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Research for saline imbibitions and diffusion during rehydration of freeze-dried pig aorta

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Abstract

The mechanism of mass transfer process for water imbibitions and diffusion of the pig aorta were analyzed. The rehydrated pig aortic which had been freeze-dried before were scanned and weighed in various stages. Then the cross-section diagrams, porosity, the ratio of the water absorption and its rate were calculated and received during the rehydration. It was demonstrated that 0.5-1h was the phase that changes about material moisture in each aspects occurred most significantly. After 3h, moisture, porosity and water absorption rate were changing dynamically and the vessels were nearly saturated. Water absorption was scaled linearly in the whole process, and the porosity declined close to the fresh from the freeze-dried vessels with higher porosity. It also pointed out that, pig aortic could be transplanted when immerged 2-2.5h in saline and normal temperature.

Keywords: pig aorta, water absorption, freeze-drying, rehydration, water content

1. Introduction

Vacuum freeze-drying technology has been widely used in the medical, food industry and other fields. It has some achievements for processing and preservation the fresh pig aorta through the vacuum freeze-drying method in theory and some related studies. Compared to other drying methods, the freeze-dried products are rehydrated because of their porous structure, so they are more close to the appearance to the fresh ones, and they can maintain their characteristics in maximum as before. Therefore, the subsequent process of rehydration such as the temperature, the properties of solution and the time for rehydration will directly affect the final quality of freeze-dried products. At present, the analysis for rehydration study is mostly focused on the areas of food such as vegetables, fruits, and cheeses, etc [1-4]. While the systematic study for aorta is rarely reported. The absorption and penetration of the rehydration process are complex, in addition, it has important practical significance for improving the related theory of vessels' freeze-drying and the effect of clinic transplantation. So the more detailed is necessary.

In this experiment, the freeze-dried vessels are weighed and scanned by CT which can analyze and calculate the porosity change, then getting optimum conditions for rehydration by the moisture content, water absorption rate and other discussions which has laid the foundation for further research on the clinical application and theory.

2. Materials and methods

2.1 Materials

The fresh aortas were bought from Shanghai slaughterhouse, washed by saline, reserved in refrigerator at 4 ℃ for one day, before the experiment.

2.2 Devices

- Advantage 2.0 Benchtop Freeze Dryer: VirTis, SP Industries Corp. U.S.
- Micro-CT：Skyscan 1074HR, Vluchtenburgstraat 3,2630 Aartselaar Belgium.

2.3 Methods

Vacuum freeze-drying

The pig aortic vessel is cut into sections of 15mm in length. In order to ensure their similar thickness and composition, 9 sections marked as A0 to A8 which are proximate on the same dimension or the location. One of the section, A0, is for Micro-CT scanning, the others, A1-A8 are used for weighing and measuring. The vessel sections are put vertically in the dish, placed on the freeze-drier shelf.

The shelf temperature of the freeze-drier on the phase of cooling, the primary drying and the secondary drying are set at -70℃, -20℃, and 10 ℃ respectively.

Selection of the rehydrated condition

In clinical transplantation, the graft material is usually put into saline at room temperature (20-26℃) rather than body temperature (35-37℃) for covenanting operation. Therefore, saline at the room temperature is used in the rehydration process, simulating the transplanting condition.

The Micro-CT tomography for pig aorta rehydration process

Sample A0 is scanned in fresh and after freeze-dried states respectively. Then it is rehydrated. It is scanned in the Micro-CT every half hour during rehydration process till to the end of the experiment.

