

# Abdominal aorta transplantation after programmed cryopreservation

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## Abstract

**AIM:** To study the morphologic and cellular immunologic changes after homologous transplantation of the abdominal aorta in rats after programmed cryopreservation ( $-196^{\circ}\text{C}$ ).

**METHODS:** Abdominal aorta was harvested from anesthetized Sprague Dawley (SD) rats for cryopreservation (group B) or immediate implantation (group A). The survival rates and apoptotic rates of aortic endothelial cells (ECs) were examined. The patency rates, histology and cellular immunologic changes of the abdominal aorta were examined on days 1, 3, 7, 14, 30, 60 after transplantation respectively.

**RESULTS:** The survival rate of ECs after programmed cryopreservation was  $90.1\pm 1.79\%$ , about 3.4% lower than that of uncryopreservation ( $93.5\pm 1.96\%$ ). The apoptotic rates of ECs was increased after cryopreservation (7.15% vs 4.86%,  $P<0.05$ ). The patency rate of group B was significantly higher than that of group A ( $91.6\pm 12.9\%$  vs  $62.5\pm 26.2\%$ ,  $P<0.01$ ). CD4/CD8 ratio, TCR  $\alpha\beta$  and CD11b/CD18 ratio of group B were significantly lower than those of group A ( $P<0.05$ ). Revivification of the cryopreserved abdominal aorta showed normal adventitia and intact smooth muscle cells.

**CONCLUSION:** Cryopreservation can reduce homologous abdominal aortic antigenicity. Even if without administration of immunosuppressive agents, it is still feasible to implement homologous artery grafting in rats.

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## INTRODUCTION

Since the first implantation, human allograft vessels have been widely used for surgical treatment of vascular diseases<sup>[1]</sup>. Approximately 1.5 million vascular reconstitutions are done each year in the United States<sup>[2]</sup>. Great sphenoid veins are commonly used in coronary artery bypass and peripheral

vascular surgery<sup>[3]</sup>. Arteries are candidates in the management of arterial infection<sup>[4]</sup> and offer superior patency rates compared with veins<sup>[5]</sup>. The availability of autologous fresh material is often limited and there is a definite need for cryopreserved substitutes. The long-term preservation of small-caliber arterial allografts has benefited from the improvement in organ harvesting and can be further improved by the advance of other cryobiology techniques<sup>[6]</sup>.

Preservation of vascular grafts at low temperatures using dimethyl sulfoxide (DMSO) as a cryoprotectant (CPA) has been repeatedly reported to be satisfactory after long-term storage. It has been reported that the mechanical properties of grafts and the functions of smooth muscle cells could be adequately preserved<sup>[7]</sup> but the endothelium was often somehow compromised<sup>[8]</sup>. Both the intact smooth muscle and endothelial cells are required to obtain full patency<sup>[9]</sup> and impairment of either of these structures will alter the complete vessel functions. Relations between the viability of vascular endothelial cells at time of implantation and immunologic rejection are the subject of debate and remain poorly understood.

We therefore designed an abdominal aorta allograft model in rats, replicating and comparing in the same animal model most clinical and experimental situations, from fresh aortic autografts to cryopreserved allografts and studied the effects of cryopreservation on the survival rates and apoptotic rates of aortic endothelial cells (ECs), histology and the cellular immunologic changes.

## MATERIALS AND METHODS

### Experimental animals

Male or female Sprague Dawley rats (Laboratory Animal Center No. SCXK2002-0031 Jiangsu Province) weighing 250 g to 300 g were used as donors and recipients. They were housed in pathogen-free conditions with a 12 h light-dark cycle and were allowed to drink water. Animal care and the experimental protocol were in compliance with the guidelines of the European Community Standards on the Care and Use of Laboratory Animals (No.28871-22A9). The SD rats were fixed in the supine position<sup>[10]</sup>, and anesthetized with 100 mg/kg ketamine and the abdomen was opened with a median incision. The abdominal aorta with a diameter of 1.5 mm was dissociated. Then the blood stream was blocked, and the abdominal aorta was transected and washed with heparin saline. The aortic anastomosis was performed with 8/0 proline. The incision was sewed up and the rat was given 200 000 units of penicillin intramuscularly qd for 3 days.

