Video Article

Transplantation of Pulmonary Valve Using a Mouse Model of Heterotopic Heart Transplantation

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Abstract

Tissue engineered heart valves, especially decellularized valves, are starting to gain momentum in clinical use of reconstructive surgery with mixed results. However, the cellular and molecular mechanisms of the neotissue development, valve thickening, and stenosis development are not researched extensively. To answer the above questions, we developed a murine heterotopic heart valve transplantation model. A heart valve was harvested from a valve donor mouse and transplanted to a heart donor mouse. The heart with a new valve was transplanted heterotopically to a recipient mouse. The transplanted heart showed its own heartbeat, independent of the recipient's heartbeat. The blood flow was quantified using a high frequency ultrasound system with a pulsed wave Doppler. The flow through the implanted pulmonary valve showed forward flow with minimal regurgitation and the peak flow was close to 100 mm/sec. This murine model of heart valve transplantation is highly versatile, so it can be modified and adapted to provide different hemodynamic environments and/or can be used with various transgenic mice to study neotissue development in a tissue engineered heart valve.

Video Link

The video component of this article can be found at https://www.jove.com/video/51695/

Introduction

Congenital cardiovascular defects are one of the leading causes of infant mortality in the western world[1,2]. Among them, pulmonic valve stenosis and bicuspid aortic valve defects are a frequently occurring form[3]. Heart valve replacement surgery is a routine choice of reconstructive surgeries; however, complications including stenosis and calcification of the heart valve, and lifelong dependence on anticoagulants are a significant source of chronic ill health and death[4-7]. Moreover, the lack of growth potential requires revision surgeries, which further increases the mortality of those young patients[4,8,9].

In an attempt to develop a functional replacement heart valve with growth potential, Shinoka *et al.* seeded autologous cells onto a biodegradable synthetic heart valve[8]. The synthetic valve transformed to a native heart valve like structure with growth potential. Preliminary large animal studies demonstrated the feasibility of using this methodology to create a functional heart valve[10]. However, long term implantation studies demonstrated poor durability due to progressive thickening of the valve neotissue resulting in narrowing of the heart valve. Work from Sodian *et al.* used the Shinoka methodology, but ultimately replaced the PGA matrix with a biodegradable elastomer, which gave the biomechanical properties of the tissue engineered valve construct a more physiological profile[9,11,12]. In the *in vivo* study, despite the success of the implantation, a confluent endothelial cell lining was not formed which could limit the long term success of this scaffold[12].

In order to rationally design an improved second generation synthetic heart valve, a murine model of heart valve transplantation was created to investigate the cellular and molecular mechanisms underlying neotissue formation, valve thickening, and stenosis development. Murine models offer a vast array of molecular reagents, including transgenics, which are not readily available in other species[7]. In this heart valve transplantation model, an *ex vivo* syngeneic pulmonary heart valve replacement was performed first; and then the heart with the implanted heart valve was implanted heterotopically into a syngeneic host using a microsurgical technique. This model enables heart valve replacement without the need for cardiopulmonary bypass.

In this paper, a detailed explanation of a heart valve harvest, donor heart preparations, heart valve transplantation, and heterotopic heart transplantation is described. The results showed a continuous heartbeat from the donor heart, which was independent of the recipient heartbeat. The blood flow through the implanted pulmonary valve was measured using a high frequency ultrasound system with a pulsed wave Doppler.

Protocol

Note: All animal procedures were approved by the Nationwide Children's Hospital Institutional Animal Care and Use Committee.

1. Pulmonary Heart Valve Harvest from a Heart Valve Donor Mouse

1. Autoclave all the surgical tools before the surgery: 1x fine scissors, 3x micro forceps, 2x micro vascular clamps, 1x clamp applying forceps, 1x micro needle holder, 1x spring scissors, 1x retractor.
2. A 6-8 week old female C57BL/6 mouse is used as a pulmonary heart valve donor. Remove the mouse from its cage and weigh it, then euthanize with a ketamine/xylazine cocktail (Ketamine, 200 mg/kg and xylazine, 20 mg/kg, IP) overdose.
3. Clip the chest area and place the mouse in a dorsal recumbence position on a pad. Then make the thoracotomy. Expose the heart, make a small cut on the right atrium, and perfuse the left ventricle with ice cold saline.
4. Bluntly dissect the pulmonary artery (PA) from the ascending aorta. Cut out the pulmonary valve (PV) along with 2 mm cuff of pulmonary artery. Dispose of the remainder of the heart.
5. Store the PV in cold heparin and saline solution (100 units/ml). Note: The PV can be kept in the solution for two hours before transplantation to the donor heart.