Weigh

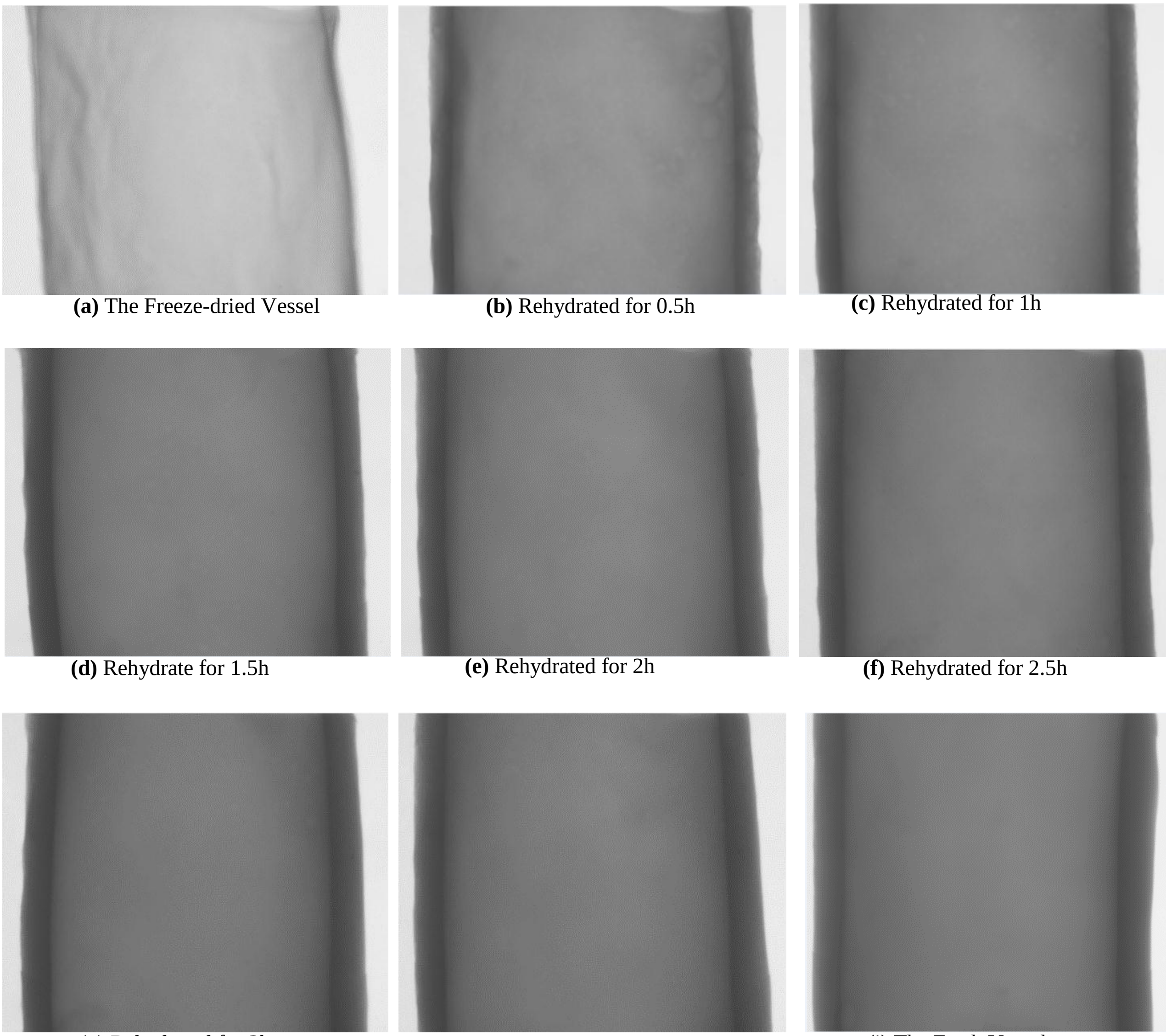
A1-A8 are weighed before and after freeze-drying. When rehydration is started, they are taken out of the solution, drained and weighed, until the end of the experiment.

3. Results

Micro-CT scanning

Vessel sections are vertically scanned in the Micro-CT, choosing the same scanning parameters such as brightness and contrast in each operation. Figure 1 shows the scanning diagrams for freeze-dried, rehydrated and fresh vessels.

In this figure, the freeze-dried vessel is more transparent, with significantly thinner vessel wall and a little shrink. After rehydration, the vessels color was deepened gradually while the images of at 0.5h and 1h are little lighter than the others. It can be seen from the figure, the wall is thickening, the color is deepened which indicates that water is absorbed and the vessel is close to the fresh state gradually during the entire 3.5h.



