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Use of cadaver skin in the treatment of wounds

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The article has reviewed the world experience and main trends in the preparation of cadaveric skin for use in the treatment of patients with wounds of various etiologies. The history of the question is described from the first attempts of transplantation of the native skin to the creation of specialized banks of allogenic decellularized tissue grafts. Presented are the modern approaches of donor material conservation, specifically, to the principle and topical question: whether the viability of the cells should be preserved in the graft, or it is more efficient to transplant the skin devoid of cellular elements. The advantages and disadvantages of lyophilized grafts have been described, namely the possibility of long-term storage, but loss of elasticity, after rehydration. The methods of cryoconservation of cutaneous allografts, their properties, and acceptable methods of sterilization have been discussed. A perspective technology of graft decellularization has been

assessed and the methodologies of their manufacturing have briefly been presented.

Keywords: allogenic skin, lyophilized and cryopreserved cadaver skin, dermal matrix, wound treatment

HIV-1 - Human Immunodeficiency Virus 1

The attempts to use cadaveric skin grafts, or allografts were preceded by a long history dated back to about 3,000 years ago, although modest by case number, of using patients' native skin, and animal skin.

All tries to apply autologous and allogenic dermal grafts, as well as the skin of animals (xenografts) are of equal importance for the history of the science development. However, only modern science makes it possible to study in detail and justify the use of such treatment techniques, to implement them into practice. Failures of predecessors disappointed their followers for a long time, but the severity and extent of skin injuries again and again forced enthusiasts to turn to studying the skin grafting potential.

The purpose of this review was to study the world experience and determine the prospects for the use of allogenous skin grafts in the treatment of wounds in Russia.

The history of skin grafting for the treatment of wounds

The first allografts in the form of very thin epidermal flaps obtained from a living donor were used by Reverdin in 1869 [1]. Those splitted flaps contributed to faster healing of granulating wounds. Two years later, Oilier further developed the successful method by applying dermal-epidermal grafts and observed less severe scarring and a quick healing of wounds [2].

The first skin grafting procedures up to Reverdin's methodology were performed in Russia in 1870 by S.M.Yanovich-Chainsky who proposed to use small flaps of the epidermis containing the papillary layer. The method of free grafting of small full-thickness skin flaps is termed the Reverdin-Yanovich-Chainsky method [3].

Pollock implemented the idea of treating burn wounds with allo-skin grafts using his own small skin flaps applied onto his patients' burn wounds [4]. Those were the attempts to apply skin from living donors. The history of using skin allografts in the treatment of burns is considered to begin with those episodes. It was followed by the attempts to apply the skin taken from amputated limbs. The most significant for the evolution of the method was Girdner's work in 1881 when cadaveric skin grafts were used to cover skin defects [5]. In 1890, Doctor S.S.Ivanova who used cadaveric skin for transplantation noted that certain body tissues retain their vitality for some time after the body death, and she had based her method on that concept [3].

In the XX century, the tasks of preserving the viability of tissues and their possible preservation were formulated. In the middle of the century, frozen skin grafts began to be used for the temporary cover of wounds, an electro-dermatome was invented [6], and the effect of the immune system on allograft rejection was identified [7], the first American Skin Bank was established, and a preservative solution was developed for pre-treating the skin before freezing to preserve the viability of some of its cells [8]. The time came when hopeless burn patients got a chance to survive using available biological wound coverage.

Further advances in using allografts were reported in many experimental and practical studies in the XX and early XXI centuries and

showed an inexhaustible interest of various branches of medicine and applied sciences to the issue of using cadaveric skin allografts.

The evolution of methods for the preparation and use of cadaveric skin has led to the formulation of a crucially important and relevant question to date: whether the skin viability should be maintained or the skin can be transplanted without viable cell elements. It has been proven that non-viable skin can be used as a temporary biological coating or as a supplement in the process of skin autografting [9]. Some authors insist on the need to preserve the vitality of cadaveric skin, explaining it with a better ability to temporarily perform the required functions [10]. In many countries, the preserved viable allografts have been used; and the stimulating properties of non-viable allografts are implemented in the properties of artificial wound coatings that do not require special storage conditions.

