[Frontiers in Bioscience 17, 575-582, January 1, 2012]

Effect of pre-freezing rate on porosity ratio and mechanical property of pig aorta

Meng Yin[1], Mengfang Liu[2], Qing Cao[3], Jianqing Wu[2], Leren Tao[2], Jinfen Liu[1]

[1]Department of Pediatric Cardiothoracic Surgery, Shanghai JiaoTong University Affiliated Shanghai Children's Medical Center, Shanghai, 200127, China; [2]Shanghai University Of Technology, Institute of Cryogenic Technology and frozen foods,shanghai,200093, China; [3]Department of Pediatrics, Shanghai JiaoTong University Affiliated Shanghai Children's Medical Center, Shanghai, 200127, China

TABLE OF CONTENTS

1. ABSTRACT

Vacuum freeze-dried blood is a good material for vascular grafts. However, studies on this technology are few, particularly on physical performance change of freeze-dried blood vessel at different pre-freezing rate. In our study, pig aortas were non-invasively scanned by micro-CT in each stage of freeze-drying at different pre-freezing rates, then comparing the porosity ratio and grey level under different conditions with each other to analysis the influence of different methods on the aorta. The mechanical properties of rehydrated pig aorta and fresh one were compared by texture profile analyzer to investigate the influence of different pre-freezing rates on mechanical properties in pig aorta. Our results showed that the proper pre-freezing rate for freeze-drying were 1°C/min. The changing rates of porosity rate and the average gray scale value were 16.6% and 3.64% respectively after freeze-dried. The puncture tolerance (PT) and circumferential tensile stress were increased about 20% and 30% respectively, and the axial tensile stress (ATS) were decreased about 20% in rehydrated aorta compared with fresh aorta. We otherwise conclude that under optimized process conditions, freeze-dried aorta with proper porosity rate and mechanical properties approximate fresh aorta could be preparation.

2. INTRODUCTION

Cardiovascular disease (eg coronary heart disease, vasculitis, and some congenital cardiovascular diseases) is a common disease in clinic and could lead to stenosis, ischemia, rupture and hemorrhage, or congenital absence of vascular, affecting nutrition, metabolism and growing development of tissues supplied by those vascular, or even threatening to life. In addition to medical treatment, the alternative treatment of vascular graft is an effective method for the disease. Transplanted blood vessel could choose biological vessels (such as autologous vessel, allogeneic vessel or treated xenograft vessels) or artificial vessels. Artificial vessel has the possibility of rupture and high incidence rate of thrombus in long term, and is liable to calcify. Thus, its therapeutic effect is restricted (1-4). Though the biological vessel has good biological properties, its source is limited. And the immunogenicity of allogeneic vessel and treated xenograft vessels should not be ignored. Thus, it's particularly important to expand the source, reduce the immunogenicity and well preserve the structure and function of biological vessel through researching preserving techniques. Vacuum freeze-drying is increasingly used in medical and health care fields as a new conservation method. Previous study has showed that

vessel treated with freeze-drying has a great possibility to be used in transplantation (5).

Most previous studies on freeze-drying in blood vessel treated freeze-drying as a method of reducing immunogenicity in medical immunity field. Refrigerating equipment they used mostly are medical refrigeraion, in which vessels were directly frozen in -60 °C around 12 to 24 hours till completely frozen and then sublimating drying and secondary drying in a certain vacuum.

However, freeze-drying is actually a complex process of heat and mass transfering. In recent years, studies on vascular freeze-drying technology is few. In the freeze-drying process, sublimation of ice crystal shapes pore spaces in the final material. The pore size and distribution directly affect the mass transfering process and drying rate (6,7). The ratio of porosity is a key parameter in a successful vascular graft, which would affect the regeneration of host cells and the formation of tissue and blood vessel when is unreasonably low. And if the porosityis too high, it would affect mechanical properties of vascular skeleton. In the entire pre-freezing stage of freeze-drying, ice crystal is forming diversely at different pre-freezing rate. Therefore, it is significant to study the pre-freezing rate in the process of vacuum freeze-drying pig aorta.

Vessel is a biological material. The crackle would appear on the wall when the pre-freezing rate is too high (8,9), which would turn into larger cracks by long-term lashing and oppressing of blood in circulatory system, resulting in that blood vessels are required to have a ability to stand a certain pressure and have a certain flexibility, pressure resistance, and extended (10). Therefore, testing quality of the freeze-dried vessels is very important.

This present study investigates the influence of different pre-freezing rates on the porosity ratio and mechanical propertyies of blood vessel from the parameters of freeze-drying technology.