(a) The Freeze-dried Vessel **(b)** Rehydrated for 0.5h **(c)** Rehydrated for 1h

(d) Rehydrate for 1.5h **(e)** Rehydrated for 2h **(f)** Rehydrated for 2.5h

(g) Rehydrated for 3h **(h)** Rehydrated for 3.5h (i) The Fresh Vessel

Fig 1: Scanning Diagrams for Freeze-dried, Rehydrated, and Fresh Vessels

The cross-section diagrams of the vessel are obtained by reconstruction software. A specific height is selected to ensure getting the images from the same cross-section.

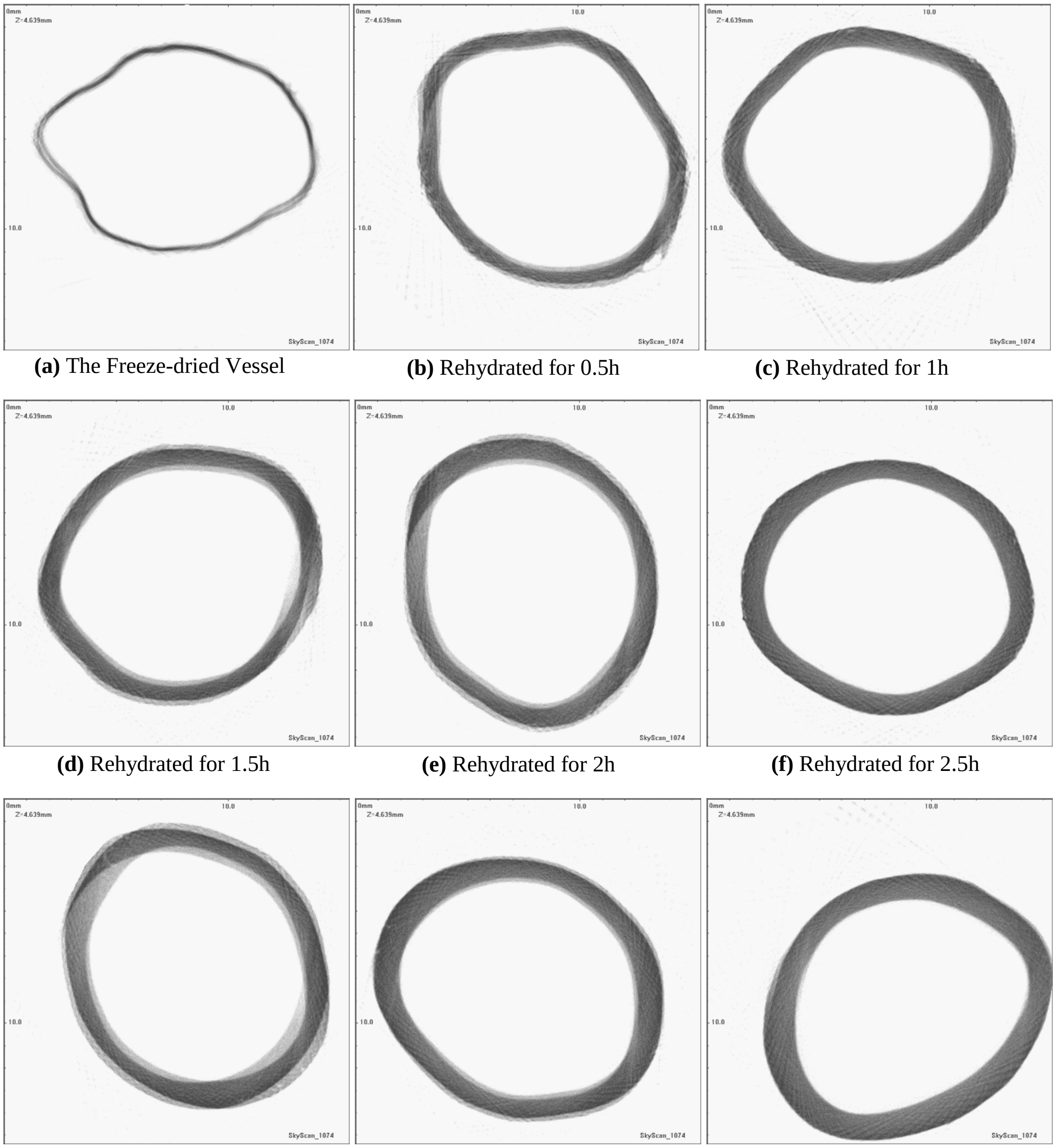
In figure 2, the changes on the thickness and the margin of the wall can be clearly observed. 0.5-2h, the wall is thickening which instructs the phenomenon of water absorption is obvious during the 2 hours; 2.5-3.5h, the wall seems like processing a dynamical adjustment until the balance state, in the end, the rehydrated vessel shows a very similar appearance to the fresh, the wall is overall uniform and the margin is rounded while the thickness of the wall is slightly narrowed.

