A Comparative Study on Suture Versus Cuff Anastomosis in Mouse Cervical Cardiac Transplant

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Abstract

Objectives: **To compare the cuff technique to traditional suture technique in establishing cervical heart transplant model in mice.**

Materials and Methods: **Eighty transplants were performed by 1 surgeon, 40 using the cuff technique, and 40 using the suture technique, under the same circumstances.**

Results: **The cuff approach was significantly superior to conventional suture anastomosis in higher surgical successful rate, less surgery, and less ischemic time ($P < .05$). Suture anastomosis required an intensive microsurgical training and at least a 16× surgical microscope, while the cuff anastomosis required less learning time and a 10× surgical microscope.**

Conclusions: **The cuff technique is the preferred method in cervical heart transplant model in mice.**

Key words: *Murine model, Heart, Heterotopic transplant, Vessel anastomosis, Complications*

Vessel anastomosis in mouse organ transplant is often a challenge obviously owing to the tiny size of the blood vessels. However, mouse organ transplant models are useful for many research projects with several advantages: the availability of hundreds of inbred and transgenic strains, abundant supplies of biological reagents, and clearer immunologic and genetic background. Since of the establishment of mouse cervical transplant by Chen in 1991 (1), great efforts have been made to improve and simplify the microsurgical procedure, forming 2 main techniques, including improved suture technique based on Chen's model and cuff technique. Although the anastomosis with cuff technology has a few advantages compared with the conventional suture approach, it has not been widely accepted. In the present study, 80 cervical heart transplants were performed by 1 surgeon—40 using cuff and 40 using suture anastomosis under the same circumstances. Comparative studies included graft ischemia time, surgery success rate, surgical feasibility, equipment requirement, postoperative endangium integrity, complications, and graft outcome.

Materials and Methods

Animals

Male or female C57BL/10 (B10; H2b) mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China), and used at 7 to 9 weeks of age, either as donors or recipients. Recipient mice were slightly bigger than donors. Animals were maintained in the animal facility at Hua Shan Hospital, Fudan University, and used under the guidelines set by Chinese Government.

Equipment

Desktop operating microscope (WS2-286-82 Shanghai Medical Apparatus and Instruments, Shanghai, China), microsurgical instruments (Ningbo Chenghe Instruments, Ningbo, China) were used in this study. Cuffs were made of an intravenous catheter (Teflon, B. Braun, Melsungen,

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Germany), 24-G (1 mm long, external diameter of 0.8 mm, and internal diameter of 0.6 mm for external jugular vein anastomosis) and 22-G (2 mm long, external diameter of 0.7 mm, and internal diameter of 0.5 mm for arterial anastomosis).

Surgical Procedure

Cuff

Recipient mice were anesthetized with pentobarbital (75 mg/kg, ip), had their hair shaved, and were then placed in a supine position with their limbs immobilized. The skin of the operative region was sterilized. A longitudinal incision from the right mandibular angle to the middle point of the right clavicle was made. The right submaxillary gland was removed to expose the right external jugular vein, the proximal tributaries of which were then ligated and cut. The right, common, carotid artery was exposed by cutting the right sternocleidomastoid muscle and mobilized to the bifurcation of the internal and external carotid artery. A midline abdominal incision was made in the donor, and 1 mL heparinized saline (100 U/mL) was injected into the inferior vena cava. A bilateral thoracotomy along the anterior axillary line to the inferior margin of clavicle was performed, and the anterior chest wall was pulled in a cranial direction. Small pieces of ice were put into the thoracic cavity for cardioplegia. Blunt dissection of the aorta and pulmonary artery was performed above the bifurcation of the preserved pulmonary artery. The aorta was freed retrogradely from the descending aorta, and the ascending aorta was freed at the level of the initial part of the innominate artery. All vessels, except the aorta and pulmonary artery, were ligated toward the posterior surface of the heart. The donor heart was then removed and stored in cold normal saline (4°C).

The proximal portion of the carotid artery was occluded with a small vessel clip, and the distal portion was ligated at the level of the bifurcation with 9-0 silk. The carotid artery was then incised between the clip and the distal ligature, and the proximal end irrigated with heparinized saline (100 U/mL). The carotid artery was then passed through the artery cuff with a retention suture on the free edge.

