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Dear Editor,
Please find our online revised manuscript (MS # JoVE59431R2) entitled "**Right Ventricular Failure Induced by Pulmonary Artery Constriction and Evaluation of Right Ventricular Function in Mice**" to the ***Journal of Visualized Experiments(JoVE)***, which we wish it to be considered for publication.

Thank you very much for giving us the opportunity to have this manuscript revised. Along with the valuable, constructive and very suggestive comments of the editor on our revised version of the manuscript (MS # JoVE59431R1), we have responded all the issues proposed by the editor point by point as best as we can and carefully revised the manuscript, the changes are indicated by red fonts and the protocol text (including headers and spacing) to be featured in the video have been highlighted in yellow. Therefore we believe that this paper has been largely improved and wish it would satisfy you and merit for publication.

All of the authors have made important contribution to the study and are thoroughly familiar with the original data. We confirm that: 1) the manuscript, or part of it, has neither been published nor is currently under consideration for publication by any other journal, 2) the co-authors have read the manuscript and approved its submission to ***JoVE***, and 3) there is no industry relationship. I would be grateful if the manuscript could be reviewed and considered for publication in ***JoVE.***

I am looking forward to hearing from you soon.

Very sincerely yours,

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TITLE:

Induction of Right Ventricular Failure by Pulmonary Artery Constriction and Evaluation of Right Ventricular Function in Mice

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KEYWORDS:

Pulmonary artery constriction, right ventricular failure, right ventricular hypertrophy, echocardiography, right heart catheterization, mouse model, surgical instrument made inhouse, pressure overload

SUMMARY:

Here, we provide a useful approach for studying the mechanism of right ventricular failure. A more convenient and efficient approach to pulmonary artery constriction is established using surgical instruments made inhouse. In addition, methods to evaluate the quality of this approach by echocardiography and catheterization are provided.

ABSTRACT:

The mechanism of right ventricular failure (RVF) requires clarification due to the uniqueness, high morbidity, high mortality, and refractory nature of RVF. Previous rat models imitating RVF progression have been described. Compared with rats, mice are more accessible, economical, and widely used in animal experiments. We developed a pulmonary artery constriction (PAC) approach which is comprised of banding the pulmonary trunk in mice to induce right ventricular (RV) hypertrophy. A special surgical latch needle was designed that allows for easier separation

of the aorta and the pulmonary trunk. In our experiments, the use of this fabricated latch needle reduced the risk of arteriorrhexis and improved the surgical success rate to 90%. We used different padding needle diameters to precisely create quantitative constriction, which can induce different degrees of RV hypertrophy. We quantified the degree of constriction by evaluating the blood flow velocity of the PA, which was measured by noninvasive transthoracic echocardiography. RV function was precisely evaluated by right heart catheterization at 8 weeks after surgery. The surgical instruments made inhouse were composed of common materials using a simple process that is easy to master. Therefore, the PAC approach described here is easy to imitate using instruments made in the lab and can be widely used in other labs. This study presents a modified PAC approach that has a higher success rate than other models and an 8-week postsurgery survival rate of 97.8%. This PAC approach provides a useful technique for studying the mechanism of RVF and will enable an increased understanding of RVF.

INTRODUCTION:

RV dysfunction (RVD), defined here as evidence of an abnormal RV structure or function, is associated with poor clinical outcomes. RVF, as the end stage of RV function, is a clinical syndrome with signs and symptoms of heart failure that result from progressive RVD[1]. With differences in structure and physiological function, left ventricular (LV) failure and RVF have different pathophysiological mechanisms. A few independent pathophysiological mechanisms in RVF have been reported, including overexpression of β2‑adrenergic receptor signaling[2], inflammation[3], transverse tubule remodeling, and Ca^{2+} handling dysfunction[4].

RVF can be caused by volume or pressure overload of the RV. Previous animal models have used SU5416 (a potent and selective inhibitor of the vascular endothelial growth factor receptor) combined with hypoxia (SuHx)[5,6] or monocrotaline[7] to induce pulmonary hypertension, which results in RVF secondary to pulmonary vascular disease[2]. The researchers conducting these studies focused on the vasculature instead of the pathological progression of RVF. Moreover, monocrotaline has extra-cardiac effects that cannot precisely represent cardiogenic disease. Other models have used arteriovenous shunts to induce volume overload and RVF[8]. However, this surgery is difficult to perform and inappropriate for mice, who require long induction periods for the production of RVF.

Rat PAC models using banding clips also exist[9,10]. Compared with rats, mice have many advantages as animal models of cardiac diseases, such as easier reproduction, more widespread use, reduced costs, and access to gene modification[11]. However, the diameters of the banding clips usually range from 0.5 mm to 1.0 mm, which are too large for mice[9]. In addition, the banding clip is hard to produce, imitate, and popularize in other labs.