3. MATERIALS AND METHODS

3.1. Reagent and instruments

3.1.1. Reagent

D-Hanks solution (self-made), PBS solution (self-made)

3.1.2. Instruments

S450 scanning electron microscope (Hitachi, Tokyo, Japan); Optical microscope (Olympus, Tokyo, Japan); texture profile analyzer (TRAPEZIUM LITE, Hong Kong, China); Feeze dryer (SP, NY, USA), micro-CT (Skyscan1074, Aartselaar, Belgium)

3.2. Methods

3.2.1. The sources of experimental samples

A total of 20 thoracic aortas from freshly slaughtered pigs in Shanghai Slaughter house, were placed in Hank's solution at 4 °C with warm ischemia time less than 10min and arrive in laboratory within 60min. Vessels were freed of adventitia and then washed with PBS. We cut aortas into 15mm long and 10mm internal diameter without branches as samples. Blood vessels were divided into 3 groups. Every group included 5 segments. Considering the instability of vessel which is heterogenicity of axia deformation in aorta, 5 segments scanned were from the same pig aorta and adjacent and were moderate size in order to guarantee the comparability of experiments.

3.2.2. The Vacuum freeze-drying on blood vessel

In our study, rising-and-reducing procedure lower temperature instrument controlled by computer was used to reduce the temperature of vessels by a fixed rate to study the influence of three different pre-freezing rates on the quality of stored freeze-dried vessels. In the pre-freezing stage, blood vessels were divided into 3 groups (I, II, III), and each group included 5 vessels. The pre-freezing rates were 0.5 °C / min, 1 °C / min and 2 °C /min for group I, group II and group III respectively. Vessels were pre-freezed to -43 °C (This temperature is the lowest that vessel can get to when the lowest shelf temperature was -70 °C in vacuum freeze drier) Vertical vessels were placed at low temperature for one hour to dry after programmed cooling. The first drying temperature was set at -20 °C, and the secondary is 15 °C The first and secondary process kept 2 hours and 4 hours respectively. The drying pressure maintained at 3Pa to 10Pa (absolute pressure)

3.2.3. Rehydration

The dried blood vessels were put into the normal saline to rehydrate for about two hours, and then removed the blood vessel for the experiments later.

3.3. Scanning pig aorta by micro-CT

3.3.1. Scanning blood vessels before and after freeze-drying by micro-CT

Fresh vessels, freeze-dried vessels and rehydrated vessels were scanned by micro-CT and then got directly scanning images and reconstructing two-dimensional images in order to analyse micro-vascular structural changes after freeze-drying from images.

3.3.2. Porosity ratio and gray value measurement in vacuum freeze-dried vessel

Porosity ratio was Porous solid volume/ Total volume (11). And the Gray value was calculated from two-dimensional reconstruction image by CT scanning. In our study, Micro-CT system software was employed to analysis the vascular porosity ratio. The Porous area/Total area ratio indicated certain cross-section images of reconstructing vessels. We set a threshold of gray value. The gray value above this threshold was regarded as pores, while under this threshold was structure. Then the porosity ratio ε could be High threshold value/Total value in the region. Figure 1A shows the vascular reconstruction of an original two-dimensional height when scanned by CT. Figure 1B indicates the interface of software analysis. The upper left captured picture is the CT scan images, and the lower left shows the results of Figure 1A using this software. When the gray value of 178 was set as the boundaries of the value of the pore and the skeleton, The backbone area formed by the black spots (showing the pore) and white area (showing

