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Skin Graft Preservation

Liangpeng Ge¹, Zhenggen Huang² and Hong Wei³

¹Chongqing Academy of Animal Sciences
²Northern Hospital
³Third Military Medical University
China

1. Introduction

Skin transplants have long been of interest to plastic surgeons for the treatment of burns and other wounds. Skin transplants provide a better micro-environment for wound healing and provide physiological barrier which greatly decreases water, electrolyte, protein and heat loss through the wound. However, skin grafts are not always available in adequate quantities for the complete duration of treatment, making skin graft preservation important in meeting the need for skin transplantation.

This chapter highlights progress in the field of skin graft preservation including basic knowledge of skin preservation, harvesting, processing and preservation, and quality control methods and is intended to provide a reference for skin preservation techniques for doctors and researchers.

2. Thermal physics of skin low-temperature preservation

The low-temperature preservation with viable cells and tissues is a complicated procedure, which needs to undergo multiple processes including pretreatment, freezing, preservation under low temperature and thawing in proper order. In these processes, there is unavoidably evident change in the functions and morphology of the tissues. An ideal preservation procedure should be that viable cells and tissues can restore to the original functions and morphology prior to preservation after undergoing all of these processes and then be used for grafting and other purposes like fresh cells and tissue. So far, the effect of low-temperature preservation does not meet the above ideal conditions. Also, doctors and researchers are not fully aware of the change in viable tissue in the preservation processes, especially in the processes of freezing and thawing.

Water occupies a considerable proportion of viable cells and tissues. The moisture content of skin is about 70%. The quality of skin is closely related to the phase transition of water during low-temperature preservation, which including the solidification in freezing, ebullition in freezing of viable tissue using liquid nitrogen, thawing during freezing and so on.

Water or water vapor can generate more than 10 kinds of solid phase structure, nevertheless, most of them need to be formed under high pressure environment (at least 200 million Pascal (Mpa)), and only hexagonal ice, cube ice or amorphous ice can be
generated under low pressure. Water vapor deposits on a cold surface within the temperature range from 0°C slightly below to about -80°C, or forms a hexagonal symmetric ice crystal in a suspended state in the air, snow is a typical case in point, and cubic crystal with diamond structure will be generated under lower temperature (about -80°C~130°C) and amorphous solid (also called glassy, vitrification) will be formed below -140°C. Liquid water usually only forms a hexagonal symmetric ice crystal upon freezing, however, solutes-bearing water solution more easily realizes vitrification upon freezing compared with pure water. Therefore, some components shall be added before freezing, in order to promote the suspension of cells or tissues to more easily reach vitrification (Zhu et al., 2002).

It is generally believed that there are two kinds of cell damaged in the freezing process: one is intracellular ice crystals damage and the other is solution damage. Ice crystals cause the change of cell wall and ultra-structures in the cells. Even death can occur by intracellular ice crystals growing to a certain degree during freezing. This damage is more serious in the case of quicker freezing. Solution damage causes cell damage by extracellular high-concentration solution because water freezes outside of the cells. The longer cells stay in high-concentration solution, the more serious the solution damage is. For certain types of cells and freezing processes, ice crystals and solution damage exist simultaneously; nevertheless, solution damage dominates in the case of slow cooling and intracellular ice damage dominates in the case of quick cooling. And the solution damage and intracellular ice damage shall be avoided or reduce if ice crystals are avoided (e.g. amorphous or glassy solid are obtained), or ice crystals are formed but fail to grow to change the structure of cells (e.g. partly vitrification) in case of snap freezing.