We provide a protocol to develop a modified reproductive RVF mouse model based on reported studies, which uses PAC to mimic the tetralogy of Fallot and Noonan syndrome or other

pulmonary arterial hypertensive diseases[12–19]. This PAC approach is created by ligating the pulmonary trunk of mice using a latch and padding needle made inhouse to control the degree of constriction. The latch needle is made of a 90° curved injection syringe with a braided silk suture passed through the syringe. The needle is made from common materials using a process that is easy to master. The padding needle is curved 120° from the gauge needle. Padding needles with different diameters (0.6–0.8 mm) are used, depending on the mice's weight (20–35 g). Additionally, we establish an evaluation criterion to determine the stability and quality of the RVF model by echocardiography and right heart catheterization. We use mice as the model animal because of their widespread use in other experiments. The needles made in the lab are easy to reproduce and can be widely used in other labs. This study provides a good approach for researchers to investigate the mechanism of RVF.

PROTOCOL:

All procedures were performed in accordance with institutional guidelines for animal research, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised in 1996). C57BL/6 male mice (8–10 weeks old, weighing 20–25 g) were provided by the Animal Center of South Medical University. After arrival, the mice were housed under a 12/12 h dark/light cycle, with sufficient food and water.

1. Preparation of the surgical instruments and fabrication of the needles

1.1. Prepare the sterile surgical instruments (**Figure 1A**), a 6-0 braided silk suture (**Figure 1B[a]**) for ligation, and a 5-0 nylon suture for incision closure (**Figure 1B[b]**).

1.2. Pass the 6-0 braided silk suture (**Figure 1B[a]**) through a 25 G needle disassembled from a 1 mL injection syringe. Then, curve the needle 90° with hemostatic forceps to make a latch needle (**Figure 1C[a]**). Curve the 22 G needle 120° (**Figure 1C (b)**) to make a padding needle.

2. Preparation for surgery

2.1. Autoclave all surgical instruments before surgery. Adjust the heating pad to 37 °C. Anesthetize the mice by intraperitoneal injection with a mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg) for pain relief. Place the mice in individual boxes to wait for narcotic onset.

NOTE: It is also recommended to use 1.5% isoflurane for inhalant anesthesia.

2.2. Monitor the adequacy of anesthesia by the disappearance of the pedal withdrawal reflex. Keep the mice in the supine position on the heating pad by fixing the incisors with a suture and fixing the legs with adhesive tape. Check the reflex again to ensure the depth of anesthesia.

2.3. Apply the depilatory paste on the skin from the neck to the xiphoid process. Disinfect the area with iodine followed by 75% alcohol.

2.4. Perform endotracheal intubation.

2.4.1. Adjust the animal miniventilator's (**Figure 1D**) parameters and set the respiratory rate to 150/min and the tidal volume to 300 µL.

2.4.2. Pull out the tongue slightly by using pointed pliers, elevate the mandible with a lab made spatula **Figure 1C[c]**) to expose the glottis, and softly insert a lab made trachea cannula (**Figure 1C[d]**) through the glottis while a cold light source is directed on the larynx.

2.4.3. Connect the tube and the ventilator to verify whether the cannula has been inserted into the trachea. Fix the trachea using adhesive tape if the cannula has been inserted correctly.

3. Surgery

3.1. Open the chest.

3.1.1. Make an incision in the skin parallel to the second rib, about 10 mm in length, with ophthalmic scissors. Ensure that the incision starts from the sternal angle and ends on the left anterior axillary line. Identify the second intercostal space by counting the ribs from the sternal angle.

3.1.2. Separate and cut the pectoralis major and pectoralis minor muscles with scissors and elbow tweezers above the second intercostal space to expose this space.

NOTE: It is also recommended to bluntly separate, mobilize, and move the pectoralis muscles to the right and cranially.

3.1.3. Bluntly penetrate the second intercostal space with elbow tweezers and open this space. Then, bluntly separate the parenchyma and thymus until the pulmonary trunk is visible.

3.2. Constrict the pulmonary artery.

3.2.1. Bluntly separate the pulmonary trunk and the ascending aorta with elbow tweezers. Pass the suture through the connective tissue between the pulmonary trunk and the ascending aorta with a latch needle.

3.2.2. Place the padding needle (see step 1.2) on the pulmonary trunk and, then, ligate the pulmonary trunk together with the padding needle, using the 6-0 braided silk suture. Remove the padding needle immediately after the filling of the pulmonary conus is observed and cut the ends of the suture.

3.2.3. Observe the filling of the pulmonary conus to evaluate whether there is a constriction present. Evaluate the animal's reflex again to ensure the success of the ligation.

NOTE: Perform a sham surgery by following all of the above steps except for the constriction.

3.3. Close the chest and the skin with the 5-0 nylon suture. Disinfect the skin again with 75% alcohol.

3.4. Inject 0.5 mL of saline subcutaneously to replace any fluid lost during the surgery. Place the mouse in the cage separately with heating pad to promote recovery. Return the mice to their cages in a 12/12 h light/dark cycle room when consciousness returns. Treat the mice with buprenorphine via their drinking water for the following 3 days[20].

3.5. Pay special attention to the healing of the thoracotomy wound by monitoring the mice 2x a day during the first week to detect any signs of insufficient healing, impaired mobility, or weight loss.

4. Echocardiographic assessment of the RV function after surgery

NOTE: Echocardiographic changes can be detected 3 days after surgery.

4.1. Anesthetize the mice with 3% isoflurane through inhalation and maintain the depth of anesthesia with 1.5% isoflurane. Fix a mouse on the platform, tape its claws to the electrode, and maintain the animal in a supine position. Maintain the mouse's heart rate between 450–550 beats/min by adjusting the concentration of isoflurane between 1.5% and 2.5%.