Since a large part of the rehydration process is completed by the tiny pores caused by the sublimation of the ice crystals during the primary drying which can absorb water, the porosity changing during the rehydration is calculated by means of analysis software, which determine the skeleton and pore area with the black and white sub-boundaries.

In fig.3, 0h refers to the freeze-dried state with maximum porosity then the value decreased gradually; after 2 hours it is basically close to the fresh, of which the change is greatest during the first half hour. Rehydration adjustments during 2.5-3.5h in Fig. 2 are represented through the porosity fluctuations in this figure.

Weighing results

Weigh each section of A1-A8 when they are fresh, after freeze-dried and rehydration in different periods. Here, we define α as the ratio of water absorption. α = (rehydrated mass of n hours (g) - freeze-dried mass (g))/ (fresh mass (g) - freeze-dried mass (g)), where n = 0.5; 1; 1.5; 2; 2.5; 3; 3.5. The results of calculated α are listed in Table 1.



(a) The Freeze-dried Vessel **(b)** Rehydrated for 0.5h **(c)** Rehydrated for 1h

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(g) Rehydrated for 3h **(h)** Rehydrated for 3.5h **(i)** The Fresh Vessel

Fig 2: Cross Section for Freeze-dried, Rehydrated and the Fresh Vessel

From the table, value of α for 8h is quite close to that for 3h and 3.5h which means the vessel's rehydration is near to saturation in 3.5h, so in terms of clinical feasibility and theoretical analysis, the duration of rehydration could be regarded as within the 3.5 hours. The average result of all the 8 section vessels is showed in Fig. 4. Similar to most of the researches, water absorption of the freeze-dried vessel displays a linear growth trend from 1h to 3h and 3 hours later, the rehydrated vessel is basically saturated, but the final water content is not as high as fresh because α is always less than 1.

For table 1, rehydration rate could be calculated by minus between the adjacent average value, namely, before and after half an hour, then we get figure 5.

Compared with other time periods, vessel's rehydration rate is extremely high in the first 0.5 hour, then drastically reduced, while when compared with the situation after 1.5h, rehydration rate of 1h, therefore it can be analyzed the first 1h is the main stage for rapid absorbent. The general trend of absorbent is still in carried on after 1.5h but it appears in high or low adjustment tends before saturation as in Figure 5. The rate of 3.5h is negative, indicating the desorption in vessel.

Table 1: α at different Phases

	0.5h	**1h**	**1.5h**	**2h**	**2.5h**	**3h**	**3.5h**	**8h**
A1	0.328	0.430	0.470	0.550	0.592	0.646	0.654	0.670
A2	0.378	0.506	0.568	0.653	0.694	0.799	0.757	0.810
A3	0.367	0.471	0.523	0.592	0.634	0.713	0.698	0.720
A4	0.313	0.404	0.456	0.519	0.563	0.619	0.629	0.670
A5	0.318	0.440	0.471	0.551	0.585	0.623	0.643	0.667
A6	0.351	0.477	0.526	0.587	0.631	0.711	0.681	0.710
A7	0.392	0.560	0.585	0.677	0.703	0.753	0.685	0.727
A8	0.424	0.564	0.596	0.669	0.694	0.753	0.730	0.765
Average	0.359	0.482	0.524	0.600	0.637	0.702	0.685	0.718