There are two different protocols used in skin low-temperature preservation: slow freezing and quick freezing/snap frozen. Slow freezing reduces the temperature of the skin treated with antifreeze fluid from about 4 to -60°C at preset rate (e.g. 1-3°C/min) and balanced for 4h using a controlled-rate cooling apparatus, which adopts liquid nitrogen gas to absorb the heat of specimen mainly by convective heat transfer, and then put skin into liquid nitrogen for preservation. It is worthy of note that, the moisture contained in those tissues with greater mass will releases a large amount of latent heat in a cold room at near 0°C, however, liquid nitrogen gas can’t rapidly absorb the latent heat of water phase transition due to lower convective heat transfer coefficient, so it makes a probable great difference between the displayed temperature by a controlled-rate cooling apparatus and the actual temperature in the skin tissues, and it is difficult to reduce this temperature difference by increasing cold gas volume. In case of snap frozen, the skin is incubated with antifreeze solution and then transferred directly to liquid nitrogen, and the temperature can be cooled from >0°C to liquid nitrogen temperature within 30s. Being simple, snap frozen eliminates special instrument and reduces cost. However, liquid nitrogen may generate grave ebullition during snap freezing, and the big temperature difference between liquid nitrogen and skin can not be fully utilized at the initial stage of freezing, thus, “thermo-zone platform” (as shown in Fig.1) will be form near zero degree. To avoid this defect, on one hand, antifreeze fluid needs to be optimized, on the other hand, a copper plate pre-cooled with liquid nitrogen shall be used for directly contacting skin graft for snap frozen using heat conduction style. in order to obtain better cooling effect (as shown in Fig.2).

At present, there is inadequate researche on the thawing process of frozen skin. Most standards advocate rapid warming of the skin to optimise viability. This can be achieved by
3. Skin harvesting and processing

Processing methods used for banking of skin for subsequent therapeutic use depend on whether the skin is to retain viability or not. For viable skin grafts, sterilisation techniques cannot be applied, however antibiotics and antifungals may be used to disinfect the tissue with respect to bacteria and fungi (Kearney, 2005; Li, 2001; Ninnmam et al., 1978).
3.1 Screening of donor

One of the major concerns with the use of skin grafts from donors is the risk of disease transmission. A case involving the transmission of HIV from a skin donor to a recipient has been reported (Clark, 1987). To reduce the risk of disease transmission, skin donors should be screened before skin harvesting. If skin grafts obtained from cadaveric donors, medical records held by hospitals, family doctors, and others must be screened for conditions that would exclude donation (Delmonico, 1998). This may include current systemic infection, diseases of unknown etiology, and previous infections with the possibility of current carrier status (hepatitis, malaria, HIV, syphilis antibodies etc). It may also be prudent to exclude a history of malignant disease particularly if the skin might be used on immunosuppressed patients, for example, those suffering massive burn injuries. Behavioral history may also be grounds for rejection of the donor owing to the high correlations between activities such as intravenous drug abuse and viral infections such as HIV and hepatitis.

3.2 Reducing the population of microorganism

Skin tissue possesses a mixed commensal population of bacteria and fungi that lives primarily in or around the hair and sebaceous follicles (Kearney et al., 1984). Even the commensal bacteria may damage the skin if subsequently allowed to grow during the skin storage period to reach massive population densities. Therefore, it is important to try to eliminate or minimize the microbial population of skin before retrieval. For viable skin allografts, it is not possible to apply a sterilization technique because sterilization methods tend to inactivate cells at an equal or greater rate than for the bacterial cells. The best that can be achieved is a combination of serology screening for viruses and bacterial disinfection using antibiotic cocktails (Holder et al., 1998). This is generally achieved using skin prepping techniques similar to those used on the skin of patients undergoing operations in hospital. When banking skin in a viable state, it is important to validate that the prepping agents have been adequately removed before harvesting the skin, and that any residuals are not toxic to the skin cells. Although effective in reducing the bioburden, skin prepping is unlikely to totally eliminate bacteria. It is therefore essential to prevent proliferation of any residual bacteria after retrieval, during transportation, and during subsequent processing. Maintaining the skin in a refrigerator or at wet ice temperatures prevents the proliferation of most bacteria. To eliminate residual bacteria and fungi in viable skin, an antibiotic/antimycotic cocktail may be used. If used at reduced temperatures (4°C), the skin may be incubated for up to 24 hours in the cocktail; however, only antibiotics that are effective at this temperature should be included. At normothermic temperature (37°C), most antibiotics can be considered; however, the exposure period should be minimized.