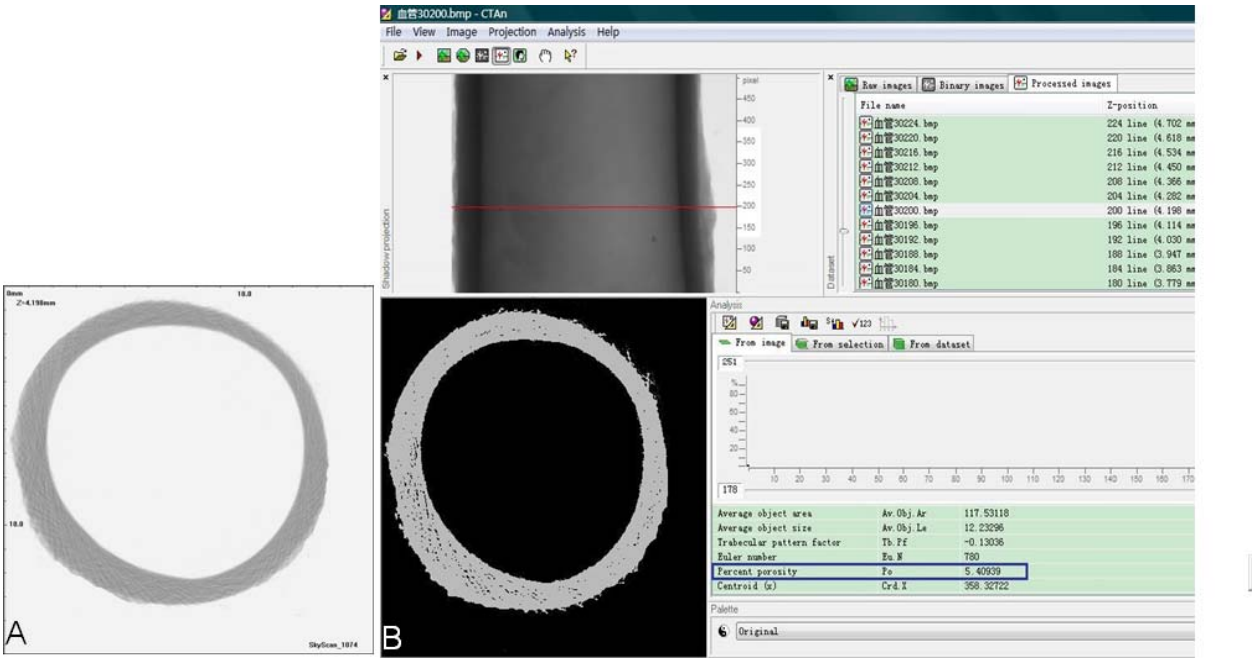

Figure 1. Two-dimensional reconstructed image of vascular cross section (A) and CT software analysing porosity ratio surface (1B).

the vessel) appeared. The ratio of black spots and total vessel area is the porosity.

3.3.3. Tomographic scanning of freeze-driedpig aorta by micro-CT

The pressure and temperature were recorded every five minutes during the freeze-drying process. And every 60 minutes, took the sample, using micro-CT scan to record the morphological changes of the blood vessels. Then the axial sections of pig aorta were analyzed to research the freeze-drying sublimation process. (pre-freezing rate was 1 °C /min, first drying temperature -20 °C, secondary drying temperature 15 °C, times of first and secondary drying were 2 hours and 4 hours respectively.)

3.4. Detecting vascular mechanical properties

Tensile test of aorta was done in the texture analyzer and a cubic block of arteries with width of 7mm was made for puncture test. Set the texture analyzer instrument with force 500N and speed 1mm/min to punctuate and stretch respectively. We immersed the freeze-dried blood vessels into distilled water for 2h to make sure the vessels are rehydration, and used the aortic artery with similar condition to the freeze-dried one to make tensile tests and puncture tests for a comparison. Changes of vascular mechanical properties after freeze-drying at different pre-freezing rates were analysed.

In data analysis, "α puncture" represented change rate of punctuate stress of fresh vessels after freeze-drying and rehydrating.

α puncture = [(the largest punctuate stress value of freeze-dried and rehydrated vessel the largest punctuate stress value of fresh vessel) / the largest punctuate stress value of fresh vessel]*100%. The "α rounding-stretched direction" and "α stretched axial direction" represented the change rate of rounding-stretched direction stress and stretched axial direction stress respectively of fresh vessels after freeze-drying and rehydrating. The α value was 0 meaning no change of vascular mechanical properties after freeze-drying and rehydrating. When the α value was less than 0, vascular mechanical properties had reduced after freeze-drying and rehydrating (ie, the corresponding stress value decreased) In contrast, if the α value was greater than 0, vascular mechanical properties had improved (ie, the corresponding stress value increased)

The highest point value of each curve were taken as the maximum values of puncture stress, rounding-stretched direction stress and stretched axial direction stress with vessels on our experimental conditions. Each measurement item (including puncture experiments, circumferential and axial tensile tests) measure five samples in parallel and their average values were the maximum values of puncture stress, rounding-stretched direction stress and stretched axial direction stress on that condition in our study.

3.5. Statistical analysis

All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) All data are expressed as mean ± Std.Deviation (SD)

4. RESULTS

4.1. Morphology

Gross morphology: vascular structure took shape of unique sponge-like porous structure after freeze-drying and preserving. After rehydrating (2 hours and 35 °C),