### Cryopreservation and thawing processes

The arteries were harvested according to sterile principle and "non-touch" technique<sup>[11]</sup> and immersed in the culture medium RPMI1640 (Life Technologies, Gibco BRL.) with 20% fetal bovine serum (FBS) and 15% DMSO (Amresco Co.) contained in a beaker and kept at  $4^{\circ}\text{C}$  for 1 h. Programmed cryopreservation was performed in a biological freezer (Thermo Forma Co.) from  $20^{\circ}\text{C}$  to  $-100^{\circ}\text{C}$ , following the cooling curve established by Gournier *et al*<sup>[12,13]</sup>. The curve involved several stages of controlled time and temperature, and was designed to achieve a mean cooling rate of  $1^{\circ}\text{C}/\text{min}$ . Once frozen, aortic segments were stored in liquid nitrogen

(-196°C).

Before operation, frozen aortic segments were taken out of the liquid nitrogen and stored at RT for 5 min. Then they were incubated at 37°C in a water bath for 5 min and stored in RPMI1640 with 20% FBS.

### Experimental design

The following groups were established. Control group (group A) included 24 fresh abdominal aortic segments and cryopreservation group (group B) included 24 abdominal aortic segments cryopreserved. Every four rats of two groups were sacrificed on days 1, 3, 7, 14, 30 and 60 after transplantation and the patency rate of the transplanted arteries was observed. The blood samples were collected from inferior vena cava and the donor arteries were harvested for examination.

### Morphology

All transplanted aortae were observed for vascular patency<sup>[14]</sup> and processed for pathologic study by light microscopy (LM). Specimens for LM were fixed by immersion in 10% formaldehyde and embedded in paraffin to obtain 5 μm-thick cuts. Sections were deparaffinated, hydrated, and stained with hematoxylin-eosin, Masson's trichrome stain and orcein.

### Flow cytometric analysis

The survival and apoptotic rates of ECs were determined by propidium iodide (PI) and annexin V staining. Endothelial cells of abdominal aorta in rats were digested by 0.25% trypsin at 37°C for 20 min and fixed with ice-cold 70% ethanol at a cell density of  $1 \times 10^6 \text{ ml}^{-1}$ . PI and annexin V were then added and incubated with cells in the dark for 30 min until detection by flow cytometry<sup>[15]</sup>.

Monoclonal antibody (10 μl) (Simultest anti-CD4 and anti-CD8, anti-TCR α β<sup>[16]</sup>, Immunotech Co., anti-CD11b and anti-CD18<sup>[17]</sup>, Serotec Co., Simultest control (Becton-Dickinson)) was added to tubes with 25 μl whole blood (heparin anticoagulated). The tubes were incubated at 37°C in the dark for 30 min and 1 ml of lysing solution was added. After 15 min lysing time, the cells were washed twice with PBS and then 200 μl of fixation buffer (2% formaldehyde + 0.1% sodium azide in PBS (pH 7.4)) was added. The samples were thereafter kept at 4°C until they were analyzed by flow cytometer (FACSCalibur, BD Co.)<sup>[18]</sup>.

### Statistical analysis

The data were expressed as mean±SD. Besides a two-sided Student test with a level of significance at 5%, a multiple-factor ANOVA (Tukey's test) was performed to analyze the difference between the two groups. All the data were analyzed with the statistical software SPSS10.0.

## RESULTS

### Survival rate and apoptotic rate of ECs in two groups

The survival rate of ECs without cryopreservation was  $93.5 \pm 1.96\%$  and that of ECs with cryopreservation was  $90.1 \pm 1.79\%$ . But the apoptotic rate of uncryopreserved ECs was  $4.86 \pm 0.252\%$  and that of cryopreserved ECs was  $7.15 \pm 0.422\%$ . There was a significant difference between the two groups ( $n=4$ ,  $P<0.05$ ).

### Patency condition at each time point of two groups

Generally, all the external appearances of the arteries were normal, but the dissepiments were stiff and the intima was thickened, especially in group A. The inner-walls of vessels were smooth and had no mural thrombus. The patency rates of all six time points in group B were higher than those in group A (Table 1).