For nonviable grafts, a range of sterilization techniques can be considered. Dry heat and autoclaving damage the structure of the skin tissue, including denaturation of collagen, and hence are not suitable. Techniques that have been successfully used include ethylene oxide gas and γ irradiation; however, concerns and limitations of these methods are beginning to appear. Ethylene oxide gas and its reaction product with chloride—ethylene chlorohydrin—are very toxic. Although acceptable levels for these compounds had been proposed, more recent data suggest that there is no safe level of ethylene oxide with respect to genotoxicity. Questions have also been raised about the effectiveness of γ irradiation. Many small viruses and spore-forming bacteria are fairly resistant to γ irradiation. The high doses that would be required to inactivate HIV in a window-period donor (more than 80 kGy) would cause
extensive damage to the tissue matrix including collagen denaturation. Therefore, a current consideration is whether “sterilization” can be achieved instead by using combinations of microbial inactivation procedures (Kearney, 1989; 2005; Prolo et al., 1980).

3.3 Time control
Viable skin grafts may be obtained from living donors or, more frequently, from cadaveric donors. In the latter case, the skin becomes ischemic immediately after circulatory arrest. This leads to progressive cell death over a period of days, which is accelerated at higher temperatures. Even if the cadaver is immediately placed into a refrigerator, the body cools at a slow rate, leading to at least some warm ischemia time. Nevertheless, viable skin can still be retrieved up to circa 24 hours after death. If the body is not quickly refrigerated, the skin will be subjected to a much longer warm ischemia period resulting in an accelerated deterioration in viability. Once the skin is removed from the body, it can be cooled rapidly by immersion into a refrigerated solution, thus immediately reducing the rate of cell death. In the other hand, tissue banking standards generally set limits on the maximum warm and cold ischemia times permitted after the death of the donor, because it is known that bacteria from the gut can be released into the vascular system postmortem and migrate to the internal organs and tissues (Kearney, 2005).

3.4 Sample of preparation skin graft from cadaveric donors
3.4.1 Donor selection
Donor skin procurement must comply with local legislation. To ensure skin grafts quality, donors were aged between 8–60 years. The exclusion criteria for harvesting skin are basically the same as for donors of parenchymatous organs. Further exclusion criteria comprise skin diseases, injuries in the areas from which skin may be harvested, and blood samples were tested for HIV, HbA, HbB, HbC and syphilis antibodies.

3.4.2 Skin harvesting
Skin is cleaned with povidone-iodine 10% solution and skin with adipose layer harvested with a knife. Areas from which skin is harvested are limited to the following regions: ventral and dorsal trunk, left and right upper arm, and left and right thigh. Immediately after the removal, grafts were placed in saline, refrigerated and transferred to the processing room. The hair was removed and skin was soaked in 0.1% benzalkonium bromide for 15 min to sterilize. Split skin of 0.2–0.3mm was harvested under sterile conditions by a dermatome. Thereafter split skin was washed extensively and soaked for 10 min 3 times in saline with Penicillin/Streptomycin; then in Neomycin–saline (0.5%) for 10 min, 3 times. All the following procedures are carried out under sterile conditions.

3.5 Sample of preparation skin graft from porcine
Domestic pigs were sacrificed and the skin was washed extensively with soap and water. The hair was removed by razor and the carcass was cleaned with povidone-iodine 10% solution. After cleaning, 0.3 mm to 0.6 mm partial-thickness skin from the pig`s back was harvested with a dermatome. Immediately after the removal, the harvested skins were placed into sterile boxes in normal saline supplemented with penicillin/streptomycin and sent to the laboratory, then soaked in 0.1% benzalkonium bromide for 15 min to sterilise and rinsed thrice in normal saline supplemented with penicillin/streptomycin in a laminar flow hood (Chiu&Burd, 2005).
4. Viable skin grafts preservation

Fresh cadaver allograft is still considered the ‘gold standard’ biologic dressing for closure of burns. Unfortunately, the use of fresh allografts is severely impeded by their inadequate availability. Skin graft preservation for the purpose of delayed application is still a basic tool in burn treatment and plastic and reconstructive surgery. Even if the skin is placed into an oxygenated nutrientrich medium, ischemic necrosis of the tissue still occurs because the diffusion path from the tissue periphery to the central cells is extensive, oxygen and nutrients cannot diffuse fast enough to supply the cells, and toxic metabolites cannot be removed quickly enough. Therefore, method for the viable storage of skin is to reduce the temperature hence reduces the metabolic rate of the cells and the nutritional demands and metabolite production (Bravo et al., 2000; Ge et al., 2010; Robb et al., 2001; Sterne et al., 2000).

