Current vascular allograft procurement, cryopreservation and transplantation techniques in the Asan Medical Center Tissue Bank

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Received April 1, 2021
Revised May 2, 2021
Accepted May 10, 2021

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Vascular allografts are important materials to facilitate partial liver graft reconstruction in living donor liver transplantation (LDLT). Tissue banks are essential in providing vascular allografts used in LDLT. This study is intended to present the details of vascular allograft procurement, cryopreservation, and transplantation techniques, which are currently used in the Asan Medical Center Tissue Bank according to the standard operating procedure (SOP). Vascular allografts can be procured from the deceased organ donors or tissue donors. In practice, the majority of vessel donors are deceased multiorgan donors, thus the vessel grafts are harvested following multiorgan donation. The vascular allografts are cryopreserved according to the SOP and stored at -150°C in the vapor phase of liquid nitrogen. They can be kept for 10 years at the tissue bank. For clinical use, the cryopreserved vessel grafts are melted rapidly. At our institution, the majority of cryopreserved vascular allografts have been used for LDLT operations and some of them are also used for various hepatobiliary and vascular surgeries. In conclusion, the supply of vascular allografts through cryopreservation at the institutional tissue bank is an essential preparation to facilitate adult LDLT operations requiring various vascular reconstruction that use patches or conduits.

Keywords: Living donor liver transplantation; Tissue bank; Deceased donors; Cryopreservation; Vascular allograft

INTRODUCTION

Vascular allografts are important materials to facilitate partial liver graft reconstruction in living donor liver transplantation (LDLT). Vascular autografts from liver transplantation (LT) recipients themselves are very limited, including the greater saphenous vein, dilated paraumbilical vein and

enlarged portal veins. Vascular allografts harvested from deceased organ donors or tissue donors are the main source of vascular grafts. They include the iliac artery and vein, femoral artery and vein, inferior vena cava, aorta, greater saphenous vein, and other sizable arteries and veins. These vascular allografts are cryopreserved at the tissue banks [1,2].

Handling of human tissues has been strictly controlled by the Ministry of Food and Drug Safety (MFDS), Korea. Only licensed tissue bank personnel can be involved in handling human tissues by law. The Asan Medical Center (AMC) Tissue Bank has been maintained since March 2005 and has been audited annually by the MFDS. Because solid organ transplantation has been actively carried out at the AMC, its institutional tissue bank has been focused on vascular allografts that are necessary for LDLT operations.

This study is intended to present the details of vascular allograft procurement, cryopreservation and transplantation techniques, which are currently used in the AMC Tissue Bank according to the MFDS-approved standard operating procedure (SOP).

CANDIDATES FOR VASCULAR GRAFT PROCUREMENT

Vascular allografts can be procured from deceased organ donors or tissue donors. In deceased organ donors, the iliac artery and vein have been routinely procured as a part of the transplanted organs (liver, kidney and pancreas) because they are often necessary for graft implantation.

These vascular allografts cannot be preserved at the tissue bank unless a formal consent from the family members of the deceased donor is obtained before organ donation. If the family members agreed to tissue donation, the vessels procured during organ harvest can be preserved at the tissue bank. Unless the family members consent to additional incisions other than in the abdomen and chest, neither the greater saphenous vein nor femoral artery and vein can be harvested. Some deceased organ donors have been sent to public or commercial tissue banks for tissue donation after obtaining the corresponding consent from the family members, where various human tissues, such as bone, skin, vessels and other transplantable tissues, are harvested and processed.

Tissue donation is possible after cardiac death. To keep the human tissue fresh, the tissue should be procured within 24 hours if the body has been refrigerated within 4 hours after death. If the body has been refrigerated between 4 and 12 hours after death, the tissue should be procured within 12 hours. The time point of patient death for tissue donation after organ donation in deceased organ donors is the aorta cross-clamping time.

Fig. 1. Intraoperative photographs of harvest for iliac artery and vein allografts following multiorgan procurement. The iliac artery and vein stumps (A) are pulled out from the inguinal areas (B). These vessels are dissected within the pelvis and inguinal areas (C). The harvested vessels are placed into an aseptic container (D). The harvested vessels are placed into an aseptic container filled with cold preservation solution (D).