2. Donor Heart Preparation

1. A 6-8 week old female C57BL/6 mouse is used as a heart donor. Remove the mouse from its cage and weigh it, then euthanize with a ketamine/xylazine cocktail (ketamine, 200 mg/kg and xylazine, 20 mg/kg, IP) overdose. This is a terminal procedure.
2. Clip the chest area and place the mouse in a dorsal recumbence position on a pad. Then make the thoracotomy. Bluntly separate the heart, inferior vena cava (IVC), superior vena cava (SVC), ascending aorta, PA, and pulmonary vein. Perfuse the IVC with ice cold sterile saline.
3. Ligate the IVC, SVC, and pulmonary vein with 6-0 silk suture then cut superior to the ligatures.
4. Cut the aorta and PA with 2 mm cuff.
5. Cut out the PV and dispose of it.

3. Heart Valve Transplantation onto a Donor Heart

1. Immediately after step 2.5, place the heart valve from step 1.5 into the donor heart and orient the heart valve.
2. Secure the PV with a stitch on the right side of the valve using 10-0 monofilament sutures on tapered needles and start to suture continuously with 5-6 stiches from the other side of the PV.
3. After finishing the front side, rotate the heart horizontally and start to suture the back side of the PV onto the donor heart.
4. Store the heart in a cold sterile heparin/saline solution. Note: The donor heart can be kept in the solution for two hours before implantation to the recipient mouse.

4. Heterotopic Heart Transplantation on to a Recipient Mouse

1. A 6-8 week old female C57BL/6 mouse was used as a recipient. Remove the mouse from its cage and weigh it, then anesthetized with ketamine/xylazine cocktail (ketamine, 100 mg/kg and xylazine 10 mg/kg). Ketoprofen (5 mg/kg) is used as preanesthesia analgesic.
2. After checking the level of sedation by tail pinching, clip the abdominal and chest hair. Lubricate the eyes with sterile ophthalmic ointment, and place the mouse in a dorsal recumbence position on a pad. Disinfect the abdomen with betadine and alcohol pads. Then cover the mouse with a sterile drape and expose the incision area only.
3. Make a midline laparotomy incision from below the xyphoid to the suprapubic region, and insert a self retaining retractor. Wrap the intestines in saline moistened gauze. Bluntly define the infrarenal aorta and vena cava.
4. Place two 6-0 silk sutures proximally and distally around the aorta and IVC to restrain blood circulation.
5. Place the donor heart on the right side of the abdominal aorta and cover it with sterile gauze. Moisturize it with saline.
6. Make an aortotomy in the abdominal aorta using a 30 G needle and extend the opening with scissors to the size of the donor aorta.
7. Perform an end-to side anastomosis using sterile 10-0 monofilament sutures on tapered needles. Secure the donor aorta with one stitch on proximal end of the opening in the abdominal aorta and start to suture continuously with 4-5 stiches from the distal end of the abdominal aorta.
8. Flip the heart to the left side, cover it with saline infused gauze, and start to suture continuously with 4-5 stiches from the distal end of the abdominal aorta.
9. Make a venotomy in the IVC using a 30 G needle and extend the opening to the size of the donor pulmonary artery.
10. Perform an end to side anastomosis using sterile 10-0 monofilament sutures on tapered needles. Secure the donor PA with one stitch on the proximal end of the opening in the IVC and start to suture continuously with 4-5 stiches from the distal end of the inferior vena cava. This time, because the aorta is in the way, make sure suturing of the left wall of the donor's PA is on the inside of the IVC.
11. Flush the IVC lumen with heparin and saline solution (100 units/ml). Close the right wall of the donor PA and recipient IVC by suturing them continuously to the distal end.
12. Remove the distal ligature and control the hemorrhage by applying a topical absorbable sterile hemostat agent. When the hemorrhage stops completely, remove the proximal suture and control the hemorrhage the same way.
13. Return the intestines and close the abdominal musculature and skin in two layers using a 6-0 black polyamide monofilament suture.
14. Inject 0.5 ml saline subcutaneously and place the mouse in a recovery cage on a warming pad until the mouse is fully mobile. Upon recovery, return the mouse to a new cage with paper bedding. Give pain medication (Ibuprofen, 30 mg/kg, drinking water) for 48 hr. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.

Representative Results

Figure 1 illustrates the schematics of the heart valve transplantation model using heterotopic heart transplantation. The heart valve was harvested from a donor heart and implanted onto a heart from a second donor mouse. Then the heart with the new heart valve was implanted to the abdomen of a recipient mouse. **Figure 2** shows an illustration of the implanted heart on the abdominal space (**A**), right after heart transplantation (**B**), and 5 min after transplantation. Upon removing sutures on both sides of the aorta and IVC, the heart starts to beat 1-2 min later and becomes pinker with more blood circulation. Note that the right atrium is more dilated in (**C**) than (**B**). The heart gradually beats stronger and is stable after 24 hr.