4.1 Storage in 4°C

As early as 1903, Wentscher reported the successful storage and grafting of skin autografts at temperatures near 0°C for 14 days (Wentscher, 1903). Further studies by Carrel using normothermic and hypothermic storage conditions confirmed the utility of the latter (Carrel, 1912). Eventually, refrigerator storage for skin autografts became the norm in burn units. The simplest technique was to fold the skin so that the cut surfaces were in apposition and then wrap the skin sandwich in tulle gras and/or saline gauze to prevent desiccation. Using this method, autologous skin can be used after around 2 weeks of storage, although cell viability is very low at this point. Unlike autograft, allograft has already lost some viability by the time it is retrieved; therefore, maximum storage times using this method are reduced to 7 or 8 days. Many attempts have been made to extend the refrigerator storage period for skin. The addition of homologous serum at 10% to 33% was found to be beneficial because it provided nutrients and diluted and buffered acids produced as byproducts of metabolism (Allgower & Blocker, 1952). Alternatively, tissue culture media as a source of nutrients and various buffering systems have been evaluated. The buffering systems, however, only extend slightly the period of useful storage. Different methods for storing skin grafts around 4°C are shown in Table 1 and show a large variance in storage time although the storage methods are similar. The main reason for this variance may be criteria incompatible and the absence of reliable detection methods of early research especially before 1970. The storage of skin grafts in a 4°C refrigerator is a simple and practicable technique; however, the skin showed a very slow viability decrease with time stored at 4°C (Chang et al., 1998; DeBono et al., 1998; DeLW, 1980; Matsuka et al., 1993; May & Wainwright, 1985). The preservation time of fresh skin should not exceed 3 days when stored in normal saline and must be used within two weeks even when stored in tissue culture media with 10% serum.

4.2 Storage in -20°C

Wang has success develop non-freeze complex cryoprotectants( 10% DMSO, 10% propylene glycol. 10% PEG. 10% glycerin) to avoid ice crystal formation when skin stored in -20°C, the skin remain 76% activity in 14th day, higher than that of stored at liquid nitrogen (60-70%) , then skin activity lower than that of stored at liquid nitrogen in the following time, but still remain 50% viability(the ultimate rate to survival post-transplantation) in 60 days, so skin grafts stored at -20°C should be first consider if skin will be used within 2 weeks. (Wang et al., 2002).
Skin Graft Preservation

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Category</th>
<th>Stored method</th>
<th>Stored time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1903</td>
<td>Wentsch</td>
<td>Human</td>
<td>Exsiccatus ice-box</td>
<td>3-14d</td>
</tr>
<tr>
<td>1912</td>
<td>Carrel</td>
<td>chicken</td>
<td>0°C, normal saline with serum, paraffin oil</td>
<td>Several weeks</td>
</tr>
<tr>
<td>1912</td>
<td>Carrel</td>
<td>Dog</td>
<td>0°C, normal saline · blood</td>
<td>Several months</td>
</tr>
<tr>
<td>1922</td>
<td>Filatov</td>
<td>Human</td>
<td>0°C</td>
<td>Several days</td>
</tr>
<tr>
<td>1945</td>
<td>Matthews</td>
<td>Human</td>
<td>3-6°C · normal saline · oxygen deficiency</td>
<td>3-8 weeks</td>
</tr>
<tr>
<td>1949</td>
<td>Hanks · Wallace</td>
<td>Rabbit</td>
<td>0-8°C · 10% serum</td>
<td>2 weeks</td>
</tr>
<tr>
<td>1952</td>
<td>Allgower · Blocker</td>
<td>Human</td>
<td>5°C, 10% serum</td>
<td>14 days</td>
</tr>
<tr>
<td>1952</td>
<td>Hyatt</td>
<td>Human</td>
<td>4°C, 10% serum</td>
<td>185 days</td>
</tr>
<tr>
<td>1954</td>
<td>Skoog</td>
<td>Rat</td>
<td>3°C</td>
<td>3 weeks</td>
</tr>
<tr>
<td>1957</td>
<td>Perry</td>
<td>Human</td>
<td>4°C · Earle medium · 10% serum</td>
<td>84 days</td>
</tr>
<tr>
<td>1963</td>
<td>Grasham</td>
<td>Human</td>
<td>4°C · 10% serum</td>
<td>6-8 weeks</td>
</tr>
<tr>
<td>1971</td>
<td>Bondoc · Burke</td>
<td>Human</td>
<td>4°C · serum</td>
<td>14 days</td>
</tr>
<tr>
<td>1972</td>
<td>Lawrene</td>
<td>Human</td>
<td>4°C · normal saline</td>
<td>1 week</td>
</tr>
<tr>
<td>1985</td>
<td>May · Wainwright</td>
<td>Pig</td>
<td>4°C · Earle medium</td>
<td>1 week</td>
</tr>
<tr>
<td>2002</td>
<td>Alotto</td>
<td>Human</td>
<td>4°C · RPMI 1640, 1% human serum albumin</td>
<td>72 hours</td>
</tr>
<tr>
<td>2003</td>
<td>Castagnoli</td>
<td>Human</td>
<td>4°C · RPMI 1640, 1% human serum albumin</td>
<td>4 days</td>
</tr>
<tr>
<td>2010</td>
<td>Ge</td>
<td>Pig</td>
<td>4°C · normal saline or DMEM</td>
<td>72/96hours</td>
</tr>
</tbody>
</table>