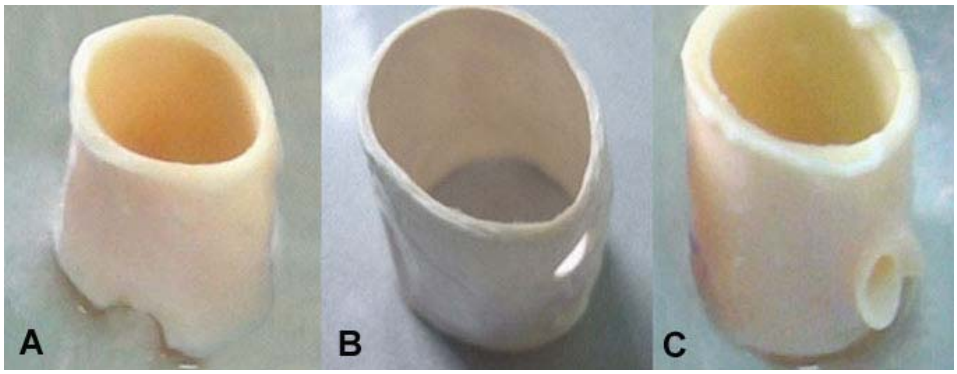

Figure 2. Fresh vessel (A), freeze-dried (B) and rehydrated vessel (C).

freeze-dried vessel could achieve a higher rehydration ratio. Rehydrated vessel was close to fresh one in the appearance, shape and colour. Figure 2 shows fresh vessel, freeze-dried vessel and rehydrated vessel, which demonstrated the changing course from fresh vessel to freeze-dried state and then to rehydrated one.

4.2. Micro-CTscanning images analysis before and after vascular freeze-drying

Freeze-dried vessels and rehydrated vessels were scanned by micro-CT in the same parameters getting images of freeze-dried vessel and rehydrated vessel. Figure 3 showed images of one vessel in fresh , freeze-dried and rehydrated state scanned by micro-CT.

4.3. Vascular two-demension axial section image

Figure 4 showed the axial section of vessel in fresh, rehydrated and freeze-dried state.

4.4. Vascular porosity ratio and gray value variation diagrams at different pre-freezing rates

Peritoneal exudates, accompanied with unpleasant odor and distal cecum binded-up and encapsulated, aggravated significantly after the CLP surgery 24 h and deteriorated afterwards. In the ileum, there was hyperemia and edema, an increase in inflammatory cells, loss of mucosa, and hemorrhage-induced vomica. The pathological changes were similar in both the THI and the CLP groups. However, the pathological damage was significantly milder in the THI group than in the CLP group at all corresponding time points (Figure 5) There were no pathological changes in ileums of the HC group.

Figure 5 and 6 showed vascular porosity ratio and gray value variation diagrams at different pre-freezing rates in the whole course of freeze-drying. The gray value variation diagrams showed changes of gray value changes in the whole layers of vascular wall analysed by computer on the basis of vascular axial section images. In the micro-CT directly scanned images, the dark colour represented small gray value and more X-ray absorption. In contrast, the light colour represented large gray value and less X-ray absorption (12). The lightest colour is in the freeze-dried vessels maybe due to lossing most water of vessel after freeze-drying (13).

In the data analysis, the change ratio of porosity ratio (gray value) meant the the change ratio of porosity ratio of fresh vessels after freeze-drying. The change ratio of porosity ratio (gray value) = [gray vale (porosity ratio) of rehydrated vessel - gray vale (porosity ratio) of fresh vessel / gray vale (porosity ratio) of fresh vessel] ×100%. When the pre-freezing rates were 0.5 °C min, 1 °C /min, 2 °C /min, the change ratio of porosity ratio were 11.9%, 16.6%, 29.7% and average change ratio of gray ratio were 2.27%, 3.64%, 6.36%, respectively. The more the cooling rate increases, the more evident changes.

4.5. Micro-CT tomographic scanning pig aorta freeze-drying

When the blood vessels were freezed, we clamp out a sample every one hour to carry out micro-CT tomography. After two-dimensional reconstruction of the original scanning, we arranged the reconstructed section in the order of time and obtained the vascular axial profile which was shown in Figure 7. The left was a picture of the outer vascular wall. The right was the inner wall of blood vessels. And 1h-4h for the pre-freeze phase, 5h-7h as a drying phase, 8h-13h for the second drying phase.

In Figure7, during the pre-freeze phase of 1h - 3h, there is no significant difference about the gray level of the three diagrams. Figure 4h was less gray, and the overall color seemed deeper because at 4h the water in artery has been completely frozen and ice had larger X-ray absorption capacity than water. In the drying phase 5h-7h the ice in external and internal layer of the arteries had sublimated first. The layer of sublimation gradually moved toward the center. In the secondary drying stage, arteries gradually lost bound water, the gray level increased. Figure 11h-13h showed phenomenon of arteries stratification which indicated the mechanical properties of each layer of blood vessels changed. However, each layer has different amount of volume change. That resulted in the stratification of blood vessels.