4.2. Remove the hair on the mouse's chest with depilatory cream and apply ultrasound gel to the skin of the chest.

4.3. Assess the pulmonary trunk constriction with a 30 MHz probe.

4.3.1. Keep the probe at 30° counterclockwise relative to the left parasternal line, while orienting the notch in the caudal direction. Regulate the *y*-axis and *x*-axis under the B-mode until the mitral valve, aorta, and LV chamber are clearly visible.

4.3.2. Rotate the probe 30°–40° on its *y*-axis toward the chest. Regulate the *y*-axis and *x*-axis until the pulmonary conus is clearly visible.

4.3.3. Place the cursor at the tip of the pulmonary valve leaflets to measure the peak flow velocity. Use the Color Doppler mode by pressing **Color**, followed by **PW**, and then moving the cursor to place the PW-dashed line parallel to the direction of the blood flow.

4.3.4. Measure the pulmonary artery peak velocity. Save the data and image with **Cine Store** and **Frame Store**.

4.4. Assess the RV parameters with a 30 MHz probe.

4.4.1. Adjust the left side of the pad so that it is lower than the right side. Keep the probe oriented at 30° to the horizon along the right anterior axillary line with the notch pointed in the caudal direction. Regulate the *y*-axis and *x*-axis until the RV is clearly shown.

4.4.2. Press **M-Mode** 2x to show the indicator line. Measure the RV chamber dimension, fractional shortening, and RV wall thickness. Save the data and image with **Cine Store** and **Frame Store**.

4.5. Stop the isoflurane inhalation for 20 s to allow the mice to recover their consciousness, and then, return the animals to their cages in a 12/12 h light/dark cycle room.

5. Right heart catheterization for assessing the RV function

NOTE: Right heart catheterization was performed 8 weeks after surgery to assess the RV function, using a 1.0 F catheter and a monitoring system.

5.1. Autoclave all surgical instruments. Anesthetize the animal via intraperitoneal injection with a mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg).

5.2. After the pedal withdrawal reflex disappears, fix the mouse on the platform, tape its claws to the electrode, and maintain the mouse in supine position. Remove the hair in the surgical area with depilatory cream.

5.3. Disinfect the skin of the surgical area with 75% alcohol. Using pointed pliers, pull the tongue out slightly, elevate the mandible with a spatula made inhouse to expose the glottis, and softly insert the trachea cannula made inhouse through the glottis while a cold light source is directed on the larynx. Use a ventilator (**Figure 1E**) to assist with ventilation.

5.4. Open the chest cavity by means of a 1.5 cm bilateral incision below the xiphoid process through the diaphragm with ophthalmic scissors and forceps. Cut through the diaphragm and ribs with ophthalmic scissors to expose the heart. Penetrate the RV free wall with a 23 G needle and remove the needle; press the puncture point gently with a cotton swab to stop any bleeding. Puncture the ventricle with the catheter tip through the wound.

NOTE: It is also recommended to perform right heart catheterization via the right jugular vein[6]. When the catheter tip is in the ventricle, the monitor will display the RV pressure curve.

5.5. Record the RV systolic blood pressure, the RV end-diastolic pressure, the RV dP/dt, the mouse's heart rate, and the RV exponential time constant of relaxation (Tau) for 10 min when the curve is stable. Using the software, click **Select** and then click **Analyze**.

5.6. Regulate the tip of the catheter toward the RV outflow tract. Pull the catheter out after the recording is complete. Place the catheter in saline when the measurements are finished.

5.7 Euthanize the mice by intraperitoneal injections of pentobarbital sodium 150 mg/kg, followed by cervical dislocation. Then, harvest the heart, lungs, and tibia for histomorphological and molecular biological analyses.

REPRESENTATIVE RESULTS:

In this study, mice were randomly assigned to the PAC group (n = 9) or the sham operation group (n = 10). Echocardiography was performed at 1, 4, and 8 weeks after the surgery. Eight weeks after surgery, following the last echocardiography and catheterization assessments, the mice were euthanized, and their hearts were harvested for a morphological and histological assessment.

Pulmonary trunk constriction caused RV hypertrophy (**Figure 2**). Compared with the sham group, a higher peak velocity (**Figure 2A, C**), greater pressure gradient (**Figure 2A,D**), and greater RV wall thickness (**Figure 2B,E**) from the parasternal long-axis view were obtained at 8 weeks after surgery in the PAC group. Additionally, the systolic function of the RV (RV ejection fraction and RV fraction shortening) was significantly reduced in the PAC group when compared with the sham group 8 weeks after the surgery (**Figure 2F, G**).

We found that the RV systolic and diastolic function were impaired 8 weeks after PAC (**Figure 3A–E**). The PAC group had a higher RV pressure in the systole and diastole, and the contractility index was reduced in the PAC group compared with that of the sham group. The RV Tau was greater in the PAC group than in the sham group, and RV dP/dt was also greater than that in the sham group. These results showed that RV dysfunction was induced in mice after 8 weeks of pulmonary artery banding. When we performed invasive hemodynamic testing in the RV, the heart rate, which was

determined using a physiological recording system, remained stable before and after catheter monitoring (**Figure 3F**).