Table 1. Effect of skin stored around 4°C

4.3 Deep freezing

Deep freezing is preservation of skin grafts in ultra-low temperature refrigerator, which generally maintain temperature under -80°C, and now -150°C ultra-low temperature refrigerator has been made in China. The advantage of this method is simplicity of operator, low maintain cost and relative long preservation time (not exceed 1 year) (May et al., 1985, 1988).

To achieve high viability of skin grafts, cryoprotective agents (CPAs) should be added before freezing. The general properties of cryoprotectants are that the molecules can pass through the cell membrane into the cell and be relatively nontoxic at very high multimolar concentrations. Several cryoprotective chemicals have been identified. CPAs are glycerol and dimethyl sulfoxide. Cryoprotective agents are able to prevent solution effect injury. CPAs mode of action is probably 2-fold. First, they act as solvents for the salt, thus reducing the salt concentration that the cells are subjected to at the high subzero temperatures (where solution effects are most damaging). Secondly, the presence of CPAs within the cells prevent excessive shrinkage of the cells during this cooling phase. Therefore, in the presence of CPAs, it is possible to use very slow cooling rates that minimize intracellular ice formation while protecting the cells against solution effects. High viabilities of all cell types can thus be achieved using this slow cooling rate.

Although CPAs are relatively nontoxic at low temperatures, the toxicity can become significant at higher temperatures. In addition, the rate at which CPAs enter the cells is temperature and CPA dependent, being faster at higher temperatures. Therefore, the
optimum temperature and the exposure time need to be validated. The basal medium into which the CPA is dissolved for incubation of the skin tissue should be a balanced salt solution, in which the zwitterionic buffers hydroxyethyl piperazine ethanesulfonic acid (HEPES) and trimethylamino-ethanesulfonic acid (TES) have been shown to work well (Basaran et al., 2006). To avoid osmotic lysis of the cells, either the saline can be added gradually or an impermeant solute such as sucrose can be added to the saline to reduce the difference in osmolality.

4.4 Cryopreservation

A better method for the long-term preservation of skin grafts is cryopreservation. The cryopreservation technique facilitates the cooling of the tissues to ultralow temperatures while protecting the viability of the cells (Aggarwal et al., 1985; Bondox & Burke, 1971; Cui et al., 2007; Fujita et al., 2000; Marrel et al., 1986). Once the skin is at a temperature lower than -130°C, no further loss of cell viability is incurred. The boiling point of nitrogen gas is -196°C, so skins immersed into liquid nitrogen should be able to keep viably indefinitely in abstracto.