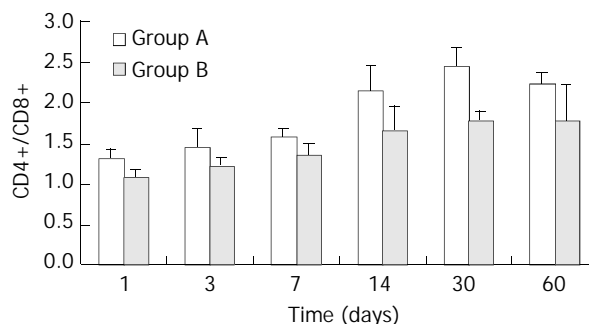
**Table 1** Aortic patency of groups A and B after transplantation

Group	Patency/total number of aortae at each time point (days)						Patency rate (%)
	1	3	7	14	30	60	
A	4/4	3/4	3/4	2/4	2/4	1/4	62.5±26.2
B	4/4	4/4	4/4	4/4	3/4	3/4	91.6±12.9

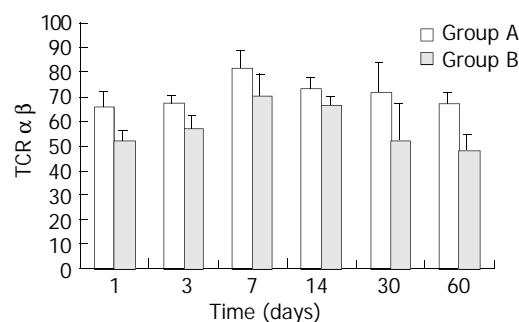
Group A vs group B,  $P<0.01$ .

### Subpopulation of T cells and TCRαβ

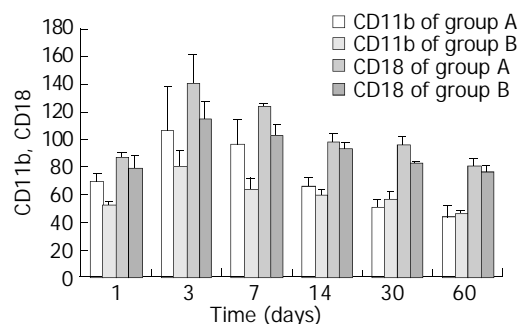
After transplantation, the number of CD4<sup>+</sup> T cells was dramatically increased and reached the peak at 4 weeks post-transplantation. In the following days, the number of CD4<sup>+</sup> T cells was maintained at a higher level. On the contrary, the number of CD8<sup>+</sup> T cells was decreased. The ratio of CD4<sup>+</sup>/CD8<sup>+</sup> was the highest at 4 weeks and there was a significant difference between groups A and B ( $n=4$ ,  $P<0.05$ , Figure 1). The expression of TCRαβ was up-regulated soon after transplantation and the tendency of which was similar to that of the ratio CD4<sup>+</sup>/CD8<sup>+</sup>. There was also a significant difference between groups A and B. ( $n=4$ ,  $P<0.05$ , Figure 2).