4.6. Difference

Figure 8 shows mechanical properties of blood vessels changed differently on different pre-freezing rates. In figure 8, PT value and circumferential tensile stress value that the freeze-dried blood vessel could bear were increased, and increased most (20.10% and 32.03%,

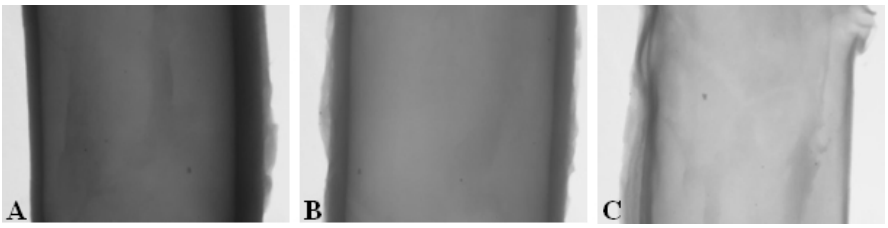

Figure 3. Directly scanned images of blood vessles: (A)fresh blood vessel; (B)rehydrated blood vessel; (C)freeze-dried vessel.

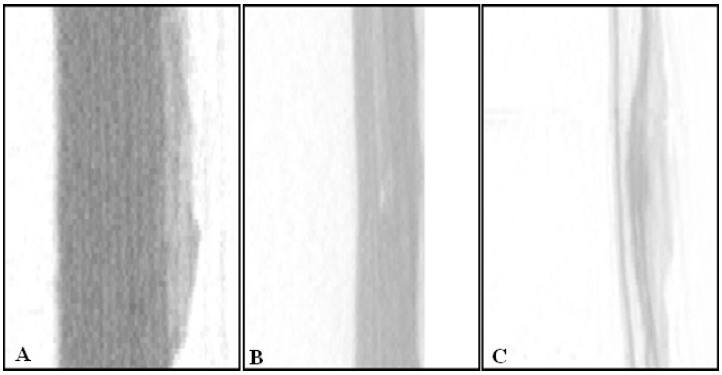

Figure 4. Vascular two-demension axial section image: (A)fresh blood vessel; (B)rehydrated blood vessel; (C)freeze-dried vessel.

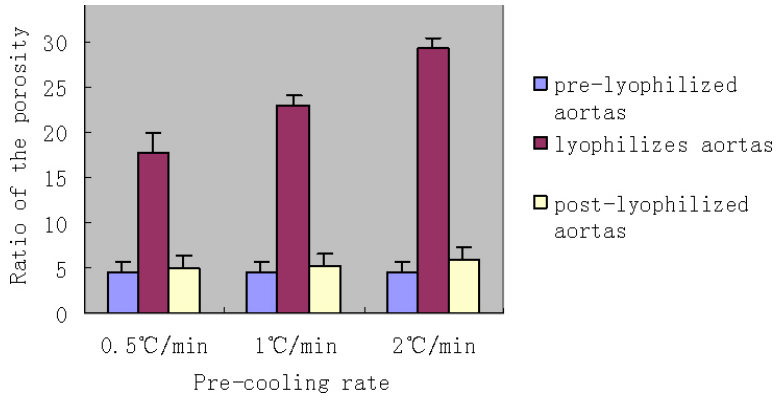

Figure 5. Vascular porosity ratio analysing at different temperature decreasing rates.

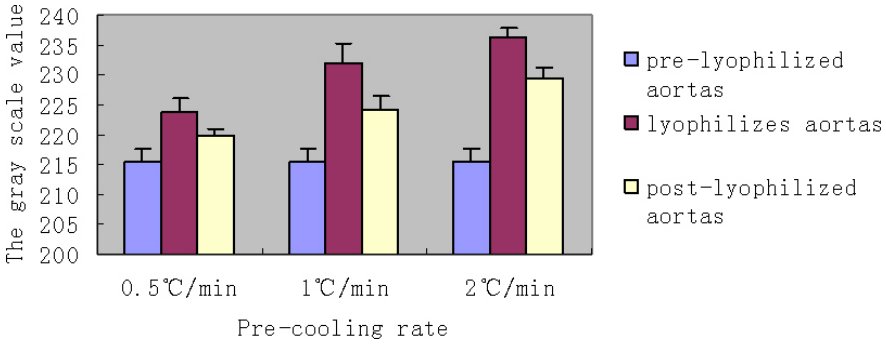

Figure 6. Gray value analysing at different pre-freezing rates.