VASCULAR ALLOGRAFT PROCUREMENT TECHNIQUES

To meet SOP requirements with the aim in achieving the quality and safety of cryopreserved allografts, it is necessary to retrieve the blood vessels within multiorgan harvest [3]. The responsible person, an experienced trans-

plant surgeon, guarantees that all surgical procedures are performed according to the SOP. The usual surgical techniques for procurement of the iliac and femoral arteries and veins following multiorgan harvest are presented in a video clip (Fig. 1; Supplementary Video 1). As soon as the vascular allografts are procured, they are immersed in a 4°C histidine-tryptophan-ketoglutarate (HTK) solution con-

Fig. 2. Temperature curves of the programmed curve within the freezing chamber (red line) and actual temperature of the vascular graft specimen (blue line).

Fig. 3. Process of cryopreservation of the vascular allografts. (A) A vascular graft is trimmed in a clean bench. (B) The vessel is dipped into a dimethyl sulfoxide-RPMI solution. (C) The aseptic plastic bags are closed using heat sealing. (D) The packed vessels are put into the computer-controlled freezing chamber.

taining a cocktail of antibiotics (vancomycin, cefazolin, and gentamycin). These vessels are incubated for 24 hours in the refrigerator of 4°C.

CRYOPRESERVATION

Cryopreservation of the vascular allografts is performed according to the SOP. The grafts are trimmed in a clean bench in a class 100 clean room at the AMC tissue bank. The vessels are put into double sterile disposable plastic bags containing 20 mL of a pre-cooled 10% dimethyl sulfoxide (DMSO) solution (10% DMSO in 90% RPMI 1640 medium). DMSO is a cytoprotectant that reduces intracellular ice crystallization. RPMI 1640 is a cell-culture medium containing L-glutamine and sodium bicarbonate. The samples of the solution for bacteriological and mycological tests are taken from the collection solution and from the final package. The plastic bags are closed using heat sealing (Fig. 2). The bags packed into an outer paper package are put into the freezing chamber of the programmable freezer and

frozen at a rate of $-0.5^{\circ}\text{C}/\text{min}$ to -20°C and then $-1.35^{\circ}\text{C}/\text{min}$ to -70°C (Fig. 3). These cryopreservation processes are presented in a video clip (Supplementary Video 2). Until clinical use, the grafts are stored in the vapor phase of liquid nitrogen (-150°C) in the biological container equipped with an automatic filling system and continuous temperature monitoring.

QUALITY ASSESSMENT OF GRAFTS FOR CLINICAL APPLICATION

The vascular allografts should be passed the assessment for clinical use. If not passed, the graft should be abandoned. The assessment items are listed below:

- Absence of contraindication for harvest in the clinical and anatomical diagnoses and patient's medical history.
- Good quality of the harvested tissue reported by the responsible person.
- Absence of laboratory signs of infection as determined by the serology tests of the donor.

Fig. 4. Process of thawing the vascular allografts. (A) A packed vascular graft is taken out from the liquid nitrogen freezer. (B) The information at the outer package is identified. (C) A vessel in the plastic bag is melted in 40°C saline. (D) Dimethyl sulfoxide DMSO infiltrated in the vessel graft is removed with immersion into the serially diluted solutions.

Fig. 5. Clinical application of vascular allografts. (A) An iliac vein is prepared after thawing. (B) The graft middle hepatic vein branches of the right liver graft are reconstructed with an iliac vein allograft conduit. (C) The graft middle hepatic vein branches of the right liver graft are reconstructed with an iliac artery allograft conduit. (D) The right portal vein is reconstructed using an iliac vein allograft conduit after segmental resection of the portal vein in a patient with perihilar cholangiocarcinoma.

- Absence of contamination of recovered grafts by pathogenic bacteria, molds or fungi.
- Proof of sterility at the output control.
- Absence of serious deviations from the SOP during retrieval, transportation, processing, and storage of grafts.

THAWING

After delivery from the storage container, the outer package with the inner bags is rapidly transported to the operating room. In the operating room, the outer package is removed and the vessel-containing plastic bag is placed in 40°C saline for rapid melting of the ice. Immediately after the ice melts, the vessels are aseptically removed from the bags and DMSO is removed by immersing them in the serially diluted solutions (Fig. 4; Supplementary Video 3). Thereafter, the vascular allograft is stored in a pre-cooled HTK solution until implantation.

CLINICAL APPLICATION

At the AMC, the majority of cryopreserved vascular al-

lografts have been used for LDLT operations. Reconstruction of the middle hepatic vein branches at the right liver graft is the primary indication for vascular allografts. They have also been used for various hepatobiliary and vascular surgeries (Fig. 5).

