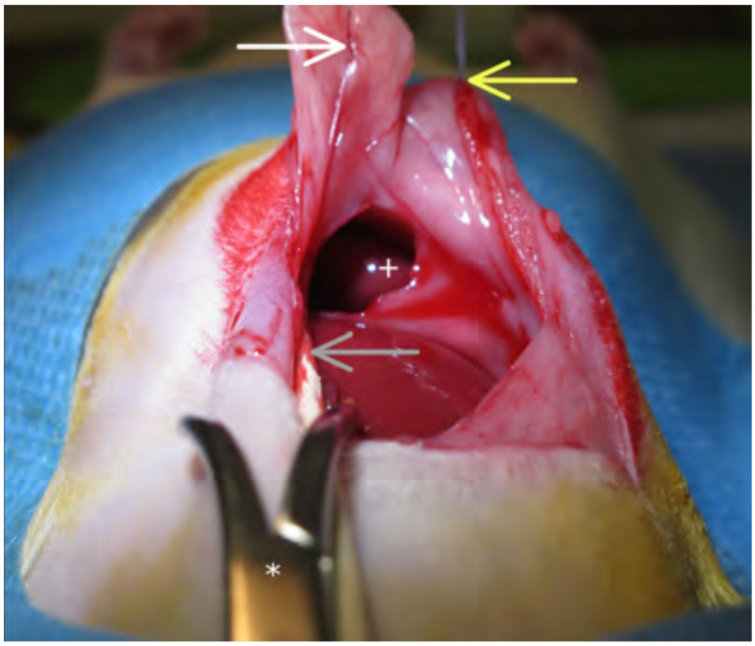

**Figure 4.**

The diaphragm is exposed by lifting xiphoid process by running half the length of an appropriate suture (Vicryl 4-0 FS-1) through the xiphoid process (white arrow) and taping the free ends of the suture to a nearby high point. Diaphragm visibility can be increased by repeating this process with another suture placed approximately 1 cm lateral left from the xiphoid process (yellow arrow). The heart apex is visible through the diaphragm (+). The diaphragm incision is made beginning dorsal to the heart apex and extends ventral 1–1.5 cm A sterile cotton swab (grey arrow, cotton head of swab not visible) anchored with a towel clamp (*) is used to keep the right lung from obscuring the heart apex.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

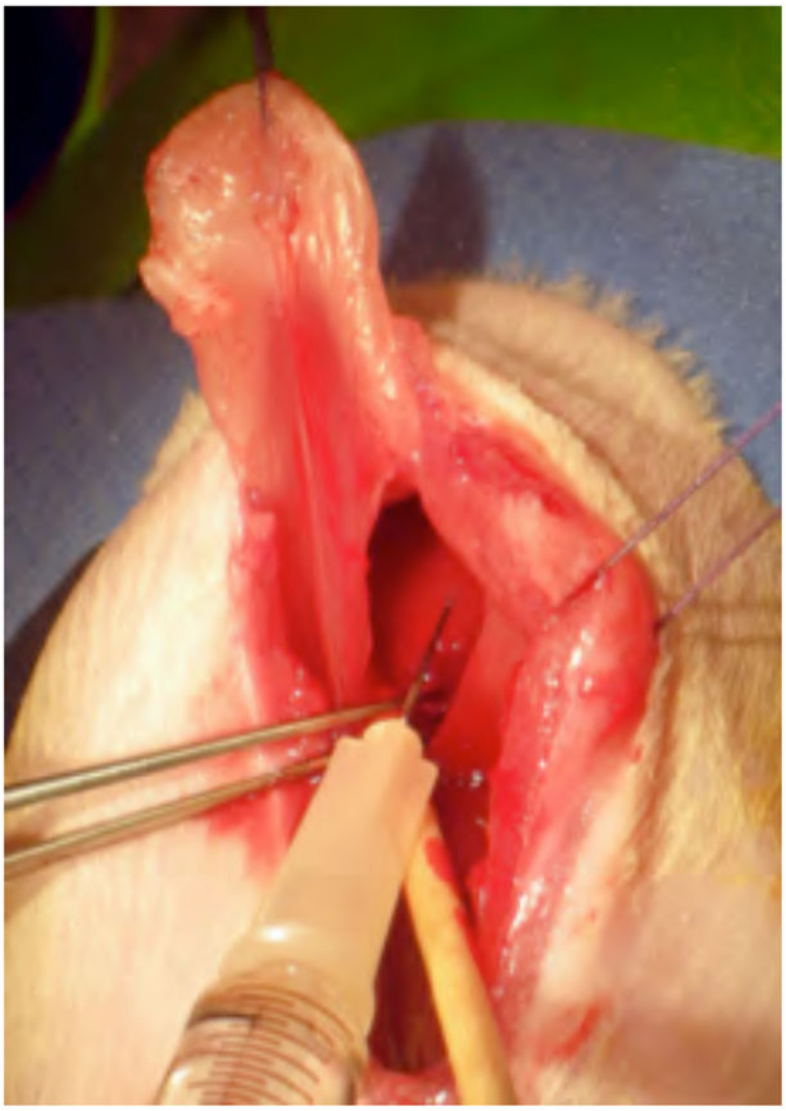

**Figure 5.**

The material is then injected into the left ventricle of the heart as shown. A 27G needle is used to deliver the material into the wall of the heart. If too much force is used the needle can fully puncture into the lumen of the ventricle, thus leading to a failed delivery.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