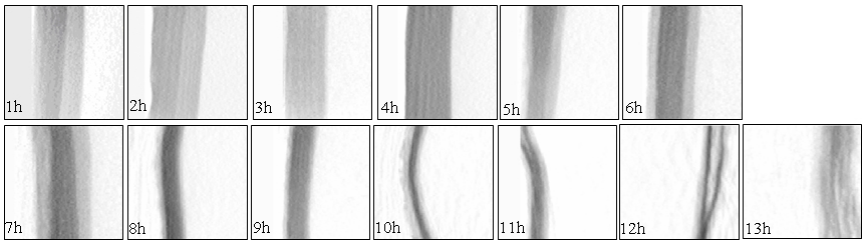

Figure 7. Micro-CT tomographic scanning pig aorta freeze-drying (axial section image in the wall)

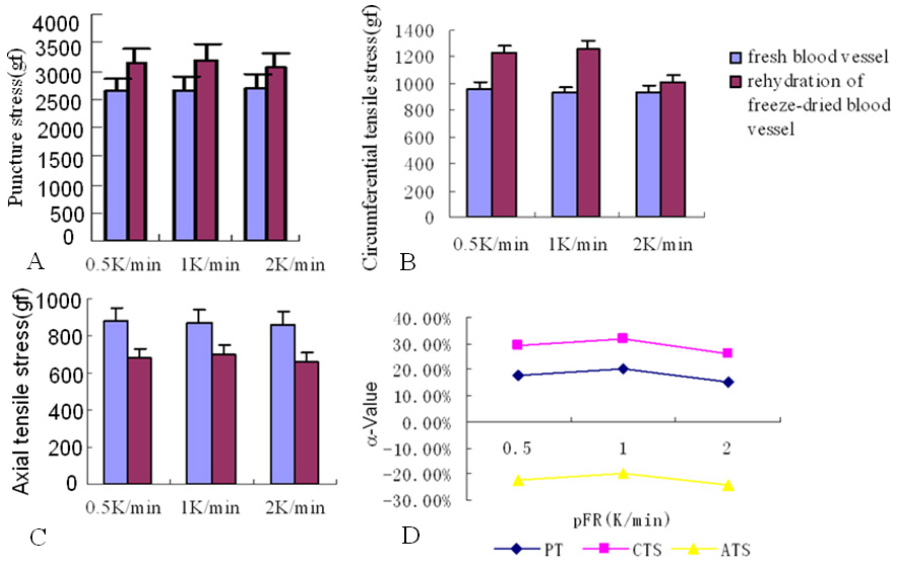

Figure 8. Different cool rate on the mechanical properties of blood vessels. (D): The curves of α values under different pFR on vessel.

respectively) at the pre-freezing rate of 1 °C / min. And at this rate, the ATS that freeze-dried blood vessel could bear was decreased and decreased to the smallest extent (-19.84%).

5. DISCUSSION

Vacuum freeze-drying technology was first used in preparation of biological materials (14,15). And until recent years, it is used to prepare porous supporting materials in tissue engineering (16). Freeze-drying technology has been in development nearly 60 years in tissue preservation and material preparation. Our study made use of freeze-drying technology in retaining and processing biological material of tubular such as blood vessel, in order to assess the feasibility of freeze-drying technology as a long-term preservation method for supporting material of vessel. In our study, the main index is the influence of different pre-freezing rates on porous characteristics and mechanical property of vessel.

The control of pre-freezing rate is most crucial in pre-freezing stage. In 2009, Nai-Yi Yuan *et al* (17) found that it was significant to achieve a suitable pore through controlling pre-freezing rate in tissue engineering. The pre-freezing rate could affect the size, shape and quantity of ice crystal and the quality of the final product. Rapid freezing of water would form more ice crystals with a smaller diameter, while slow freezing would make water to form less ice crystals with a larger diameter. The quality of pre-freezing rate controlling determines the quality of material after freeze-drying to a certain extent. From the perspective of cell freezing, under condition of slow freezing, ice crystals were produced in intercellular space and make its volumel expanse, leading to the concentration of solution in unfrozen water increasing. Unfrozen water absorb water from intracellular though osmosissing, resulting in cell shrinkage and finally cell death for high concentration in cell sap. In contrast, fast freezing produces smaller ice crystal which leads cell to less damages than slow freezing. However, fast freezing makes the outer surface of the material rapidly cool, crystallize and expanse, resulting in volume difference and temperature difference. Meanwhile, the internal heat cannot transfer timely generating great thermal stress and producing great harm on the freeze-dried material. Different freezing process of samples could cause different effects on the pore and gray value. These changes could be quantified by Micro-CT (18).