RV remodeling induced by PAC is shown in **Figure 4**. Compared to the sham group, the RV dimension was significantly enlarged, and the RV weight was higher in the PAC group. Factors that indicate the degree of RV hypertrophy, such as the heart weight/body weight ratio, RV/(left ventricle + septum), and RV/tibial length, were greater after 8 weeks of PAC than those of the sham group. Moreover, a histological examination showed that cardiac fibrosis and the area covered by cardiomyocytes were greater in the PAC group than in the sham group. In summary, we developed a reproductive, inexpensive, and easy RVF model and established evaluation criteria to successfully evaluate the RVF model.

FIGURE LEGENDS:

Figure 1: Surgical instruments, tools made inhouse, and materials required for the PAC procedures. (**A**) The surgical instruments used for the PAC procedure. (**B**) (**a**) 6-0 medical braided silk suture and (**b**) 5-0 medical nylon suture. (**C**) Tools made inhouse. (**a**) Latch needle. (**b**) Padding needle. (**c**) Spatula made inhouse. (**d**) Endotracheal intubation. (**D).** Animal mini-ventilator. (**E**) ALC–V8S ventilator.

Figure 2: Representative results of ultrasound imaging of the pulmonary trunk and the RV function of sham and PAC mice. (**A**) Color and pulsed-wave Doppler imaging of the pulmonary trunk of mice after 8 weeks. Red marks represented blood flow toward the probe; Blue marks represented the blood flow backward the probe. (**B**) B-mode and M-mode ultrasound imaging of the RV of sham and PAC mice after 8 weeks. (**a**) Right ventricle. (**b**) Left ventricle. (**C**) The RV peak velocity PLAX (V), (**D**) pressure gradient (pressure gradient = 4 x V^2), and (**E**) RV wall thickness from the parasternal long-axis view were significantly increased after 8 weeks. (**F**) The RV short axis shortening rate (RVFS) was significantly reduced after 8 weeks. (**G**) The RV ejection fraction (RVEF) was significantly reduced after 8 weeks. For panels **C–G**,**P* < 0.01 vs. sham operation (*n* = 9 in the PAC group, *n* = 10 in the sham group). PAC = pulmonary artery constriction. The data are presented as mean ± SEM.

Figure 3: Representative results of the RV hemodynamics in mice subjected to PAC or a sham operation, 8 weeks after the surgery. (**A**) Representative curves of RVP and RV dP/dt in sham and PAC mice at 8 weeks after the surgery. (**B**) Right ventricular systolic blood pressure (RVSBP) and right ventricular end-diastolic pressure (RVEDP). (**C**) RV maximum and minimum dP/dt. (**D**) RV Tau. (**E**) Contractility index. (**F**) Heart rate. RVP = right ventricular pressure; RVSBP = right ventricular systolic blood pressure; RVEDP = right ventricular end-diastolic pressure; Tau = exponential time constant of relaxation; max and min dp/dt = maximum and minimum rise and decline in right ventricular pressure; PAC = pulmonary artery constriction. For panels **B–F**, *n* = 9 in the PAC group

and *n* = 10 in the sham group. **P* < 0.01 vs. sham operation. The data are presented as mean ± SEM.

Figure 4: Pulmonary artery constriction in mice leads to RV remodeling after 8 weeks. (**A**) Representative images of the whole heart (the scale bar = 3 mm). (**a**) Right atrium, red arrow: ligation of the pulmonary artery trunk. (**B**) Heart weight/body weight ratio (HW/BW). (**C**) Ratio of the right ventricular mass to left ventricular mass plus septum mass (RV/[LV+S]). (**D**) Ratio of the right ventricular mass to tibial length (RV/TL). For panels **B–D**, *n* = 9 in the PAC group and *n* = 10 in the sham group. **P* < 0.01 vs. sham operation. (**E**) Representative images of cardiac cross-sections stained with hematoxylin-eosin (first row: the scale bar = 2 mm; second row: the scale bar = 50 μm). (**F**) Representative Masson-stained images of myocardial fibrosis in each group. The scale bar = 100 μm. For panels **E** and **F**, *n* = 4 in each group. **P* < 0.01 vs. the sham group. RV = right ventricle; PAC = pulmonary artery constriction. RV/[LV+S] = ratio of the right ventricular mass to left ventricular mass plus septum mass. The data are presented as mean ± SEM.

DISCUSSION:

Pathological increases in RV filling pressures result in a leftward shift of the septum, which can alter the LV geometry[21]. These changes contribute to reduced cardiac output and LV ejection fraction (LVEF), which can cause a hemodynamic disorder of the circulatory system[22]. Therefore, an efficient, stable, and economical model for studying the mechanism of RVF is valuable.

We developed a more effective and highly reproducible approach to PAC using a latch and padding needle made inhouse. The latch made inhouse allows for an easier separation of the aorta and the pulmonary trunk, which reduces the risk of arteriorrhexis and improves the surgical success rate. By selecting different diameters of the padding needle, we induced differing degrees of RV hypertrophy.