There are two different cryopreservation protocols used in skin preservation: slow freezing and quick freezing/snap frozen. Slow freezing can be achieved using a controlled-rate cooling apparatus (Blondet et al., 1982). There is an optimum cooling rate for any cell type that produces maximum cell survival. On either side of this optimum, the survival rate falls. In the presence of CPA, a cooling rate of -1 °C per minute will ensure survival of most of the cells within skin tissue. As the cooling rate is increased, cell populations are sequentially and adversely affected. Many cells in the body derived from leukocytes or closely related lineages are known to be exquisitely sensitive to cryogenic injury. The depletion of immunostimulatory “passenger leukocytes” was demonstrated by increasing the cooling rate for pancreatic islets of Langerhans while maintaining the viability of the insulin-producing islet cells (Ingham et al., 1993). This concept of cooling rate–dependent immunomodulation was evaluated for skin tissue. A cooling rate of -30°C/min was shown to maintain the viability of keratinocytes and fibroblasts while reducing the immunogenicity (as assessed by the mixed epidermal cell/lymphocyte response assay) of murine allografts by 95%. This was assumed to be due to an effect of the faster cooling rate on the major immunostimulatory cell in the skin—the Langerhans cell.

Quick freezing/snap frozen is skin vitrification technique. Vitrification is defined as “the instant solidification of a solution brought about by an extreme elevation in viscosity during cooling, without ice crystal formation”. In other words, vitrification is faster and lacks some of the typical disadvantages seen in traditional slow freezing (Mukaida, 2003). It bypasses the ice-crystal formation phase and instantaneously solidifies into a glass-like structure, moves the water directly into a glass-like phase. In a glass, the molecules do not rearrange themselves into grainy ice crystals as the solution cools, but instead become locked together while still randomly arranged as in a fluid, forming a “solid liquid” as the temperature falls below the glass transition temperature (Silvestre et al., 2002). This technique was simple, and no expensive equipment was needed. Initially, this method needed a 2-step procedure because the cryoprotective agent was toxic. Kasai et al., (1990) modified this impractical to a 1-step method and incubated the skin with vitrification solution at room temperature and was transferred directly into liquid nitrogen.
Ben-Bassat et al., (1996, 2001) evaluated the graft performance of cryopreserved cadaveric skin by programmed freezing (1°C/min). The results demonstrate that graft performance of cryopreserved skin decreased with time as seen in the lower percent of samples with high scores of separate histologic criteria after prolonged storage (Ben-Bassat et al., 1996). Nevertheless, paired comparison analysis between cryopreserved and fresh skin indicated that this decrease was not significant for storage of 5 years; however, it was highly significant for 6 years of storage (Ben-Bassat et al., 2001).

Table 2 shows the distinction of different preservation methods and viability of skin frozen at -20°C attenuated to 50% (the ultimate rate to survival post-transplantation) in 60 days. Skin grafts stored at -20°C should be used within two months. To be similar, the skins should be transplanted in one year of freezing in -80°C and 5 years in liquid nitrogen. Viability declines rapidly after thawing of the skin and further storage before use cannot be recommended.

Table 2. Comparison of different preservation methods

<table>
<thead>
<tr>
<th>Temperature(°C)</th>
<th>Common refrigerator</th>
<th>Freezer ultra-low temperature refrigerator</th>
<th>Liquid nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature(°C)</td>
<td>4</td>
<td>-18~20</td>
<td>-80</td>
</tr>
<tr>
<td>Equipment cost</td>
<td>Cheap</td>
<td>Cheap</td>
<td>Costly</td>
</tr>
<tr>
<td>Sustain cost</td>
<td>Cheap</td>
<td>Cheap</td>
<td>Cheap</td>
</tr>
<tr>
<td>Electricity effect</td>
<td>Great</td>
<td>Great</td>
<td>Great</td>
</tr>
<tr>
<td>Storage time</td>
<td>3~7days</td>
<td>60days</td>
<td>1 year</td>
</tr>
<tr>
<td>Viability ( % )</td>
<td>30~80</td>
<td>50~60</td>
<td>50~60</td>
</tr>
</tbody>
</table>

Table 2. Comparison of different preservation methods

5. Nonviable skin grafts preservation

With non-viable skin (or dermis) there is no requirement to maintain the viability of the skin cells. Nevertheless, it is still important that degradative changes do not adversely affect the tissue matrix and bacteria are not allowed to proliferate; therefore, many of the practices discussed earlier will still apply. A major difference from viable skin, however, is that much more effective disinfection or sterilisation methods can be applied to the skin, including virucidal treatments.