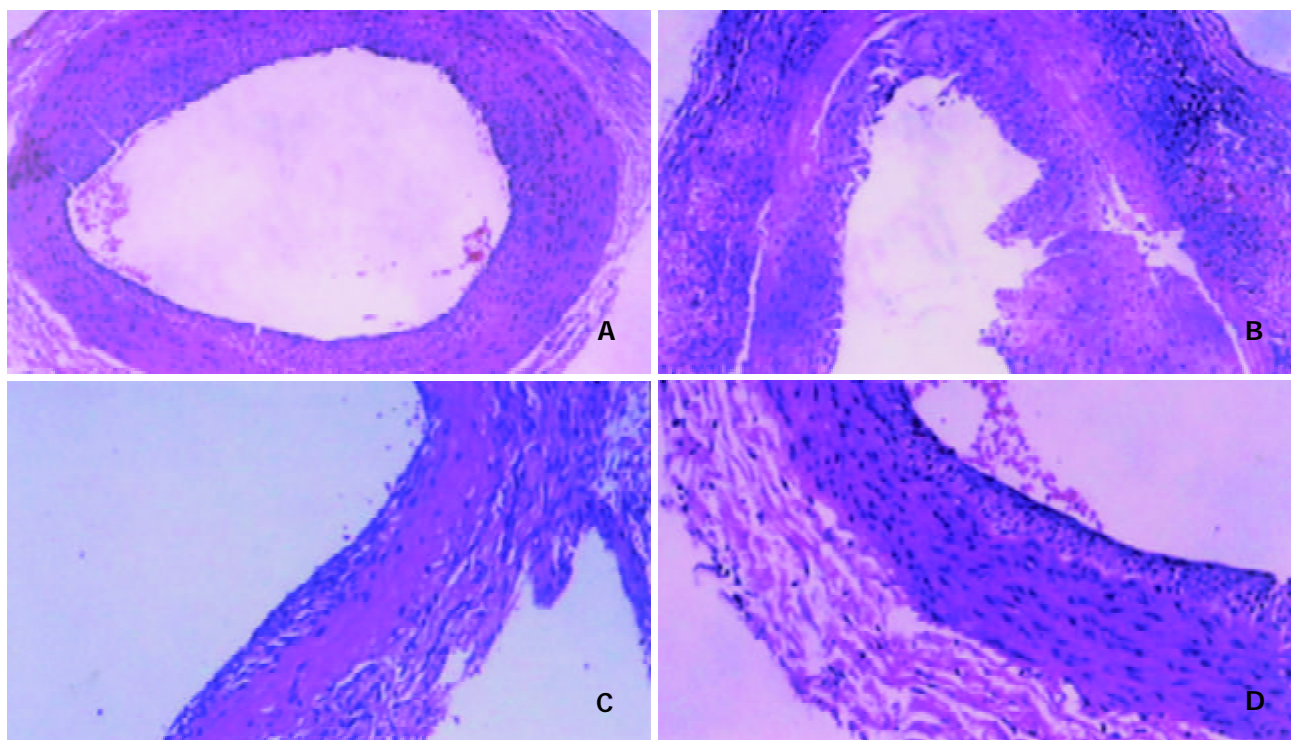
**Figure 1** CD4<sup>+</sup>/CD8<sup>+</sup> expression of groups A and B after transplantation ( $n=4$ ).



**Figure 2** TCR α β expression in T cells of groups A and B after transplantation ( $n=4$ ).



**Figure 3** CD11b and CD18 expression on PMNs of groups A and B after transplantation ( $n=4$ ).



**Figure 4** HE staining of abdominal aorta in rats after transplantation. A: Infiltration of T cells at 4 weeks in group A ( $\times 100$ ), B: Excessively thickened intima at 8 weeks in group A ( $\times 100$ ), C: Completely recovered ECs of cryopreserved aorta at 4 weeks in group B ( $\times 100$ ), D: Intact and slightly thickened intima at 8 weeks in group B ( $\times 100$ ).

#### *Ratio of CD11b/CD18*

The CD11b/CD18 ratio of the two groups after operation was increased. There was a significant difference at each time point between groups A and B ( $n=4$ ,  $P<0.05$ , Figure 3).

#### *Pathological and histological changes*

Severe fragmental necrosis was observed in the intima of group A. The distribution of inflammatory cells in arteries was intensive, most of them intruded into tunica adventitia and media. Hyperplasia occurred in the intima 4 weeks after operation, smooth muscles in the media were thickened and the lumen of blood vessels became narrow. The intima was thickened excessively 8 weeks after operation and the lumen of blood vessels was markedly narrowed. Calcification and necrosis were observed in parts of the media. In group B few necrosis and fragments were observed and there was no obvious hyperplasia of smooth muscle in the media. ECs recovered completely and the intima remained intact and the intima of aorta was thickened slightly 4 and 8 weeks respectively after operation (Figure 4).