Even though the general procedures of pulmonary trunk banding described here are like those described in previous reports[4,9,10,14,15], we made improvements to the surgical instruments. Thus, we reduced the difficulty of the operation, shortened the operating time, and increased the success rate of the surgery. The diameters of the padding needles we used ranged from 0.6 mm to 0.8 mm, and these were only suitable when the mice weighed between 20 g and 35 g. In rats, the application of the padding needles (0.6–0.8 mm) may lead to acute RVF and death. In addition, the padding needles (0.6–0.8 mm) may not easily lead to RVD if the mice weigh less than 20 g. Therefore, the correct padding needle diameter should be selected according to the weight of the animal.

Pulmonary arterial hypertension (PAH) is usually induced by a subcutaneous injection of the vascular endothelial growth factor receptor inhibitor SU5416 and feeding in a hypoxic environment for longer than 3 weeks[23–28]. These conditions mimic the pathophysiological process

of chronic ischemia and hypoxia of the pulmonary artery to induce PAH and pulmonary artery fibrosis. However, RV remodeling, hypertrophy, or RVF requires more than 12 weeks for induction by chronic hypoxia in these models. Additionally, SU5416 and hypoxic treatment may affect other organs. Furthermore, expensive machines are required to create a hypoxic environment. Therefore, a faster and more efficient model of RVF is required. Reddy et al. reported a method of RV remodeling by entrapping the two anterior-most pulmonary valve leaflets[13]. Instead of using a microscope and expensive surgical vascular clips[29], we used a latch needle and different types of padding needles made inhouse to precisely create a quantitative constriction along with an evaluation of blood flow velocity by echocardiography.

In addition, the latch and padding needle made inhouse were also used to induce transverse aortic constriction (TAC) in mice. The latch made inhouse can also be used to induce PAC or TAC in rats. During transposition of the great vessels, the LV does not encounter sufficient resistance, so it needs to be strengthened by applying pulmonary artery constriction in preparation for corrective surgery[30,31]. The PAC approach we provided can result in elevated pulmonary artery resistance, which will help studies of the underlying mechanisms. In the setting of a heart transplantation, the donor RV may be acutely exposed to elevated pulmonary artery resistance in the recipient, causing the RV to fail. The PAC method presented here can help studies of the mechanisms of post-heart transplantation complications[32,33] and congenital heart diseases[34].

The PAC approach has a few limitations. First, the RVD induced by the ligature around the pulmonary trunk cannot imitate RVD in PAH[5,7]. Second, PAC causes a very sudden increase in RV afterload that is different from the gradual increase in pulmonary vascular resistance in PAH[9,19].

Consistent with the results presented here, previous reports have shown that significant increases under echocardiographic testing in pulmonary valve peak velocity, RV wall thickness, and RV diastolic internal diameter demonstrate successful constriction and hypertrophy of the RV[13,35]. Increased RV pressure, RV dP/dt, and contractility index indicate the development of RVF and a decompensated period of RV function[36]. In conclusion, we demonstrated the application of two novel lab-made instruments to establish RVD in an inexpensive and convenient way. We used a noninvasive echocardiographic technique and invasive right heart catheterization to evaluate the quality of the RVF method.

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DISCLOSURES:

The authors have nothing to disclose.

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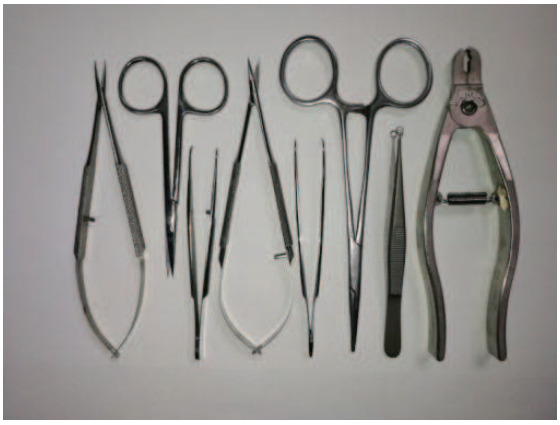
Figure 1

A



B

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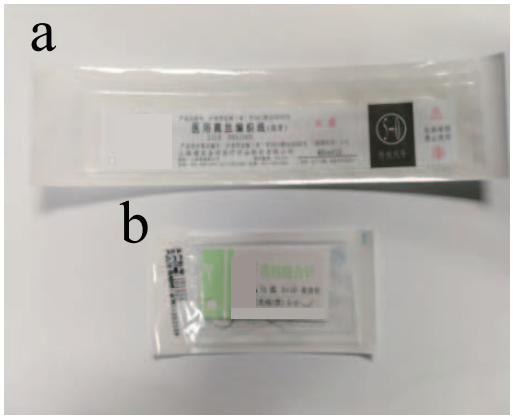

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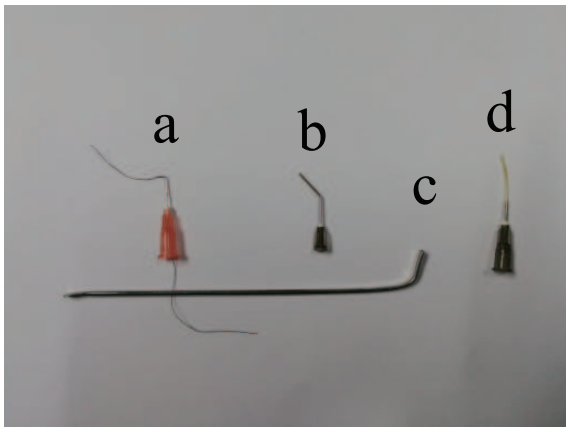