5.1 Freeze drying

In the early 1950s, the US Navy Tissue Bank was the first to commence large-scale freeze drying of human tissues for implantation. In 1955, the use of freeze-dried skin was first reported (Brown et al., 1955). It was subsequently shown that freeze drying reduced the immunogenicity of skin without interfering with its beneficial properties (Abbott & Hembree, 1970). This process involves the removal of water from skin in the frozen state by sublimation, which is achieved by applying a vacuum to the tissue and condensing the removed water molecules downstream. Sublimation from the frozen state helps to protect molecules that would otherwise be adversely affected by high salt concentrations at higher temperatures, for example, denaturation of proteins. Drying must continue until enough water has been removed to prevent degradation reactions, which equates to less than 5% residual water as measured gravimetrically. Although accepted in most tissue banking standards, “residual water” is not identical to “water activity”, which is the most appropriate measure.
5.2 Glycerolization preservation
In 1980s, glycerin was applied in clinical practice as skin graft preservation fluid, and had very good effect (Hermans, 1989; Huang et al., 2004; Richter et al., 1997; Van Baare et al., 1994,1998; Vuola & Pipping, 2002). EuroSkin Bank has done considerable fruitful work in the area and developed a solute preservation method using glycerol (De Backere & Mackie, 1997; Mackie, 1997). The skin was incubated in successively more concentrated glycerol solutions (50%, 70%, and 85%) and maintained long term in 85% glycerol. More recently, the glycerol and water flux kinetics have been characterized and a more efficient validated protocol was proposed. Study demonstrated that the 85% concentration originally chosen by the Euroskin Bank is optimal for minimizing degradative reactions (Ross & Kearney, 2004). It is very important that the glycerol is removed from the skin before clinical use. Failure to remove glycerol may lead to high systemic concentrations when used on open wounds. This is achieved by repeatedly washing the skin in physiological saline, and at least 30 to 60 minutes are required to remove most of the glycerol.

5.3 Glutaraldehyde preservation
As early as 1975, Schettnter reported the successful grafting of glutaraldehyde- preserved skin in clinical (Schettnter, 1975). The morphology of skin grafts has not evident change after treated with glutaraldehyde, and skin antigenicity was greatly reduced because antigen determinant site was closed by cross-linking of protein in the skin grafts. Glutaraldehyde-treated skin grafts can be used for escharectomy and tangential excision wounds and granulation wound covering, and has been shown to promote the formation of granulation tissues. However, such skin grafts have least elasticity, that is, with the extension of preservation duration, the skin becomes harder. Fresh allograft skin or lyophilized skin shall be immersed in 0.5% glutaraldehyde solution for 7-20min and be washed with normal saline for three times prior to use. There is no evident change in the elasticity and softness of such skin, but its antigenicity is decreased, which helps to extend the adhesion duration of grafted skin.

5.4 Irradiation preservation
Irradiated porcine skin is the most commonly used burn wound covering in China. Fresh porcine skin graft is sealed in a plastic bag and is irradiated with 60Co or an accelerator, then put into an ordinary refrigerator for preservation. After being irradiated, porcine skin can achieve integral asepsis, and its antigenicity can be decreased. For gamma irradiation, the most resistant microorganisms are viruses, D10-values as high as 13 KGy have been reported for certain small viruses. Application of the commonest used dosage (25 KGy) to these viruses would only reduce the bioburden by 2 logs10. The high doses that would be required to inactivate HIV in a window-period donor (more than 80 kGy) would cause extensive damage to the tissue matrix including collagen denaturation. There are two possible effects on the macromolecular structure of skin matrix resulting from ionising irradiation. The first is scission which results in the breaking of bonds thus weakening the matrix. In the presence of water a second process involves crosslinking by new bond formation resulting from the generation of free radicals which may lead to stiffening of the matrix.

6. Quality control
A variety of national/international organisations have issued standards or guidelines for tissue banking, including the National Blood Service, British Association for Tissue Banking,
and European Association of Tissue Banks, etc (Baxter, 1985; Ben-Bassat et al., 2000; British Association for Tissue Banking, 2011; Chua et al., 2004; European Association of Tissue Banks, 2011; Freedlander et al., 1998; Janezic, 1999; Kalter, 1997; Kearney, 1998; May, 1990; Pillipp et al., 2004; Pianigiani et al., 2005). The core principle of skin banking quality control is to avoid the risk of cross infection and provide high-quality skin grafts for clinical. Therefore, donor selection, microbial/viral tests, skin viability detection and transplantation performance will be discussed in this paper.