Vessel is a biological material. The crackle would appear on the wall under internal thermal stress when the pre-freezing rate is higher than 2 °C / min (8,9), which

would turn into larger cracks by long-term lashing and oppressing of blood in circulatory system, resulting in hemorrhage which is a great risk in transplant operation. In comparison, vessel could maintain a good contractile function in the pre-freezing rate of 0.5-2.0 °C / min, while could have a favorable diastolic function in the pre-freezing rate of 0.1-2.0 °C / min (19). For these reasons, this present study choose the pre-freezing rates of 0.5 °C / min, 1 °C / min, 2 °C / min as conditions to detect vascular changes of porosity ratio and mechanical properties after lyophilizing.

Our study showed that the changing rates of vascular porosity-ratio were 11.9%, 16.6%, 29.7% after freeze-drying under the pre-freezing rate of 0.5 °C / min, 1 °C / min, 2 °C / min respectively. The vascular porosity-ratio increased after freezing-drying as the pre-freezing rate increases. Previous study had indicated that characteristics of dry material were partly determined by the size and distribution of pore among many parameters (vacuum, heating board temperature, material thickness, etc.) (20), so the size and distribution of pore directly affected the process of delivery and drying rate. In addition, the growth rate of transplanted tissue, which is the rate of vascular endothelium, depends on the porosity and permeability of graft. Permeability with too low affects the rate of endothelium in graft and increases the risk of thrombosis. But too high permeability would cause surrounding tissue to growing into graft too fast and then obstruct the vessel. Therefore, transplanted grafts with suitable porous is very important in the success of transplantation.

The pre-freezing rate in 1 °C / min is suitable for mechanical properties of vessels. There are two reasons for this. Firstly, pre-freezing rate in 0.5 °C / min may be slow, in which cells of vessel tissue are exposed to solutions with high concentrations and then much intracellular water exosmose leading to shrinkage, dehydration of cells. As a result, cell protoplasm and organelles are damaged and further adversely affect vascular structure. Secondly, the pre-freezing rate in 2 °C / min may be fast for vascular tissue, in which water osmosis rate can not keep up with cooling rate during the course of pre-freezing and intracellular water directly form ice crystals before exosmose, resulting in physical injury on cell structure and further affecting vascular structure.

Freeze-dried vessel shown in Figure 3 was a lighter colour meaning less X-ray absorption than fresh vessel, which may be due to large amounts of water in vascular tissue loosing after freeze-drying (13). Figure 4 showed that the wall of freeze-fried vessel was loose and porous and thinner than fresh vessel. In Figure 3, the colour of rehydrated vessel was close to the fresh one, since the loose and porous structure of freeze-dried vessels had been close to the state of fresh vessel after rehydration. Figure 4 further demonstrated that the thickness of vascular wall returned to the level of fresh vessel. However, figures still showed some differences between freeze-dried vessel and fresh one. For example, vascular structure of rehydrated vessel was not as compact as fresh one, which was mainly account for the pore space and layer of vessel during the course of freeze-drying. From this perspective, although freeze-dried vessel was lack of vascular intima, endothelialization could be achieved through cell implanting and new tissue growing (21,22). And then the rejection for antigen would reduce. Large pores in the tunica media vasorum may provide condition for infiltration of smooth muscle cell suspension, and then tissue differentiate and repair and ultimately ideal vascular structure is reconstructed. Therefore, we suggest that freeze-dried vessel as supporting material in transplanting application is promising.

6. CONCLUSION

An ideal supporting material should be comprised of an ideal pore structure and mechanical strength to sustain vascular matrix. Our study showed that 1 °C / min was the best pre-freezing rate in the course of vessel pre-freezing, in which porous structure resulting from freeze-drying made the performance of rehydrated vessel access to fresh vessel and reduction of the damage of vessel from thermal stress, with significant advantages in preservation. Endothelial cells, separated from vessel and then freeze-dried and irradiated, could be used as a good source of supporting materials in tissue engineering.

7. ACKNOWLEDGEMENTS

Meng Yin and Mengfang Liu are co-first authors. Cao Qing and Jinfen Liu are co-corresponding author. This work was financially supported by Shanghai Leading Academic Discipline Project (No. S30503) and Shanghai JiaoTong Universiry Academic Project (No. YZ1012) for support.

