

Research Article

Judgment about the Endpoint of Primary Drying for Freeze-dried Pig Aorta

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Abstract: How to process and preserve these biological material has always been a concerning problem. Vascular studying and evaluating the feasibility of preparing the vessel scaffold by freeze-drying technology is expected to provide high-qualified and reliable vascular graft for the clinical treatment of the related diseases. Judgment of the end of primary drying has always been a key point on the process of freeze-dried pig aorta, because it can directly affect the final quality of the dried sample if improper judgment has been used. In this study, different methods have been adopted to determine the endpoint of primary drying and then various methods of observation on the status of the aorta are used for contrast, including Micro-CT scanning, percentage of dehydration and calculation of the rehydrated rate and so on. From the results, differences are obvious. These experiments point out how to judge the endpoint of the pig aorta's primary drying when they are freeze-dried by small laboratory equipments in which heat leakage and other external factors cannot be ignored.

Keywords: End point of primary drying, freeze-drying, micro-CT scanning

INTRODUCTION

Vacuum freeze-drying technology can well keep the compositions and structures of the heat-sensitive material because of its lower temperature in the whole process, in addition, dehydrated samples can be easily stored (Dong *et al.*, 2011; Xiao *et al.*, 2010), so it has universally applied in biological (Jong-Pil *et al.*, 1997; Todor *et al.*, 1996; Zheng and Wang, 1997; Gao *et al.*, 2011), medical (Ding and Zhao, 2011) and food (Gooding and Macdougall, 1961; Yu *et al.*, 2011; Lu *et al.*, 2010; Wu *et al.*, 2010; Chen *et al.*, 2010) fields is a universal application.

Vacuum freeze-drying process can be mainly divided into three stages: pre-cooling, primary drying and secondary drying. Some studies (Paakkonen and Mattila, 1991; Genin and Rene, 1996) consider the pre-cooling rate within a safe range has not much influence on the results of freeze-drying. For most of the freeze-drying process, the primary drying lasts longest and it can be up to several hours or even several weeks depending on the water content of the sample. Once the control of the primary drying or the end point of the primary drying is operated of judged improperly, it will cause the collapse and structure change of the sample, therefore, primary drying process is very important to the impact of sample qualities after freeze-drying (Tang *et al.*, 2006; Roos, 1987). Along with the development research of pig aorta, obtaining high-quality freeze-dried blood vessels by means of optimized experiment appears to be particularly critical. Through comparative analysis of repeated weighing, scanning and mechanical

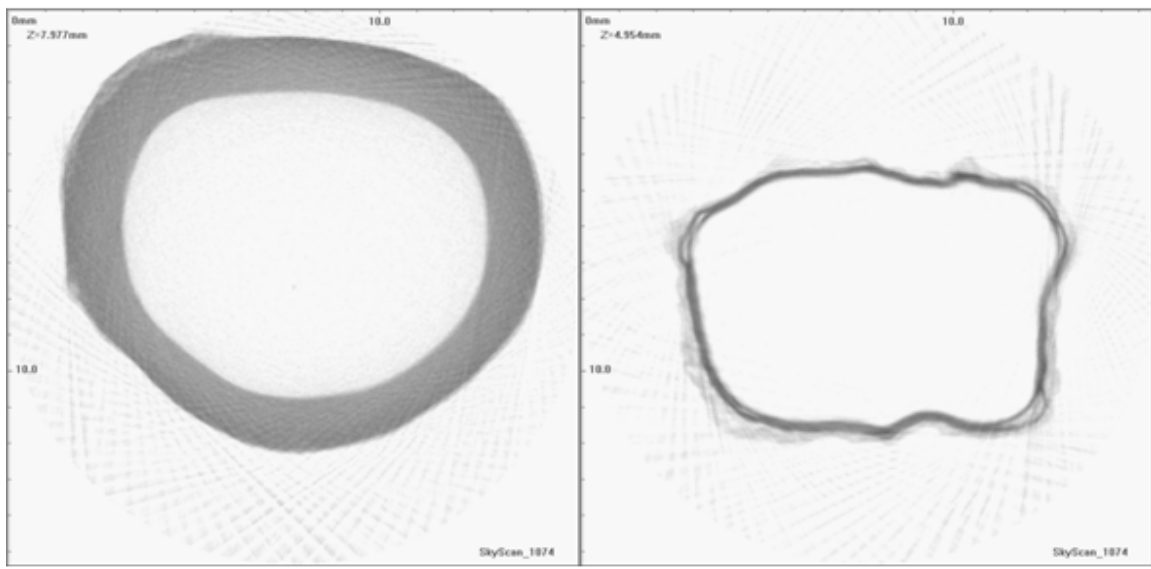
properties experiments for the freeze-dried pig aorta, it puts forward the optimized method which can reduces the pleat and collapse on vessel for estimating the end point of primary drying under the existing instruments and experimental conditions, in order to get pig aortas of better quality for application of transplant. Vascular studying and evaluating the feasibility of preparing the vessel scaffold by freeze-drying technology is expected to provide high-qualified and reliable vascular graft for the clinical treatment of the related diseases.