Today, about 10 types of skin allografts are known. Wound coating with skin allografts after necrectomy is a routine procedure that has been included in an emergency “gold standard of surgical treatment of burns [11–13]. Skin allografting is the most efficient for the treatment of burns of over 40% of the body surface area [14]. The stock of preserved skin allografts stored in the world's tissue banks is constantly being renewed and amounts to hundreds of square meters. The needs of hospitals for very large amounts of cadaveric tissues are satisfied by the tissue banks arranged in different countries [11–17], having a network in Europe (European Association of Tissue Banks [EATB]), USA (American Association of Tissue Banks [AATB]), Asia (Shanghai Bio Corporation Tissue Bank [SBC TB], Joint Tissue Banking Facility at Tianjin), Latin America (the International Atomic Energy Agency [IAEA] Program in Radiation and Tissue Banking, Brazil).

According to the American services controlling the use of cadaveric tissue, 325 hospitals of the countries got use of cadaveric tissues in 2005.

Skin banks were organized within the structure of tissue banks for the needs of burn centers and trauma departments. Cadaveric skin is also used to treat chronic ulcers [18], extensive post-traumatic defects [19, 20], in cases after surgical interventions where a large area of skin amount is required, and the patient's native donor skin resource is limited or cannot be used.

Production of lyophilized skin allografts

Cadaveric skin is procured in the countries that have The Law on Transplantation. The algorithm for the preparation, storage, and use of allografts is not complicated. Skin grafts are preserved by lyophilization or freezing [21, 22]. All skin grafts can be conditionally allocated into two groups: those with preserved viability of tissues and those deprived of viable cells.

Lyophilization is a method of skin allograft preservation, which allows them to be stored for a long time at ambient temperature [21, 22]. The epidermal layer of human skin is harvested after obtaining the permission from the forensic medical examiner and no later than 8 hours after death, provided that the corpse has previously been stored at a room temperature. The epidermal skin layer of 0.25-0.3 mm thick is removed using a dermatome, placed in a Ringer-Locke solution with antibiotics, and stored at -45° C until the laboratory test results have been obtained and the donor autopsy study has been performed by a pathologist. Then, immediately prior to the preservation, the allografts are unfreezed, treated with a 3% hydrogen peroxide solution, washed with saline, straighten on a polypropylene mesh,

covered with the polypropylene coating on the two sides, and frozen at −45° C.

The lyophilization of the prepared grafts is performed using the standard methodology until achieving the 1–5% residual moisture. Dried grafts are packed in double (inner and outer) bags of polypropylene and sterilized with ionizing irradiation at a dose of 15–20 kGy. The completed sterilization is documented by the protocol. The biosafety of lyophilized skin should be certified by a microbiological test for sterility of 1–2 samples from the series. The shelf life and storage of such human skin allografts of the epidermal layer is at least 2 years at ambient temperature in a place protected from the sunlight. Immediately prior to using the lyophilized grafts, they are soaked in sterile saline for 20–30 minutes and used as a temporary biological coating of wounds. The undoubted advantage of such grafts is the possibility of their long-term storage. The disadvantages include a certain loss of elasticity after rehydration and the absence of viable cells.

Cryopreservation of cadaveric skin

For over 30 years, the cadaveric skin preservation has been carried out worldwide by freezing in various solutions aimed at maintaining the cell viability [23–25]. The most well-known and simple option is the preservation of the skin in glycerol: at least 75% of skin cells remain viable. The cadaveric skin prepared in this way can be used in extensive burns for additional cover in case of applying perforated autografts. The glycerol-cryopreserved allogenic skin can be used to cover chronic, poorly blood-supplied wounds to improve the wound surface condition as a preparation stage for autografting and in cases of extensive excision of soft tissues and lack of available skin for autografting. Such an allograft is easy to use, it

helps to quickly relieve severe pain in a burn wound, prevents the losses of fluid, electrolytes, and proteins. The cost of such skin is relatively low.