## DISCUSSION

#### *Methods of cryopreservation and thawing*

Many methods could be used for the cryopreservation and thawing of blood vessel tissues<sup>[19-21]</sup>. In our study, we cryopreserved the aorta using the biologic freezer with controlled rate and medium containing RPMI1640, 20% calf serum, and 15% DMSO was used as the protective agent. So the formation of ice crystals in tissues and cells was decreased, the thermal stress caused by the dilution shock and the temperature were also decreased, hence the damage to the vessels was minimally reduced. Some studies indicated that vascular endothelial cells cryopreserved by the biologic freezer with controlled rate could achieve a survival rate from 70% to 90%<sup>[22-24]</sup>. Besides, these cryopreserved cells had similar proliferation and antithrombotic functions as normal cells after

revival<sup>[25]</sup>. It was indicated that the competence of abdominal aorta in rats was almost entirely preserved in our stud, while the increase of apoptosis in group B was thought to be related with cryopreservation.

#### *Cellular immunologic change after transplantation*

Among all components of vascular dissepiment including ECs, smooth muscle cells (SMCs), elastic fibers and collagen fibers, ECs have the strongest antigenicity, which can offer all essential signals that result in the activation of T cells. Therefore, ABO blood type and histocompatibility should be determined and better in combination with the use of immuno-suppressive drugs. The correlation between immunosuppression and long-term patency rate has been confirmed in animal experiments. In our study we found that cell-mediated immunity caused by allografts was reduced dramatically in group B. T cell subpopulation changed after transplantation. Many sensitized CD4<sup>+</sup> T cells entered into the peripheral blood and CD8<sup>+</sup> T cells gathered at the part of grafts to perform the cytolytic function. The pathological study indicated that cellular immunologic reaction in group B was slight as compared with that in group A. The reduction of immunogenicity in group B might be related with the decrease of antigenicity and the adhesive ability of resuscitated ECs and the shedding of ECs caused by the brushing of blood stream.

The expression of CD11b/CD18 on the surface of polymorphonuclear leukocytes (PMNs) was gradually increased after transplantation, suggesting the activation of PMN. Recent studies have shown that white blood cells play an important role in the proliferation of SMCs and activated PMNs can promote activation of platelets, proliferation of SMCs, release of oxygen-derived free radicals and inhibition of nitrogen monoxide (NO) secreted by Ecs. Besides, the decrease of NO synthesis could reduce the inhibition of CD11b/CD18 expression, which reinforces the interaction between PMNs and Ecs through a feedback mechanism<sup>[26]</sup>. Our study demonstrated that the lumens of aortas in group A were

markedly narrower than those in group B, but the expression of CD11b/CD18 on PMNs in group A was significantly higher than that in group B. It was confirmed that PMNs could take part in the process of vascular remodeling after transplantation, and the activation of PMNs was closely correlated with the restenosis and cellular immune rejection of allografts.

### Effect evaluation

There were many reports about experiments of vascular allografting<sup>[27,28]</sup>. Chaw *et al*<sup>[29]</sup> reported that arteries had a patency rate of 84% 1 to 3 months after transplantation. Pratt *et al*<sup>[30]</sup> found that arterial allografts had a patency rate of 93% 2 months after operation. Raman *et al*<sup>[31]</sup> reported only 30% arterial patency rate in homologous transplantation 8 weeks after operation with the same method. We used DMSO as the cryoprotectant in programmed cryopreservation of abdominal aorta in rats and found the survival rate of ECs was 90.1% after cryopreservation. Some ECs were brushed out because of blood stream impact, but the remaining live ECs inhibited thrombosis formation after operation. Meanwhile cryopreservation decreased antigenicity of vascular allografts and cellular immune rejection and PMN infiltration, which would greatly promote the allograft restenosis after transplantation. The patency rate of group B was 91.6% 8 weeks after operation, which was significantly higher than that of group A.

The most obvious change of allografts after preservation and thawing was the rapid recovery of vascular endothelial cells and slight proliferation of SMCs intermedium. The recovery of ECs after cryopreservation was helpful to reduce thrombosis. The relative intact endothelium partly reduced excessive repair process after injury of blood vessels and excessive proliferation of SMCs intermedium. The recovered ECs were possibly from the hyperplasia of rest live ECs in donor arteries, the constitution of hyperplasia in recipients<sup>[33-35]</sup> and the sedimentation of circulating Ecs on vascular walls. Our study showed that ECs of cryopreserved aorta recovered completely 4 weeks after transplantation and the endometrium hyperplasia was mild, while in group A the recovery of ECs was not complete 4 weeks after operation and the hyperplasia of vascular endometrium was severe and proliferation of SMCs intermedium was marked 8 weeks after operation.