EXPERIMENTAL METHODS AND RESULTS

Experimental apparatus:

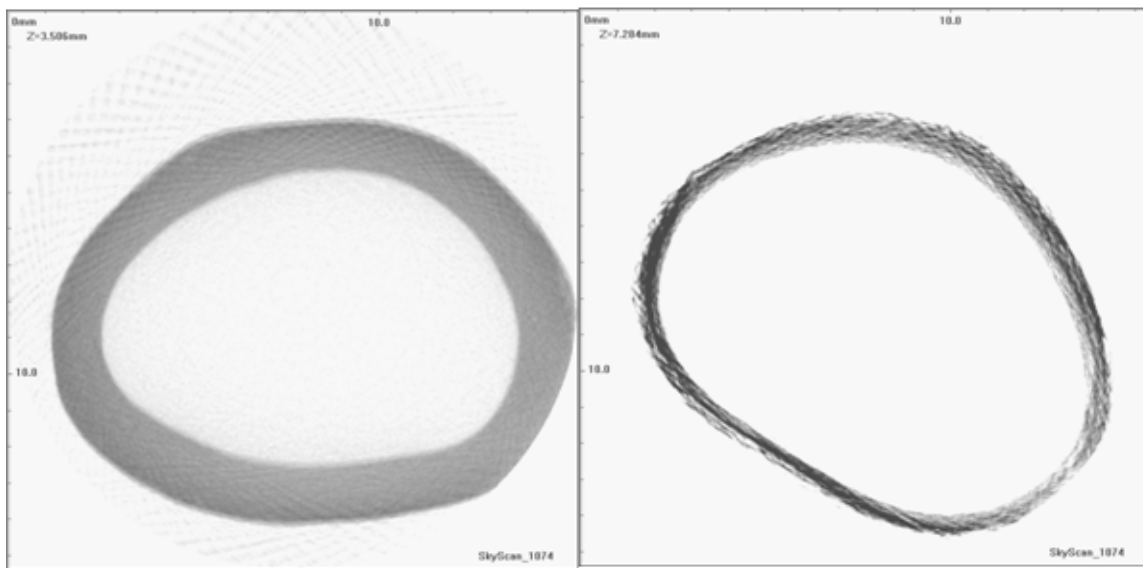
Materials and methods: The fresh pig aorta is washed and cut into the vessel sections of two groups, marked A0, A1 and A2, B0, B1 and B2 which are all around 15 mm. To reduce the individual differences of biological tissue, these sections are from the same root vessel and A0 and B0 are adjacent on the vessel, also, A1 is adjacent to B1 and A2 is adjacent to B2. Temperatures about pre-cooling, primary drying and secondary drying for both experiments are set to -70, -20 and 10°C, respectively.

Vacuum of these two freeze-drying process are maintained less than 100 Pa. When the drying is carried on, water sublimation will raise the pressure in freeze-dried chamber, so open the vacuum pump and then turn off the vacuum after chamber pressure is adjusted to 100Pa around, repeating this regulation in the following process. Group A reaches its end point of the primary drying when the temperature gets to the shelf



(a) The fresh A₁

(b) The freeze-dried A₁



(c) A the fresh B₁

(d) The freeze-dried B₁

Fig. 1: The fresh and freeze-dried cross-section diagrams for A₁ and B₁

Table 1: Time consumed in various phases of the two methods

	Pre-cooling (h)	Primary drying (h)	Secondary drying (h)	Total (h)
A	2	2	4	8
B	2	15	1	18

temperature set before, while Group B for method improved, when samples get to the shelf temperature, continue freeze-drying without any adjustment until the sample temperature and chamber vacuum pressure (under the condition of not opening the vacuum pump) no longer change (for about 1) hour as the end. A0 and B0 are inserted into thermocouple for monitoring of temperature at real-time. Table 1 lists the time

consumed in various phases of these two kinds of methods.

Micro-CT scanning results: In Fig. 1a to d, are the fresh and the freeze-dried cross-section diagrams for A₁ and B₁ respectively. From the figure, the pleated phenomenon of the freeze-dried vessel in Group A is more obvious than in Group B and Group B is relatively smooth, moreover its wall is thicker than A.