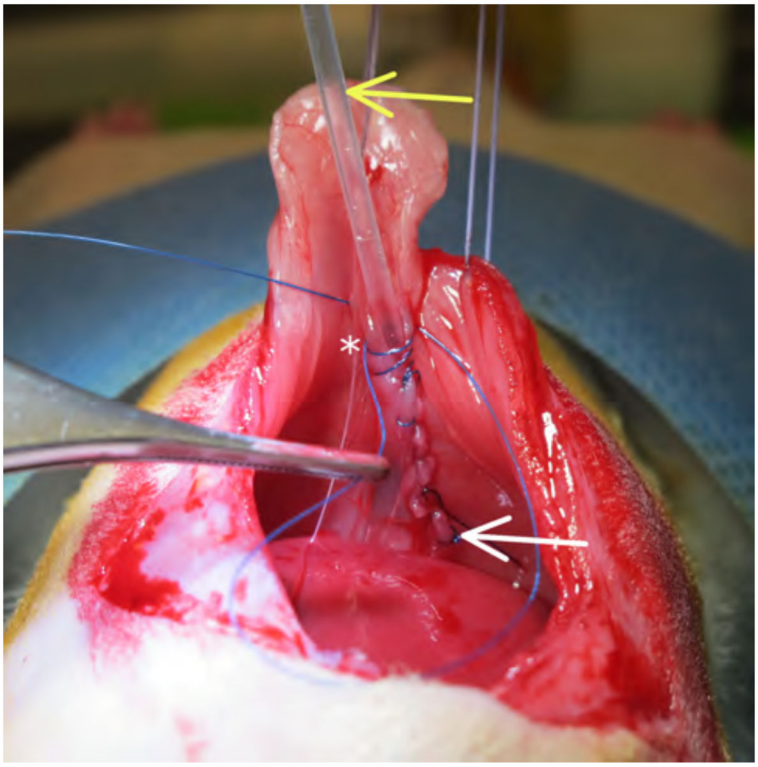

**Figure 6.**

The diaphragm is sutured closed beginning at the base of the incision using a running stitch (white arrow). The running stitch extends around the aspiration tubing (yellow arrow) and is held taught while leaving the last stitch loose (*) for the closing knot once the aspiration tube is removed.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

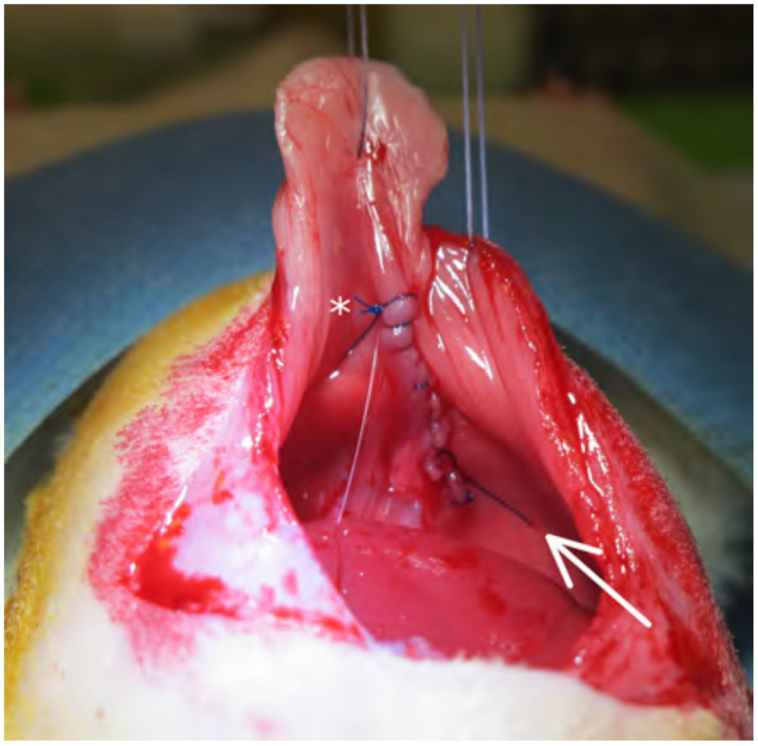

**Figure 7.**
The airtight running stitch closing the diaphragm (*) produces a concave diaphragm (white arrow) after aspirating air from the thoracic cavity.