8. REFERENCES

1. J. Zeltinger, L. K. Landeen, H. G. Alexander *et al*: Development and characterization of tissue-engineered aortic valves. *Tissue Eng,* 7 (1): 9-22 (2001)

2. G. Steinhoff, U. Stock, N. Karim, *et al*: Tissue engineering of pulmonary heart valves on allogenic aeellular matrix conduits: *in vivo*restoration of vavle tissue. *Circulation,* 102 (19 suppl 3): III 50-55 (2000)

3. P. Simon: Early, Failure of the tissue engineeredpig heart valve synergraft in pediatric patients. *Eru J Cardiothorac Surgery,* 23: 1002-1006 (2003)

4. C. Booth, S. A. Korossis, H. E. Wilcox, *et al*: Tissue engineering of cardiac valve porstheses I: Development and histological characterization of all acellularpig scaffold. *J Heart Valve Dis,* 11 (4): 457-462 (2002)

5. I. Vesely, L. Gonzalez — Lavin, D. Graf, et a1: Mechanical testing of cryopreserved aortic allografts. Comparison with xenografts and fresh tissues. *J Thorac Cardiovase Surg,*99 (1): 119 (1990)

6. D. Lei, J. H. Zhao, L.A. Tian: Analysis of Thermal Stresses Around the Whole Process of Artery

Cryopreservation. Journal Of University of Science *And Technology of China,* 34 (3):328 (2004)

7. A. Nussinovitch, N. Jaffe, M. Gillilov: Fractal pore-size distribution on freeze-dried agar-texturized fruit surfaces. *Food Hydrocolloids,* 18 (5):825–835 (2004)

8. P. T. Wang, Z. Q. Shu, L Q. He, *et al*: The Structure and Cellular Viability in Cryopreserved Rabbit Carotid Arteries. *J. Surg. Res.,* 131 (2): 241-251 (2006)

9. Y.C. Song: Cryopreservation of the common carotid artery of the rabbit: Optimaization of dimethyl sulphoxide concentration and cooling rate. *Cryobiology*, 32: 405-421 (1995)

10. G. Gherardini, A. Haegerstrand, A. Matarasso, A. Gurlek, G. R. Evans, T. Lundeberg: Cell adhension and short-herm patency in human endothelium preseeded 1.5 mm polytetrafluoroelylene vascular grafts:An Experimental study. *Plast Reconstr Surg,* 99:472, (1997)

11. Y.Cao, L Sun: Applications of Industry CT in Porosity Analysis of Composite Materia CT.*Computerized Tomography Theory And Applications CT,* 2001, 10 (4):14-17 (2001)

12. K. Steppe, V. Cnudde, C. Girard: Use of X-ray computed microtomography for non-invasive determination of wood anatomical characteristics. *Journal of Structural Biology,* 148: 11-21 (2004)

13. M. D. Bentley, M. C. Ortiz, E. L. Ritman, J.C. Romero: The use of microcomputed tomography to study microvasculature in small rodents. *Am. J. Physiol. Reg. I,* 282: R1267-R1279 (2002)

14. A. U. Smith: Freezing and drying biological materials . *Nature* ,181 (21), 1694 - 1696 (21 June (1958)

15. A. Briggs: A new freeze-drying technique for processing biological materials .*Dev Bid Stand,*36:251-260 (1976)

16. S. Ha, A. Ta, D. Ta, *et al*: Fabrication of highly porous keratin sponges by freeze-drying in the presence of calcium alginate beads. *Materials Science and Engineering,* 28 (8):1250-1254 (2008)

17. N. Yuan, Y. Lin, M. Ho. *et al*: Effects of the cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze-gelation method. *Carbohydrate Polymers,* 78 (2):349-356 (2009)

18. A. C. Jones, C. H. Arns, *et al*: Assessment of bone ingrowth into porous biomaterials using MICRO-CT. *Biomaterials,* 28 (15):2491-2504 (2007)

19. G Zhou, H. Xu, Z. Hua, *et al*: Basic function testing of cryopreserved arteries. *Journal of University of Shanghai for Science and Technology,* 24 (2):104-108 (2002)

20. T. Fukasawa, Z. Y Deng. *et al*: Pore structure of porous ceramics synthesized from water-based slurry by freeze-dry process. *Journal of Materials Science,* 36 (10):2523 – 2527 (2001)

21. N. D. Martin, P. J. Schaner, T. N. Tulenko, I. M. Shapiro, C. A. Dimatteo, T. K. Williams, E. S. Hager, P. J. DiMuzio: *In vivo* behavior of decellularized vein allograft.*J Surg Res.* 129:17 (2005)

22. R. C. Elkins, P. E. Dawson, S. Goldstein, S. P. Walsh, K. S. Black :Decellularized Human Valve Allografts. *Ann Thorac Surg,* 71:S428 (2001)

Abbreviations: PT: puncture tolerance, ATS: axial tensile stress

Key Words: pig aorta; Vacuum freeze-drying; Tomography; Mechanical properties

Send correspondence to: Cao Qing, Department of Pediatrics, Shanghai JiaoTong University Affiliated Shanghai Children's Medical Center, Shanghai ,200127, China, Tel: 86-21-38626161-5703, Fax: 86-21-58393915, E-mail: caoqingsci@yahoo.cn

http://www.bioscience.org/current/vol17.htm