The blood flow through the implanted pulmonary valve was measured percutaneously 10 days after implantation using a high frequency ultrasound system with the pulsed-wave Doppler mode (**Figure 3**). The locations of the aorta, right ventricle (RV), implanted pulmonary valve (PV), and pulmonary artery (PA) in B mode was shown in **Figure 3** (**A**). The yellow sample volume overlay is located on the implanted PV. **Figure 3** (**B**) shows a diagram of the anatomy and the location of the sample volume overlay. As shown in **Figure 3** (**C**), the donor heart QRS wave was detected rhythmically and independent of the recipient heart wave. The measured systolic and diastolic blood volume at the implanted PV matched the donor heart wave. The peak velocity was around 100 mm/sec.

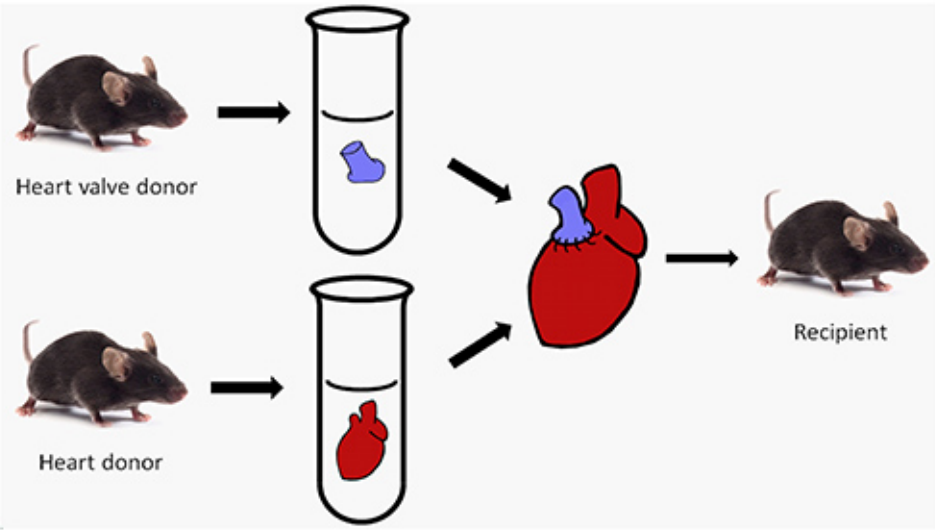

Figure 1. Schematic of heart valve transplantation. A pulmonary heart valve was harvested from a first donor mouse and implanted into a heart from a second donor mouse. Then the heart with the new valve was implanted heterotopically into a recipient mouse.

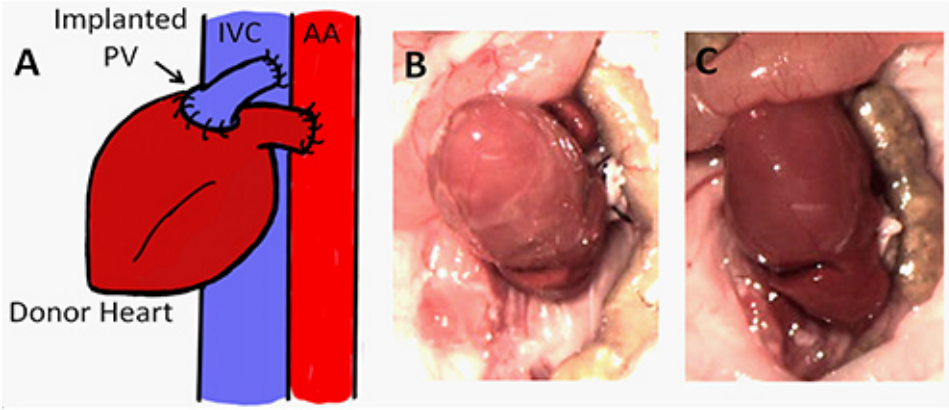

Figure 2. The transplanted heart. A) A diagram of a heart with new heart valve implanted to abdominal space (**B**) right after the implantation, and (**C**) 5 min after implantation.