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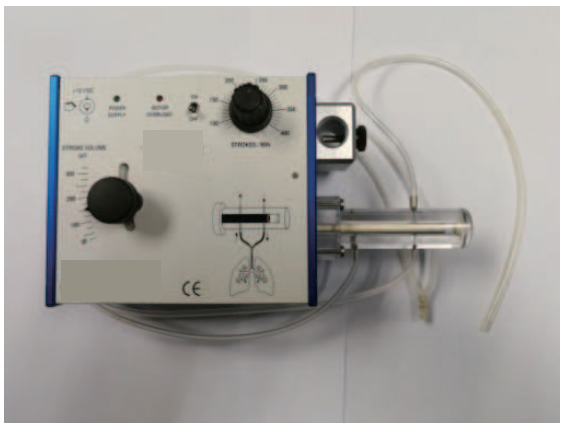

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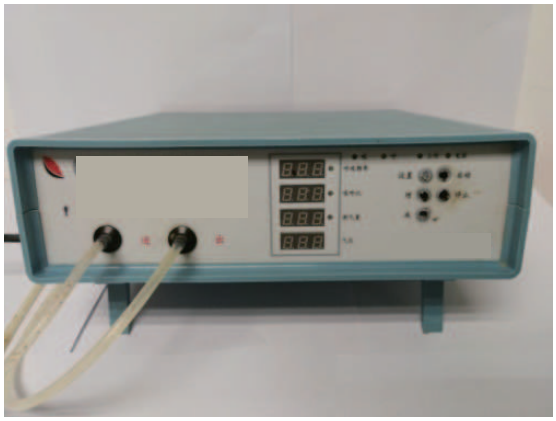