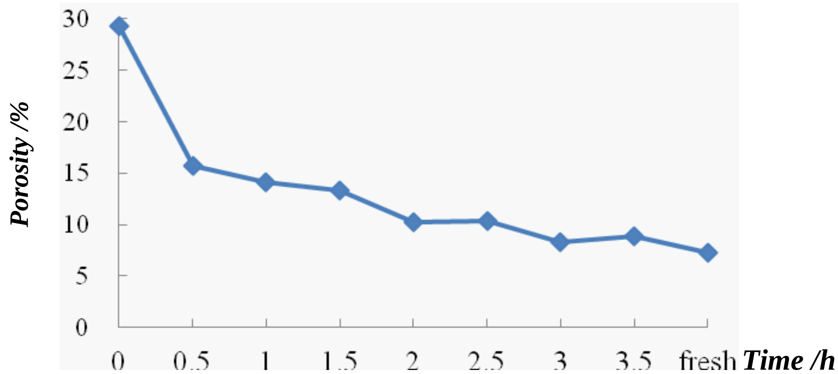

Fig 3: Porosity Changes of Rehydration Process

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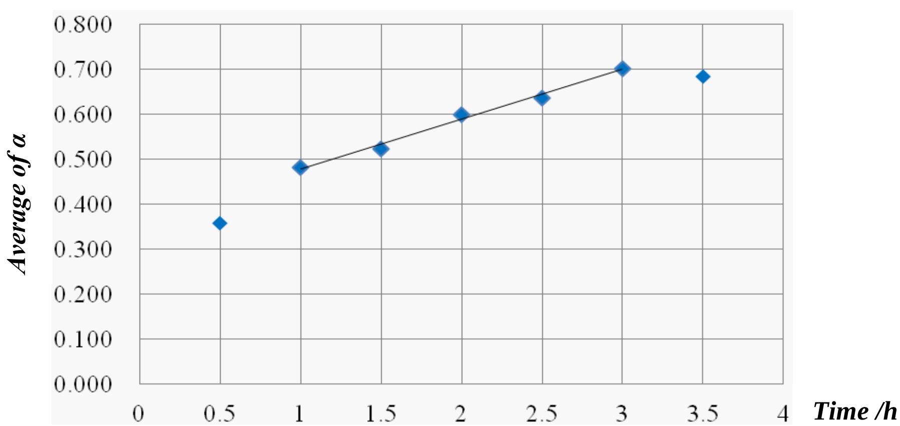