By pulling the free edge of the carotid artery with microforceps, the proximal end of the carotid artery was everted over the cuff and ligated with a circumferential ligature of 9-0 silk (Figure 1). The right, external jugular vein was prepared in the same way. The donor heart was placed in the right neck of the recipient and covered with an icy normal saline pad. The arterial cuff was inserted into the donor aorta and fixed with a preset ligature (9-0 silk). A 22-G intravenous cuff was inserted into the donor pulmonary artery and fixed with 9-0 silk ligature (Figure 2). The clamp on the external jugular vein was unclamped, followed by unclamping of the carotid artery. The heart rapidly changed from pale to red.

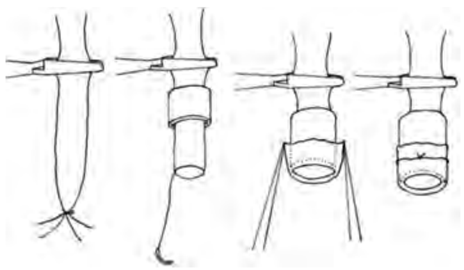

Figure 1. Vessel cuff preparation. After clamping of the proximal portion of vessel, the distal end of vessel was passed through the cuff, everted, and fastened with a 9-0 suture.

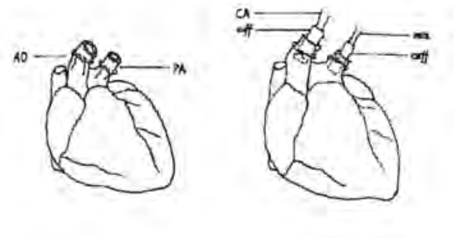

Figure 2. Cuff anastomosis. The cuffed, common, carotid artery, and external jugular vein of recipients were inserted into the aorta and the pulmonary artery of the graft and fastened.

Within 1 minute, the heart graft developed sinus rhythm. Then, the arterial cuff was again ligated with a 9-0 silk, pulled in a cranial and medial direction, and fixed to the adjacent soft tissue by suturing, bending the artery in a gentle obtuse angle arc. The venous cuff was fixed the same way, but pulled in lateral direction (Figure 3). The incision was closed with layered suture.

Suture

The anesthesia, exposure of the right carotid artery and right external jugular vein, and donor heart harvesting were done similar to the cuff. The proximal portion of the carotid artery was occluded with a small vessel clip, and the distal portion of it was ligated at the level of bifurcation with 9-0 silk.

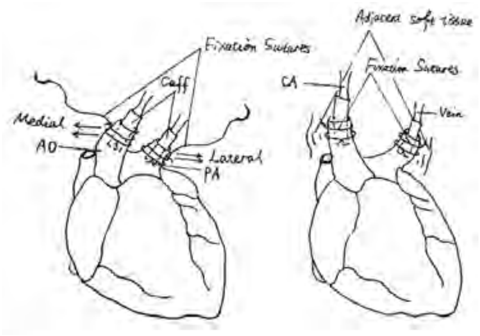

Figure 3. Graft fixation. After graft cardioversion, the arterial cuff and venous cuff were again ligated with a 9-0 silk, pulled in medial and lateral direction, and fixed to the adjacent soft tissue by suturing.

The carotid artery was incised between the clip and the distal tie. A longitudinal incision was made in the anterior wall of the carotid artery toward proximally, the length of which was about the same as the diameter of donor ascending aorta. The proximal end also was irrigated with heparinized saline (100 U/mL).

The donor aorta then was anastomosed to the recipient carotid artery using an end-to-end continuous suture technique with 11-0 silk (Ningbo Medical Suture, Ningbo, China). The donor pulmonary artery was anastomosed to the recipient's right external jugular vein using the same technique, followed by sequentially unclamping the external jugular vein and the carotid artery. Also, the donor heart turned from pale to red, at once and developed sinus rhythm within 1 minute. The position of the donor heart requires special attention to avoid twisting of the vessels owing to the absence of a proper site for fixation. The incision was closed with layered suture.

Perioperative care and observation

No special handling was needed when blood loss was less than 0.5 mL during the operation. Subcutaneous injection of normal saline (1-2 mL) was made when more blood loss occurred. Animals were kept warm until they fully recovered from the anesthesia. Graft function was monitored by daily palpation. Graft survival longer than 72 hours was defined as successful. Autopsies were done on all noninstant-death–failed cases (not clear) to confirm the cause of graft failure.

Statistical Analyses

Statistical analyses were performed using Stata 8.0 software (Stata, College Station, TX, USA), using a Fisher exact test or *t* test. *P* values less than .05 were considered statistically significant.