6.1 Donor selection

With respect to medical/behavioural history screening, the regulatory and standards documents are once again very specific with a good deal of international consensus. Sources of medical/behavioural information include the next of kin (or partner/close friend) of the deceased who must be interviewed regarding medical history and any factors, practices or behaviour that may have increased the risk of exposure to HIV/Hepatitis. In addition, the family doctor, hospital records, and autopsy report should be consulted as additional sources of medical and behavioural history. Evaluation of the risk of virus exposure must be explored in detail with other aspects of medical and behavioural history. The exclusion criteria for harvesting skin are basically the same as for donors of parenchymatous organs including cancer, diseases of unknown aetiology and various viral, bacterial and parasitic diseases. Further exclusion criteria comprise other skin diseases and injuries in the areas from which skin may be harvested.

6.2 Microbial/viral tests

There is a widespread consensus on the testing of donors to preclude certain virological diseases, including HIV, Hepatitis B, Hepatitis C, syphilis antibodies, and in general these follow the National Blood Service testing regimens for each country. Screening for CMV is important when recipients are CMV negative and will receive immunosuppressive drugs following organ transplantation. However, the natural immunosuppression associated with burns does not appear to lead to CMV related problems in skin allograft recipients. Many tissue banks do not screen for CMV. The microbial tests are generally done on allograft samples and the viral tests on blood samples. Skin samples for microbial tests are incubated in Thioglycollate and Roswell Park Memorial Institute (RPMI) 1640 without antibiotics, in a 37°C incubator for 10 days.

6.3 Skin viability

The viability of graft skin is fundamental for surgical procedures involving graft taking, and the American Association of Tissue Banks indicates that the viability of skin is an essential prerequisite for good-quality grafts (American Association of Tissue Banks, 2011). It is a necessity that a skin bank should certify, not only the sterility, but also the viability of the skin allografts used and that routine quality controls be carried out to certify that grafts are viable before transplantation. The of viability assays is two-fold: one is that of supplying an “experimental assay”: used to optimize the various parts of a preservation procedure during experimental development. The other offers a “predictive assay”: used to predict the “quality” of an individual sample that has been stored by an established tested technique. Various methods, both qualitative and quantitative, are currently used to determine skin grafts viability such as cell culture, succinate dehydrogenase, trypan blue dye, skin oxygen
consumption, Tetrazolium salts (MTT/WST-1), SYTO/EB dyes and so on (Alotto et al., 2002). The MTT method is most widely used because it is simple, quick and precise (Castagnoli et al., 2003; Klein, 1996; Pegg, 1989; Yang et al., 2000; Zieger et al., 1993).

6.4 Transplantation performance
Skin transplantation experiment is a straightforward and reliable skin graft quality evaluation method. For any skin graft and preservation method, the final result is to identify that the skin graft can survive in the living body. For evaluation of transplantation performance of preserved skin, samples of skin were often grafted onto mice and rats and the experiment animals were sacrificed after 4 or 7 days. The grafts were then assessed by gross inspection including adherence to wound bed, color and pliability and by histologic evaluation including epidermis integrity, epidermal–dermal junction, collagen organization, the presence of fibroblasts and graft adherence (Cinamon et al., 1993).

7. Conclusions
Skin graft preservation for the purpose of delayed application is still a basic tool in burn treatment and plastic and reconstructive surgery. It is therefore important to provide a framework for selecting optimal guidelines for procurement, processing, preservation, and quality control of skin grafts for doctors and researchers, thus ensuring high levels of safety and efficacy of skin grafts.

8. References

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The procedure of skin grafting has been performed since 3000BC and with the aid of modern technology has evolved through the years. While the development of new techniques and devices has significantly improved the functional as well as the aesthetic results from skin grafting, the fundamentals of skin grafting have remained the same, a healthy vascular granulating wound bed free of infection. Adherence to the recipient bed is the most important factor in skin graft survival and research continues introducing new techniques that promote this process. Biological and synthetic skin substitutes have also provided better treatment options as well as HLA tissue typing and the use of growth factors. Even today, skin grafts remain the most common and least invasive procedure for the closure of soft tissue defects but the quest for perfection continues.

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