DISCUSSION

All procedures for vascular tissue procurement and processing complied with Korean legislation and conformed to the ethical and safety concerns for therapeutic use. All activities at the tissue bank are described in the SOP, which is approved by the MFDS. Any change in the institutional SOP should be approved by the MFDS. All tissue banks over the world have their own SOP [1,2]. The AMC Tissue Bank has undergone audit annually by the MFDS since 2005.

The quality of cryopreserved vascular allograft is important because it can be closely associated with luminal patency and vascular complications. It is reported that the advantages of cryopreserved allografts include the low probability of infection transmission and low immunogenicity leading to limited cellular and humoral rejection [3].

However, it is also suggested that certain cryopreservation protocols are responsible for early ruptures of grafts that may occur even intraoperatively and are always associated with life-threatening complications [4]. It seems, however, that such serious adverse events are less likely to occur if cryopreservation protocols based on equilibrium and slow freezing are used.

It should be considered that not only the freezing protocol itself, but also the pre-freezing history of the graft may be responsible for adverse vascular complications. A study with dogs showed that hypothermic storage of vessels in physiological saline for several days can lead to considerable vessel wall edema [5]. This finding shows the enhanced probability of vessel wall injury caused by crystal formation during freezing. For this reason, it appears to be important to use organ preservation solutions such as HTK solution for intermediate hypothermic storage immediately after vascular graft harvest and to strictly control the time-span between the graft harvest and start of the cryopreservation procedure [6].

In an *in vitro* study with saphenous vein samples observed up to 35 days [7], in terms of preserving the morphology of saphenous veins, phosphate-buffered saline and isotonic saline with heparin and antibiotic were the poorest, whereas HTK solution and glutaraldehyde solution were the best. Demonstrating good retention of endothelial nitric oxide synthase staining throughout the study period, isotonic saline with heparin and antibiotic seems to have the best potential to retain vein wall functionality, despite relatively poor morphological preservation.

Achieving relatively stable ice structures by slow freezing does not guarantee complete avoidance of devitrification phenomena during thawing [8], in which the formation of microfractures in arteries caused by devitrification during fast thawing was responsible for graft rupture. This finding suggests adopting the implementation of a slow-thawing protocol. In contrast, we have used a rapid-thawing protocol because we do not use the cryopreserved artery allografts for high-pressure large arterial reconstruction. In practice, we have the cryopreserved artery allografts as substitutes for venous allografts.

Long-term storage of cardiovascular grafts up to 10 years in the tissue bank was introduced [9-11]. We think that the quality of cryopreserved vascular allografts can be well maintained for at least 5 years. The storage period of vascular allografts in the AMC Tissue Bank is usually less than 1 year because of marked supply shortages from high-volume LDLT operations.

Although there are some studies favoring the use of cryopreserved vessels in LDLT operations [12-14], we have paid special attention to using cryopreserved vein allografts for portal vein or inferior vena cava interposition, since long-term patency of such vascular reconstruction has not been fully evaluated yet. In a study on portal vein reconstruction in adult LDLT using cryopreserved vein grafts [15], their 5-year primary and secondary patency rates were as low as 58% and 79%, respectively.

One of the roles of the AMC Tissue Bank is temporary storage of the vascular allografts imported from the Korea Public Tissue Bank because we need more allografts to meet the demand for LDLT. Currently, cryopreserved femoral vein and artery allografts are commercially available through the Korea Public Tissue Bank.

Our institutional tissue bank is a very useful facility for performing LDLT operations. However, its maintenance requires high upkeep expenses, more than \$200,000 USD every year. If the supply of vascular allografts from other domestic tissue banks including the Korea Public Tissue Bank is available, it must be much more cost-effective to import vessel allografts than maintaining an institutional tissue bank. Currently, the import of vascular allografts from other countries is not practical, primarily because of very high costs for international delivery.

In conclusion, the supply of vascular allografts through cryopreservation at the institutional tissue bank is an essential preparation to facilitate adult LDLT operations requiring various vascular reconstructions using patches or conduits.

SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at <https://doi.org/10.52604/alt.21.0016>.

FUNDING

There was no funding related to this study.

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare.

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AUTHORS' CONTRIBUTIONS

Conceptualization: SH. Data curation: JHB, IOK, JJH.
 Methodology: JHB. Visualization: SH. Writing - original draft: SH. Writing - review & editing: All.

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