Porosity: Through the calculation of correlative software of Micro-CT, porosity of the fresh A₁ and B₁ are 7.22 and 7.36% respectively, as well, the freeze-

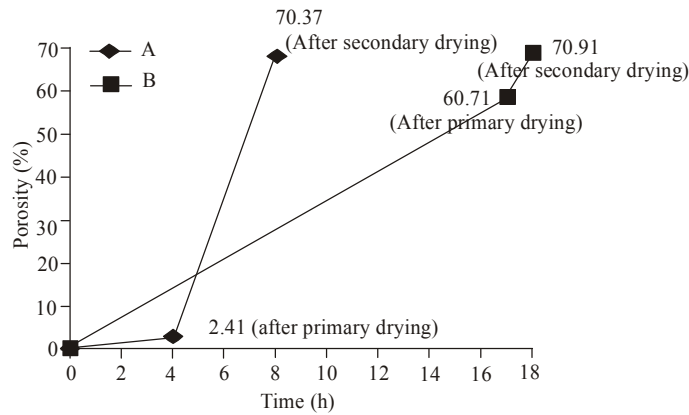


Fig. 2: Comparison of dehydration rate with two methods after primary and secondary drying

Table 2: Comparison of mechanical properties before and after rehydration

	Axial tensile strength (MPa)	Circumferential tensile strength (MPa)	Puncture stress (N)
Fresh A	1.243	1.790	2504
Rehydrated A	1.654	1.361	2884
Fresh B	1.107	1.100	3003
Rehydrated B	1.289	1.310	2736
Fresh A-rehydrated A	-0.411	0.430	-380
Fresh B-rehydrated B	-0.182	-0.210	267

dried for A1 and B1 are 29.26 and 34.42% respectively. It can be seen that porosity of freeze-dried B for improved methods is higher than the other group.

Weighing: Vessel sections of A1 and B1 are used for the weighing and scanning when they are in fresh, freeze-dried and rehydrated states, in addition, once A or B has finished the primary drying, take them out, weighing and recording.

Dehydration rate: According to the formula: $\text{Dehydration Rate} = \frac{(\text{fresh A (B) (quality, g)} - \text{freeze-dried A (B) (quality, g)})}{\text{fresh A (B) (quality, g)}} \times 100\%$, dehydration rate of A1, A2, B1 and B2 can be achieved. Make average for Group A and B respectively obtaining the Fig. 2, comparison of dehydration rate with two methods after primary and secondary drying. From the diagram, it can be observed that vessel's dehydration rate in improved method is higher than the other, besides, it has taken off most of the water after primary drying, in contrast, 2.41% of the water has removed with the other method.

Rehydration rate: According to the formula: $\text{Rehydration Rate} = \frac{(\text{fresh A (B) (quality, g)} - \text{rehydrated A (B) (quality, g)})}{\text{fresh A (B) (quality, g)}} \times 100\%$, calculate the average, then rehydration rate of Group A and Group B are 90.80 and 91.33% respectively.

Mechanical test: Shear the 15 mm vessel section open along the vertical, that is, axial direction, then cut two

artery strips into 15 mm long and 3 mm wide respectively which are adjacent. Also, cut another vessel along the transverse direction into the vessel ring about 3 mm wide and then cut the ring at both ends, getting two artery strips in circumferential direction. Strips above are used for axial and circumferential tensility by DMA. As well, cut a 7 mm vessel section into the artery piece about 7 mm long and 7 mm wide which is also adjacent. These pieces will be punctured by Texture Analyzer. One of these two adjacent strips or pieces acquired in each time is used to the mechanical test in fresh and the other is in rehydrated. The reason for this cutting means is for ensuring the comparison accuracy from the same period of the vessel before or after freeze-drying.

The working conditions of DMA is: the initial stress is 0N, increased to 17N by 3N/min, among this process, whenever the vessel strip is pulled off, it means the end of the process, furthermore, make the stress at the time vessel is broken off as the maximum tensile stress (N). For accurate calculation, measure the thickness and width of the wall, finally, calculate the maximum tensile strength (MPa) per unit of area. In the puncturing experiment, texture analyzer is set to the workload of 500N and puncturing rate 30 mm/min, when the puncture needle has penetrated the wall, the test is over, accordingly, stress at this time is the maximum puncture stress. Make the difference between fresh and rehydrated max strength or stress, receiving Table 2, comparison of mechanical properties before and after rehydration.