Results

The surgical success rate was 92.5% in cuff group (37/40) and 72.5% in the suture group (29/40) ($P < .05$). There were 14 surgical failures, 3 in the cuff group, and 11 in the suture group, all related to complications. In cuff group, 1 animal died of bleeding after revascularization of the donor heart, the other 2 deaths were related to anesthetic accident. In the suture group, 1 animal died of an anesthetic accident; 5 deaths were caused by anastomotic bleeding; graft failure occurred in another 5 animals caused by infarction resulting from vessel twisting (2) and anastomotic constriction (3) (Table 1). Vascular complications were significantly higher in suture than in the cuff group ($P < .05$). The incidence of each vascular complication also was higher in the suture than in the cuff group, but of no statistical significance owing to the small number in each group ($P > .05$). There was no statistical difference between the 2 groups regarding incidence of anesthetic accidents ($P > .05$). Time requirements for various phases during surgery also were compared (Table 2). Time for recipient preparation was longer in the cuff than in the suture group ($P < .05$); donor heart ischemic time and time for vessel anastomoses were shorter in the cuff than in the suture group ($P < .05$); there was no difference in total time for surgery, and time for cardioversion and time for donor heart harvesting (all $P > .05$).

Table 1. Comparison of complications.

	Complications	Suture % (n)	Cuff % (n)	*P* value
Vascular complications	Anastomotic bleeding	12.5 (5)	2.5 (1)	.20
	Anastomotic constriction	7.5 (3)	0 (0)	.24
	Vessel twisting	5 (2)	0 (0)	.49
	Total	25 (10)	2.5 (1)	.01
Anesthetic accidents		2.5 (1)	5 (2)	1.00
Total		27.5 (11)	7.5 (3)	.03

Table 2. Comparison of time of different phases in surgery.

Time (min)	Suture	Cuff	*P* value
Recipient preparation	25.2 ± 1.8	31.9 ± 2.2	> .05
Donor heart harvesting	20.5 ± 2.1	21.1 ± 2.2	> .05
Donor heart ischemia	51.3 ± 3.5	28.5 ± 3.8	< .05
Vessel anastomosis	30.8 ± 2.7	5.1 ± 0.9	> .05
Total surgery	83.9 ± 2.9	57.8 ± 3.9	< .05

Discussion

Mouse heterotopic heart transplant models have been used extensively in organ transplant research because the mouse has hundreds of inbred, transgenic, and gene knockout strains with clear genetic backgrounds (2). There is a wide variety of monoclonal antibodies commercially available. Moreover, drug consumption is small, and experimental costs are low in mice owing to their small size. Compared with the mouse abdominal heart transplant model first described by Corry in 1973 (3), cervical heart transplant has a few advantages, such as minor postoperative stress and high surgical achievement rate. The beating of the heart graft can easily be seen or palpated owing to the superficial position. However, it has not been popular because of the technical difficulties of vessel anastomoses. Despite the technique improvement and simplification (4), complications associated with suture anastomosis, including bleeding and thrombosis, remain a common. To overcome these problems, the cuff technique was first introduced by Matsuura and associates (5). It brings almost no injury to the endangium, and there is no requirement of difficult vascular suturing techniques, but 3 retention sutures remain needed. Tomita and associates (6) used a special cuff with fixed arms with no need of retention sutures, but the specially made cuff is hard to obtain. We used 24-G and 22-G intravenous catheter Teflon tube as the arterial and venous cuffs, which are easy to obtain.

Wang and associates (7) prepared the cuff on the pulmonary artery of the graft. The pulmonary artery wall is thin and easy to tear. Moreover, it is sometimes impossible to make the cuff when the pulmonary artery trunk is too short. The most difficult part of cuff technique is to evert the vessels to wrap around the cuffs. Feng and Sun (8) introduced a cuff with a mandril to evert the vessels, but insertion of the mandril will easily bring the danger of damage to the endangium. We made the cutoff at the bifurcation of recipient external jugular vein and carotid artery, which helped enlarge the end of the vessels; thus making eversion of the vessels easier and safer. The general weakness of preparing a cuff on the graft is prolongation of graft ischemia time. To improve surgical procedures, we tried to prepare the cuffs on the recipient cervical vessels in advance, followed by harvesting of the donor heart, thus, graft ischemia time was substantially reduced. With these improvements mentioned above, we established a new cuff technique, which is easier and safer, compared with previously reported cuff techniques.