Figure 1

Figure 2

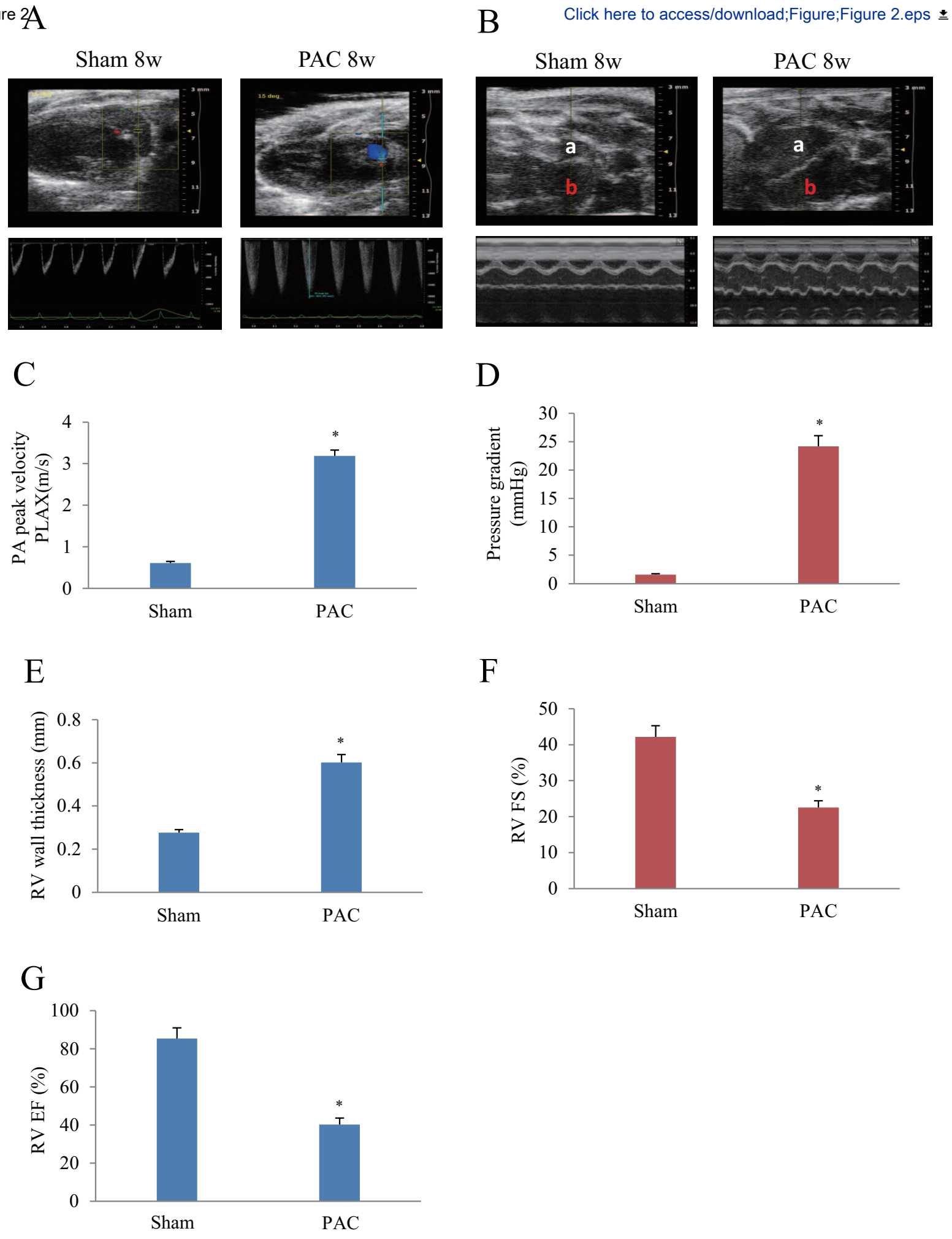

Figure 2

Figure 3

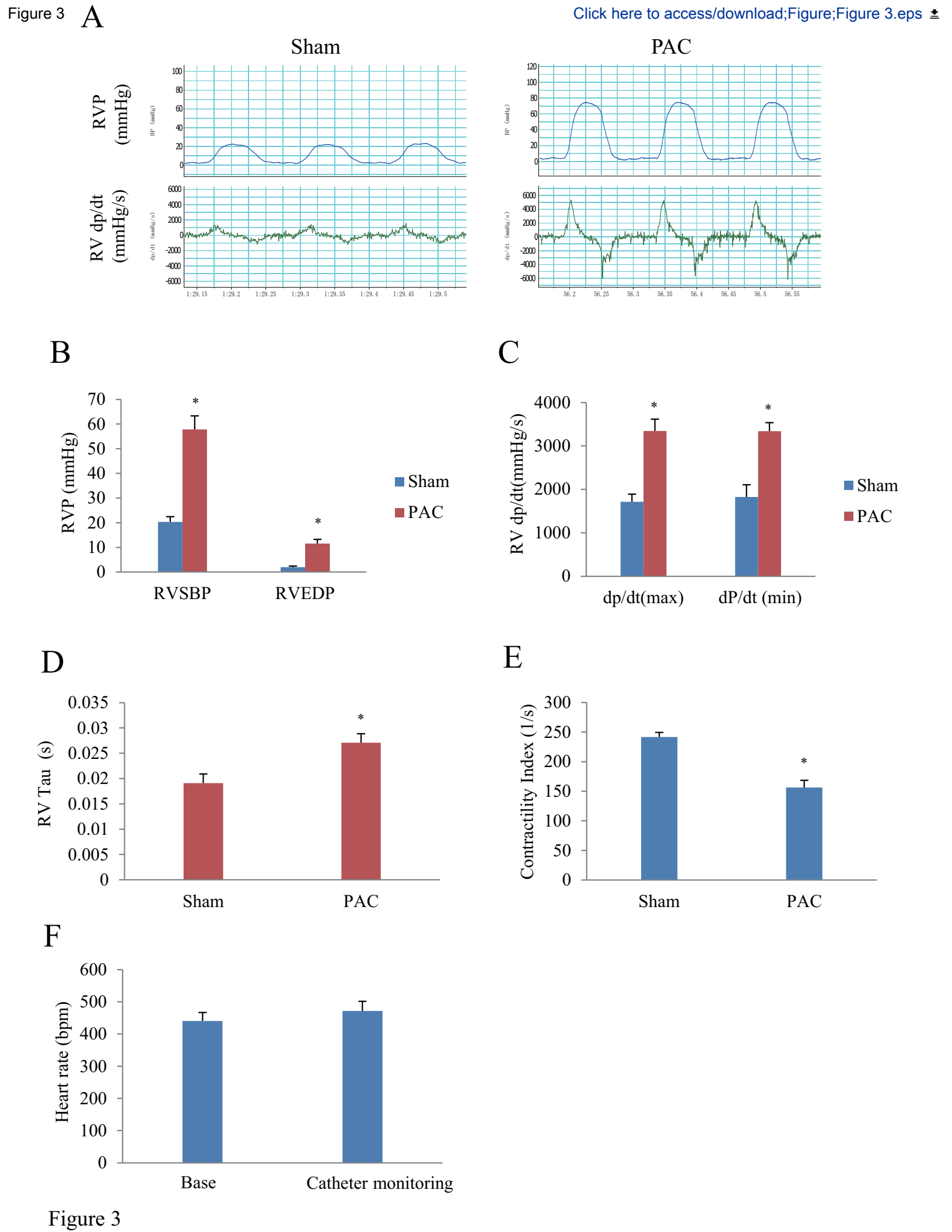

Figure 3

Figure 4

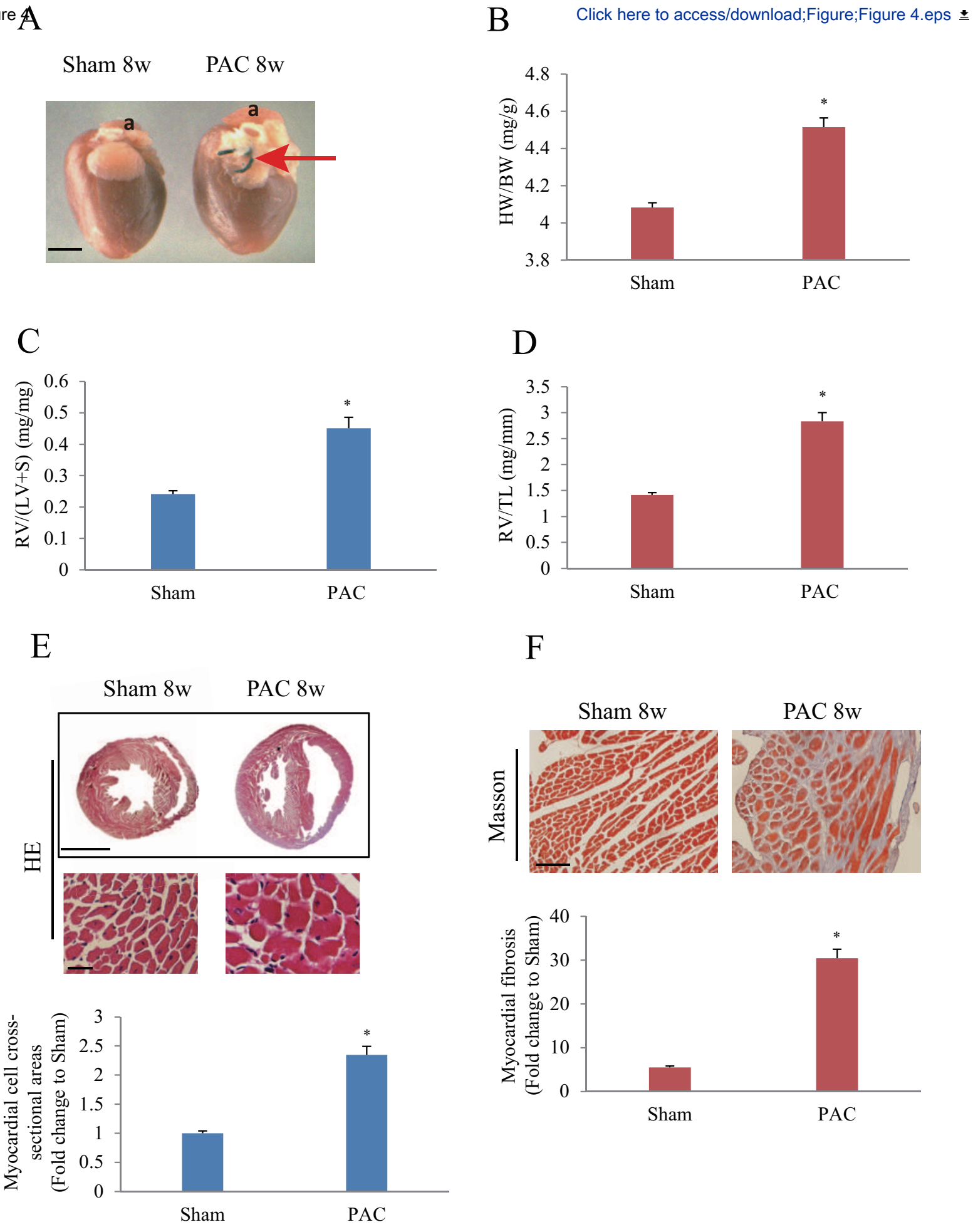

Figure 4

Name of Material/ Equipment	Company	Catalog Number
ALC-V8S ventilator	SHANGHAI ALCOTT BIOTECH CO	ALC-V8S
Animal Mini Ventilator	Haverd	Type 845
Animal ultrasound system VEVO2100	Visual Sonic	VEVO2100
Cold light illuminator	Olympus	ILD-2
Heat pad- thermostatic surgical system (ALC-HTP-S1)	SHANGHAI ALCOTT BIOTECH CO	ALC-HTP-S1
Isoflurane	RWD life science	R510-22
Matrx VIP 3000 Isofurane Vaporizer	Midmark Corporation	VIP 3000
Medical braided silk suture (6-0)	Shanghai Pudong Jinhuan Medical Supplies Co.	6-0
Medical nylon suture (5-0)	Ningbo Medical Needle Co.	5-0
Millar Catheter (1.0 F)	AD instruments	1.0F
Pentobarbital sodium salt	Merck	25MG
PowerLab multi-Directional physiological Recording System	AD instruments	4/35
Precision electronic balance	Denver Instrument	TB-114
Self-made latch needle		
Self-made padding needle		
Self-made tracheal intubation		
Small animal microsurgery equipment	Napox	MA-65
Transmission Gel	Guang Gong pai	250ML
Veet hair removal cream	Reckitt Benchiser	RQ/B 33 Type 2
Vertical automatic electrothermal pressure steam sterilizer	Hefei Huatai Medical Equipment Co.	LX-B50L
Vertical small animal surgery microscope	Yihua Optical Instrument	Y-HX-4A

Comments/Description
Assist ventilation
Assist ventilation
Echocardiography
Light
Heating
Inhalant anaesthesia
Anesthetization
Ligation
Suture
For right heart catheterization
Anesthetization
Record the result of right heart catheterization
Weighing sensor
Separate the aorta and pulmonary trunk
Constriction
Tracheal intubation
Surgical instruments
Echocardiography
Remove hair of mice
Auto clean the surgical instruments
For right heart catheterization

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Author(s): Qiancheng Wang, Kaitang Chen, Hairui Lin, Mingyuan He, Xiaoxia Huang, Hailin Zhu, and Yulin Liao.

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