DISCUSSION

Determine the sample temperature reaching to the shelf temperature as the end of drying like the way of Group A is a usual method (Patel *et al.*, 2010; Tang and Michael, 2004). For retaining the structure and function of biological materials to a large extent, the primary drying is usually at a lower temperature, so this study is also on the basis of it. However, it is found in this

experiment that when the sample temperature reaches to the shelf temperature, although there is not any adjustment, the sample temperature will continue to rise even higher than the shelf temperature, which can prove that there are errors in the experiment. Generally, mainly two aspects cause the error:

- The established premise of the theory that sample temperature is equal to the shelf temperature as the end point is, under the condition of the minimal pressure of the chamber, convective heat transfer indoors and radiation heat transfer outdoors can be ignored, that is, the sample gets the heat totally dependent on the shelf to provide the heat only through heat transfer, but in actual operation, the radiation heat transfer outdoors are so high that it cannot be ignored, which leads to the final sample temperature exceeding the shelf temperature.
- Values of temperature are read by the thermocouple inserted into the vessel, in one hand, sample is therefore destructed producing a little large gap and in the other, the speed of sample inserted thermocouple will increase more rapidly than others because of the low resistance that copper-constantan thermocouple has. As a result, method in Group A finishes its primary drying only in a short period (2h), ignoring the subsequent increases of temperature, inducing the sample dehydration rate to 2.41%. At this time, large amount of water exists as the ice crystals form, if it elevates the temperature to the secondary drying, the freezing part will be molten back that makes degradation of frame stiffness, becoming viscous thus causing the sample collapse and pleat, thus it is a great damage to the structure of the vessel.

The freeze-drying process is decided by both heat and mass transfer and that temperature and water vapor pressure are the main two driving factors for heat and mass transfer respectively, the optimization of freeze-drying principally lies in the control of temperature and pressure (Genin and Rene, 1996). Under the same temperature, the higher the vacuum degree indoors, the better the effect of heat transfer, but it is bad for escaping of ice crystals molecules, while by contraries, the lower the vacuum degree, the worse the effect of heat transfer but it is helpful for the ice to escape. Therefore, vacuum degree controlled in 100Pa can not only ensure the sublimation of ice crystals but quicken the drying rate (Chokri and Frédéric, 1997). In later drying, vacuum degree may be properly reduced (at 10Pa or so) which makes ice crystals sublimate completely. The experiment of Group B, consider the sample temperature and vacuum degree generally unchanged for a long time without opening the vacuum

pump as the ending point, eventually the water taken off accounts for 86% of the whole drying process, which accords with the general rule of freeze-drying.

From Table 1, the primary drying time of Group B has consumed is 13h longer than that of Group A, yet the secondary drying time is 3h shorter, this is caused by the principle of sublimation: the regulation of the sublimation is from the surface of the material to the center (Marques *et al.*, 1991; Rene *et al.*, 1993), ice crystals sublimation simultaneously leaving the pores which turn to the channel for the inner water vapor sublimating to the outside, but as the thickening of the dry layer, sublimation resistance is increasing gradually (Ansgar *et al.*, 2006; Tang *et al.*, 2005), consequently, the primary drying time of Group B is long, which is also corresponded with the freeze-drying law. Because the latent heat of the ice sublimation at 0°C is greater than the water evaporation at 0°C, the evaporation time is less than the sublimation time (Genin *et al.*, 1996). With the improved method, primary drying time is much longer, accordingly, the secondary drying is shorter.

In the evaluation index to sample, the dehydration rate, rehydration rate and the maintenance of the physical states of raw materials are very important to keep (Ponciano, 2002). This study find out that sample B has a better structure and higher porosity after freeze-dried, thus rehydration is easier. Two groups of the freeze-dried vessels both have changed in the tensile strengths and puncture stresses, overall, vessels of Group B are closer to the fresh, inferring that the optimized method plays a good effect on maintaining the properties for the vessel.

CONCLUSION

When pig aorta is being freeze-dried, retaining of the vessels appearance and final quality have a great relation with the judgments of the ending point of the primary drying. In most of the practical production and experiments, freeze-drier is often facilitated a glass door or window for the better observation, in this condition, effect to the temperature from radiation heat transfer cannot be ignored. If it is taken consistent of the sample and shelf temperature as end point of primary drying, a lot of errors will be caused, at this time, adding the vacuum degree is more reasonable. This experiment is based on the theory of freezing drying, determining the temperature and pressure unaltered in a certain time as the end of the primary drying, eliminating the sample collapse and shrinks. After freeze-drying, dehydration is more thoroughly and there are more higher rate in rehydration and porosity, moreover, the axial, circumferential tensile and puncture stresses are more close to the fresh vessel. It is

able to keep the various performances which will be completely significant in freeze-drying the biological materials.

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