Our study showed that ECs of the cryopreserved donor arteries still had strong activity at the time of homologous grafting. Due to the brushing of blood stream, a few ECs were shedded. But the rest live ECs still inhibited the formation of thrombus. Because of cryopreservation, the antigenicity of transplanted arteries, the cellular immune reaction and infiltration of PMNs were decreased. So the incidence of restenosis after homologous transplantation was greatly reduced.

In conclusion, the antigenicity of transplanted aorta can be decreased due to programmed cryopreservation and homologous transplantation of abdominal aorta can be performed in rats.

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### REFERENCES

- 1 **Concha M**, Casares J, Ross DN, Gonzalez-Lavin L, Franco M, Mesa D, Legarra JJ, Merino C, Garcia Jimenez MA, Roman M, Munoz I, Alados P, Chacon A. Aortic valve replacement with a pulmonary autograft (the Ross operation) in adult and pediatric patients. A preliminary study. *Rev Esp Cardiol* 1999; **52**: 113-120
- 2 **Langer R**, Vacanti JP. Tissue engineering. *Science* 1993; **260**: 920-926
- 3 **Cooper GJ**, Underwood MJ, Deverall PB. Arterial and venous conduits for coronary artery bypass. A current review. *Eur J Cardiothorac Surg* 1996; **10**: 129-140
- 4 **Vogt PR**, von Segesser LK, Goffin Y, Pasic M, Turina MI. Cryopreserved arterial homografts for *in situ* reconstruction of mycotic aneurysms and prosthetic graft infection. *Eur J Cardiothorac Surg* 1995; **9**: 502-506
- 5 **Gournier JP**, Adham M, Favre JP, Raba M, Bancel B, Lepetit JC, Barral X. Cryopreserved arterial homografts: preliminary study. *Ann Vasc Surg* 1993; **7**: 503-511
- 6 **Motomura N**, Eishi K, Kitoh H, Kawazoe K, Kitoh Y, Kawashima Y, Oka T. Cell viability of aortic allografts after long-term cryopreservation and clinical application to aortic root replacement in a patient with aortitis. *Cardiovasc Surg* 1995; **3**: 231-234
- 7 **Adham M**, Gournier JP, Favre JP, De La Roche E, Ducerf C, Baulieux J, Barral X, Pouyet M. Mechanical characteristics of fresh and frozen human descending thoracic aorta. *J Surg Res* 1996; **64**: 32-34
- 8 **Stanke F**, Riebel D, Carmine S, Cracowski JL, Caron F, Magne JL, Egelhoffer H, Bessard G, Devillier P. Functional assessment of human femoral arteries after cryopreservation. *J Vasc Surg* 1998; **28**: 273-283
- 9 **Furchgott RF**, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373-376
- 10 **Schmitz-Rixen T**, Berwanger C, Lohe-Quast A, Addicks K, Hilgers RD, Pichlmaier H. Immunosuppression and cryopreservation of arterial allografts. *Vasa Suppl* 1992; **35**: 169-172
- 11 **Song YC**, Hunt CJ, Pegg DE. Cryopreservation the common carotid artery of the rabbit. *Cryobiology* 1994; **31**: 317-329
- 12 **Gournier JP**, Favre JP, Gay JL, Barral X. Cryopreserved arterial allografts for limb salvage in the absence of suitable saphenous vein: two-year results in 20 cases. *Ann Vasc Surg* 1995; **9**(Suppl): S7-14
- 13 **Mesa F**, Serra JM, Herreros J. Vascular cryopreservation in microsurgery. *J Reconstr Microsurg* 1997; **13**: 245-250
- 14 **Saito S**, Fukushima N, Kobayashi Y, Tori M, Tsukamoto Y, Shirakura R. Effects of cryopreservation of aortic xenografts on graft patency and immunogenicity. *Transplant Proc* 2000; **32**: 2398-2400
- 15 **Park JC**, Sung HJ, Lee DH, Park YH, Cho BK, Suh H. Specific determination of endothelial cell viability in the whole cell fraction from cryopreserved canine femoral veins using flow cytometry. *Artif Organs* 2000; **24**: 829-833
- 16 **Smith E**, De Young NJ, Drew PA. Immune cell subpopulations in regenerated splenic tissue in rats. *Aust N Z J Surg* 1999; **69**: 522-525
- 17 **Ng CJ**, Chen JC, Chiu DF, Chen MF, Chen HM. Role of prostacyclin on microcirculation in endotoxin-induced gastroprotection in rats: a microdialysis study. *Shock* 2002; **17**: 334-338
- 18 **Woo EY**, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, Rubin SC, Kaiser LR, June CH. Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001; **61**: 4766-4772
- 19 **Komorowska-Timek E**, Zhang F, Shi DY, Lineaweaver WC, Buncke HJ. Effect of cryopreservation on patency and histological changes of arterial isogenic and allogeneic grafts in the rat model. *Ann Plast Surg* 2002; **49**: 404-409
- 20 **Showalter D**, Durham S, Sheppeck R, Berceli S, Greisler H, Brockbank K, Makaroun M, Webster M, Steed D, Siewers R. Cryopreserved venous homografts as vascular conduits in canine carotid arteries. *Surgery* 1989; **106**: 652-659
- 21 **Gottlob R**, Stockinger L, Gestring GF. Conservation of veins with preservation of viable endothelium. *J Cardiovasc Surg* 1982; **23**: 109-116
- 22 **Skop B**, Urbanek T, Bursig H, Ziája K, Wilczok T. The effect of prolonged ischaemia and cryopreservation on the cell viability of human aortic and femoral artery allografts. *Folia Histochem Cytobiol* 2002; **40**: 217-218