Most previous studies describe a single model, lacking comparison with a conventional suture approach. We developed an improved cuff technique and compared it with a traditional suture technique, including surgery success rate, graft ischemic time, surgical feasibility, equipment requirement, postoperative endangium integrity, complications, and graft outcome. Surgery success rate was higher in the cuff group. Indeed, surgery success rate is usually related to the incidence of complications. We further compared the vascular complications, including anastomotic bleeding, anastomotic constriction, and vessel twisting. As for total vascular complications, the incidence was much higher in the suture than in the cuff group, and it was statistically significant ($P < .05$). This was similar to comparing the total incidence of complications ($P < .05$) with use of cuff technique and is associated with a significantly lower incidence of complications, resulting in high surgery success rate.

It is intriguing to know why suture anastomosis is associated with high vascular complications. First, during the suture procedure, malpositioning of vessel edges and inappropriate suture intervals may induce stoma hemorrhage, while with the cuff technique, apposition of the vessel edges is accurate; therefore, the incidence of anastomotic bleeding is rare. In this study, there were 5 deaths from anastomotic bleeding in suture group, all due to multipoint bleeding that could not easily be controlled. While in the cuff group, there was only 1 death from bleeding, the cause of which was loosening of the ligature, which can be avoided.

Second, endangium injury is common in anastomoses of vessels during suture, resulting in thromboses and anastomotic constriction, which affect vessel patency and graft survival. Yet anastomosis cuff techniques bring almost no injury to the endangium. In this study, 3 failed cases in suture group were caused by thromboses, including 2 arterial thromboses resulting in ischemic infarction of the graft, and 1 venous thrombosis resulting in congestive infarction. This did not occur in the cuff group. The difficult part of the cuff technique is to evert the vessels to wrap around the cuffs. We cut the cuff at the bifurcation of recipient external jugular vein, as well as the carotid artery, which helped to enlarge the inner diameter of the vessels and thus, conversion of the vessels becomes easy. This modification serves as another effective method in reducing the incidence of anastomotic constriction in addition to the maintenance of endangium integrity.

Third, the ellipsoid-shape and the contraction/relaxation of cardiac graft may result in vessel twisting and strangulation. Use of the cuff provides a reliable fixation point for the graft. After cardioversion of the graft, the arterial cuff was again ligated with a 9-0 silk, pulled in a cranial and medial direction, and fixed to the adjacent soft tissue by suturing. The venous cuff was fixed in the same way, but pulled in a lateral direction. This fixation procedure enlarges the space and includes the angle between the 2 large arteries of the graft; thus, avoiding twisting and strangulation of the vessels. However, the fixation point is unavailable when using the suture technique; therefore, the arteries of the graft could not be fixed after cardioversion, resulting in higher incidence of vessel-twisting and strangulation. In this study, although special attention was paid to the positioning of the donor heart to avoid twisting of the vessels, there remained 2 failed cases in the suture group caused by vessel twisting and strangulation after infarction of the graft, while there was none in cuff group.

In terms of surgery time, our results showed that the time of recipient preparation was longer in cuff group than in the suture group, possibly owing to the extra procedure of cuff preparation. There was no difference in time of donor heart harvesting between the 2 groups. As for vessel anastomoses, the cuff technique required less time than the suture group ($P < .05$). Average time for reconnection of both vessels using the cuff technique was only 5.1 ± 0.9 minutes. Thanks to the fast anastomoses, the donor heart ischemic time also was limited in the cuff group. In addition, time for cardioversion also was shorter in the cuff group. We presume that shorter graft ischemic time may result in reduction of ischemia-reperfusion injury, which is beneficial to recovery of the cardiac graft. These results demonstrate that compared with the suture technique, the cuff technique can simplify the surgical procedure, reduce time for vessel anastomoses and graft ischemic time, which greatly improves overall outcome.

Moreover, the suture anastomoses requires an intensive microsurgical training and at least a 16× surgical microscope, while cuff anastomoses required less learning time, and a 10× surgical microscope was sufficient. The cuff technique is convenient, but it is not widely used. One possible reason may be the difficulty in obtaining appropriate cuffs. We used 24-G and 22-G intravenous catheter Teflon tube, which are easy to obtain. The cuff technique is the preferred means to be used in cervical heart transplant in mice.

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