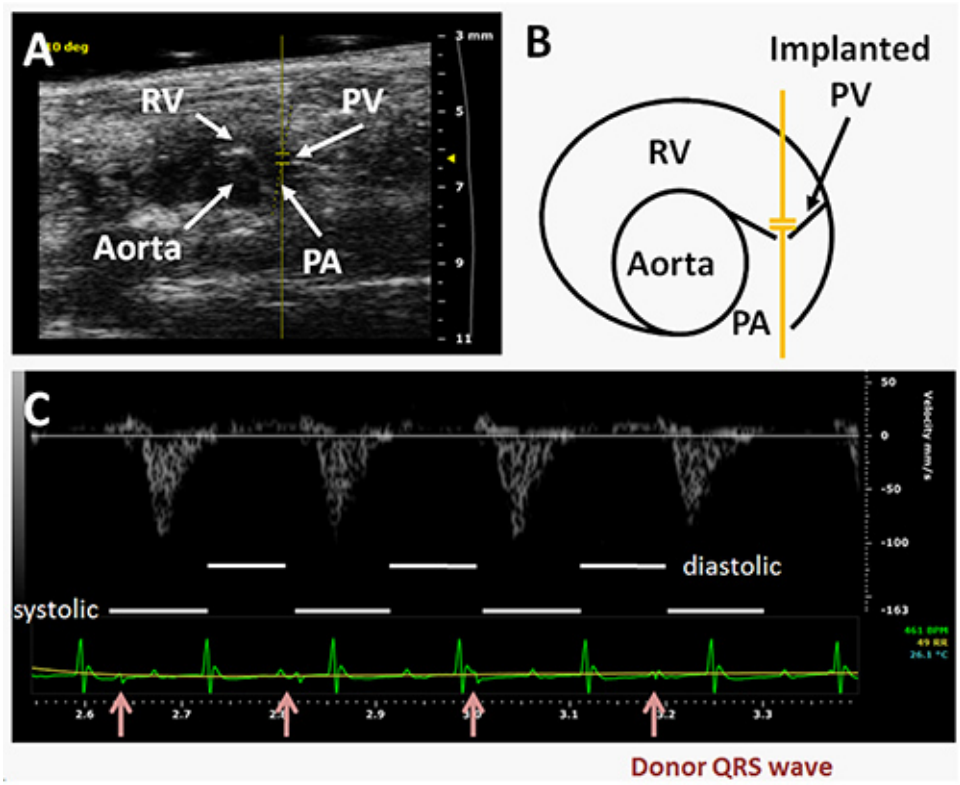

Figure 3. Blood flow measurement in implanted pulmonary valve. A) B-mode image indicating the locations of aorta, right ventricle (RV), implanted pulmonary valve (PV) and pulmonary artery (PA). **B)** A diagram of the anatomy and location of the sample volume overlay. **C)** Velocity measurement at the implanted PV with ECG wave.

Discussion

The mortality rate of this procedure is close to 20%, which was mostly caused by hemorrhage at the PV transplantation site and anastomosis on the donor aorta to the recipient abdominal aorta. In most of the cases, the mortality rate decreases significantly 48 hr post surgery. The survival mice showed strong heart beats and blood flow through the implanted PV. The entire process takes four hours for an experienced micro surgeon. It will take roughly 250 mice to master the technique. The heterotopic heart transplantation is relatively straight forward in comparison to the PV implantation to the donor heart. One of the most critical steps for a successful HV transplantation is harvest of the PV structure from a donor mouse. The PV structure should be transected around 1-2 mm below the valve. If the remaining tissue is too short, anastomosis will be challenging. If the tissue below the PV is too long (*i.e.* the PA will be too long in comparison to the ascending aorta after implantation), the implanted PA may twist or kink. Another critical step is the anastomosis between the implanted PA and recipient IVC. Since the IVC is very thin, it is extremely easy to tear during suturing.

In this model, the aortic blood comes through the aorta, flows through the coronary arteries, then exits through coronary sinus to the donor RA. So the blood volume to pass through the implanted PV is 5% of the total blood volume in estimation, which is the most significant limitation of this model in studying TEHV. To increase the blood flow though the PV, three additional models were created. First, a third anastomosis was created from the donor RV to the recipient IVC. The third anastomosis can increase blood flow by 10% to 50% of total blood volume. Second, to further increase the blood flow, after placing the third anastomosis, the IVC was ligated proximal to the third anastomosis. This method insured 50% of blood flow through the implanted PV. Third, in order to increase flow through the implanted PV and maintain more physiological circulation, the heart was transplanted with the lungs. This method could increase the flow up to 50% of total blood flow and more importantly, the left ventricle and left atrium maintain their circulation. These different physiological flow models enable us to study how the difference in physiological flow conditions affect the development of neotissue and stenosis in a transplanted heart valve.

Recently, we conducted a pilot study to transplant decellularized HV without cell seeding using the described technique in this paper. The implanted PV showed similar blood flow characteristics as the control, predecellularized transplanted PV. In the future, different types of cells will be seeded to study the neotissue formation and stenosis development of the transplanted HV. Moreover, using transgenic mice, such as green fluorescent protein (GFP) mice or a mouse model of HV disease, the process of neotissue formation can be studied mechanistically by studying the source of cells populating the decellularized or diseased heart valve using immunohistochemistry, which will aid the development of more rationally designed, second generation tissue engineered heart valves. The possibility of using different physiological flow conditions, transgenic mice, decellularized PV implantations, and possible combinations of all three show the versatility and potentially important preclinical utility of this HV transplantation model.

Disclosures

We have nothing to disclose.

Acknowledgements

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