- 23 **Wusteman MC**, Pegg DE. Differences in the requirements for cryopreservation of porcine aortic smooth muscle and endothelial cells. *Tissue Eng* 2001; **7**: 507-518
- 24 **Rudderman RH**, Guyuron B, Mendelsohn G. The fate of fresh and preserved, noncrushed and crushed autogenous cartilage in the rabbit model. *Ann Plast Surg* 1994; **32**: 250-254
- 25 **Bambang LS**, Mazzucotelli JP, Moczar M, Beaujean F, Loisan D. Effects of cryopreservation on the proliferation and anticoagulant activity of human saphenous vein endothelial cells. *J Thorac Cardiovasc Surg* 1995; **110**(4 Pt 1): 998-1004
- 26 **Page CS**, Holloway N, Smith H, Yacoub M, Rose ML. Alloproliferative response of purified CD4+ and CD8+ T cells to endothelial cells in the absence of contaminating accessory cells. *Transplantation* 1994; **57**: 1628-1637
- 27 **Callow AD**. Arterial homografts. *Eur J Vasc Endovasc Surg* 1996; **12**: 272-281
- 28 **Orosz CG**, Pelletier RP. Chronic remodeling pathology in grafts. *Curr Opin Immunol* 1997; **9**: 676-680
- 29 **Chow SP**, So YC, Chan CW. Experimental microarterial grafts: freeze-dried allografts versus autografts. *Br J Plast Surg* 1983; **36**: 345-347
- 30 **Pratt MF**, Bank HL, Schmehl M, Horton WD. Freeze-dried microarterial replacement allografts in rabbits. *Laryngoscope* 1989; **99**(10 Pt 1): 1020-1026
- 31 **Raman J**, Hargrave JC. Freeze-dried microarterial allografts. *Plast Reconstr Surg* 1990; **85**: 248-251
- 32 **Jacobsen IA**, Pegg DE, Starklint H, Chemnitz J, Hunt C, Barfort P, Diaper MP. Effect of cooling and warming rate on glycerolized rabbit kidneys. *Cryobiology* 1984; **21**: 637-653
- 33 **Boren CH**, Roon AJ, Moore WS. Maintenance of viable arterial allografts by cryopreservation. *Surgery* 1978; **83**: 382-391
- 34 **Tice DA**, Zerbino VR, Isom OW, Cunningham JN, Engelman RM. Coronary artery bypass with freeze-preserved saphenous vein allografts. *J Thorac Cardiovasc Surg* 1976; **71**: 378-382

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