Fig 4: Water Absorption Ratio during Rehydration 3.5h

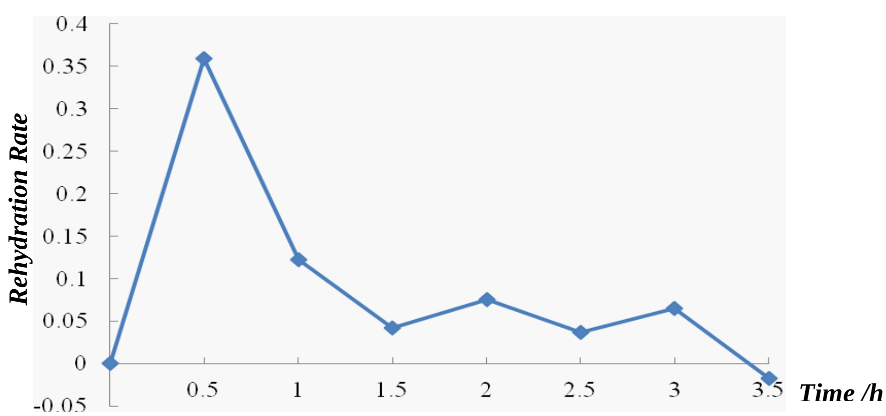

Fig 5: Rehydration Rate

4. Discussion

Rehydration is a complex water absorption and diffusion process [5-8]. The rehydrated pig aortas after freeze-dried are generally similar to others [9-12], but there are also differences. The trend of the rehydrated vessel after freeze-dried can be seen from the scanning results. The freeze-dried vessel appears transparent, and the wall is significantly thinner and wrinkled. After rehydration for 0.5h, the wall is obviously thicker because of its absorption of water, in addition, the surface of vessel becomes rounded compared with the shrinkage after freeze-dried. From the point of porosity and weighing figure (3, 4, 5), the changes of the data in 0.5h is very obvious so it means this is a rapid absorption process. From figure 2, the color of the sample at the center is significantly deeper than the margin in 0.5-1h, indicating that in the initial stages of rehydration, absorbent speed of the sample center is faster. This is consistent with the principle and the result of the freeze-dried, that is, the freeze-dried method makes samples out of water by the sublimation of ice crystals produced by the cooling stage, at the same time, it left pores at the place of ice sublimation, which causes the porous structure of the freeze-dried vessel in the end. At the beginning of rehydration, the pore from the edge of inside and outside wall to the middle of the wall causes the capillary action, resulting in the large surface tension making the absorption of water dramatically until pooling the water to the center of the sample. The mass and heat transfer in freeze-drying proves causes the wall edge shrinking, while after dehydration, these places take on a restorable trend, it means that the vessel absorbs the water not only between pore, the structure itself also can be absorbed. When the pores are filled by absorption, the structure absorption make the vessel swells gradually at shrinking place. Therefore the advanced stage 0.5-1h is the fastest stage for edge recovery in figure; meanwhile, the solid swelling for vessel itself also occurs owing to the water inhalation of the center, causing the entire vessel softened in structure. From 1.5 to 2h, the wall is still thickening, and the weight is also increasing, indicating that the vessel is always absorbing water in the former 2h. Some studies agree that the degree of volume change and water absorption in the former process is consistent [13].

From 2.5h to 3.5h, the edge of the vessel is in a state of constant adjustment which can be seen in the dynamic changes of porosity and weight. The whole vessels is uniform in 2.5h, while the change of margin status is obvious in 3h, and gradual recovery happens in 3.5h, all of which show the rehydration is going on the process from the dynamic adjustment to the balance. An important reason for this is alternant phenomenon in water absorbing is that, with the changing of pore aperture of margin, vessel's rehydration is sometimes in the decrease of capillary suction effect, or sometimes in the increase of water-swelling pore, so it is in the adjustment of changing in dynamic equilibrium. Most researchers believe that pores especially small pores determine the rehydration rate which causes the reduced water absorption rate in Figure 5. Another reason is, when rehydration is in process, three parts for dry materials sucking water, material swelling, and soluble substances separated out are simultaneously going along. In the front part of rehydration, water absorption, and material swelling play a leading factor, while the effect of third part compared to first two is too small that can be ignored. With the general saturation of absorption, capillary action has disappeared duo to water filling up in the interstitial space, and soluble substances desorption of vessel itself with the solution are gradually appeared in weight changes.

Analysis from a variety of figures and data, vessel's rehydration for 2-2.5h can basically meet the requirements and standards for surgical transplantation such as its appearance, internal quality and the degree of water absorbing are generally close to the fresh.

After rehydration, the entire vessel is comparatively uniform, and the margin is rounded, but compared with the fresh, wall thickness has a little narrowed. Mass analysis in some figures (4, 5) can be seen that water absorption rate is negative and the mass is decline in 3.5h revealing the reach of saturation. The reason for mass decrease may be the desorption of soluble substances. After absorption saturated, the final porosity is higher than the fresh, moreover, equilibrium moisture content is not as high as the fresh owing to the ice generation in freeze-drying and the complex heat and mass transfer process making some various degrees of change to the structure of the sample. All the three parts of freeze-drying will make damage to liposome. The pores after freeze-drying cause the loss of bilayer integrity in liposome leading to the irreversible cellular rupture and collapse of dense structure which induces the capillary effect dropped to a huge extent, so the water absorption is lower and rehydration is not wholly thorough.

5. Conclusion

Most of the freeze-dried material must be properly rehydrated for subsequent use. Rehydration is to retain the properties of the raw material, rehydration result is as well as an important basis in many studies for testing material damage degree on varying drying method. Rehydration is not caused by a single factor, but a rather complex water penetration and absorption phenomenon. In this experiment, we study the rehydration of pig aorta in a deep degree by Micro-CT scanning and weighing, clarifying the water diffusion, absorption and desorption mechanism and point out, under the normal temperature, freeze-dried pig aortic sucked into saline for 2-2.5h can be used for transplant. However, good or bad degree of rehydration depends on the integrity of cellular and structural, therefore, how to reduce freeze-dried destruction will another point of research.

6. References

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