It is known that glycerol has antibacterial and antiviral properties. Studies have shown that the antiviral properties of glycerol depend on its concentration, temperature, and the duration of exposure. Although some viruses can be neutralized during the production process, further studies should determine the optimal conditions for their elimination. According to literature reports, no cases of viral disease transmission were noted with the annual use of about 1,550,000 cm^2 of cadaveric skin in clinical burn centers in Europe [26, 27].

The use of glycerol for the cadaveric skin preservation is justified by its antibacterial and antiviral effects. Glycerol is a slow-acting but effective bactericidal agent; 97% of bacteriologic cultures from the glycerol-preserved skin were negative for 3 months.

Concentrated glycerol has a marked antiviral activity. Van Baare reported that at 37° C, the herpes simplex virus was inactivated with 85% glycerol within 6 hours, while the polio virus was inactivated within 24 hours. At ambient temperature, the inactivation time increased to 8 and 22 days, respectively, and viral inactivation was not observed at 4° C [28].

Due to the concerns about the potential transmission of human immunodeficiency virus 1 (HIV-1), studies have been conducted to investigate the effect of glycerol various concentrations on HIV-1. Free HIV-1 is inactivated with 70% glycerol within 30–60 minutes at any temperature, and intracellular HIV-1 was inactivated within 3 hours at 37 ° C at 70% or 85% glycerol concentration. The storage of cadaveric HIV-1 infected skin in 85% glycerol at 4° C resulted in a complete inactivation of the virus after 5 days [29–31].

Other methods for preserving cadaveric skin are known: using the dimethyl sulfoxide solution in various concentrations, the low glycerol concentration solution, and sterilization with gamma irradiation [32–36].

The main disadvantage of lyophilized or cryopreserved skin allografts is the preservation of their cells or cell membranes carrying the main histocompatibility complex antigens, which leads to the rejection reaction development at 10-21 days after the allograft application. That is why such grafts are used only as a temporary wound cover (for no more than 10–12 days) [21, 41]. On the other hand, even during this period, the use of skin allografts allows us to reduce wound losses, prevent secondary wound infection and pain during dressings. However, occasional cases of the partial adherence of allografts in some patients led to the need to remove those tissues at 6 to 12 months later due to an inevitable development of the rejection reaction.

Dermal matrix

A very promising trend in the treatment of deep burns is the use of decellularized (cell-free) cadaveric skin [38-40]. The main advantages of such biological material include the composition and organization of the derma used in a way similar to the patient's own derma, allowing it to be used as a temporary biological coating. The cell-free cadaveric skin has no immunogenic factors that can lead to the rejection of donor material. Meanwhile, the native structure and composition of the skin matrix are preserved.

In the Sklifosovsky Research Institute for Emergency Medicine, there was developed a method for obtaining a dermal matrix of the splitted human skin up to 1 mm thick harvested from a cadaverous donor [41]. After the

skin has been collected using a dermatome up to a standard procedure with respect to the aseptic and antiseptic rules, the biological material is placed in a sterile container filled with an aqueous solution of a broad-spectrum antibiotic agent. The material is stored at –40^{o} C until the results of the autopsy study, and the study of the biological safety of the donor's tissues have been obtained. At the first stage, the epidermis is removed. To do this, a piece of skin is frozen several times to –96^{o} C within 15 minutes, defrosted to +37^{o} C for 5 minutes, and then incubated in a hypertonic solution of sodium chloride at an ambient temperature and continuously stirring until the complete detachment of the epidermis occurs. At the next stage, the derma is decellularized using detergent solutions (0.2% sodium dodecyl sulfate solution). Then the graft is repeatedly washed with saline to completely remove toxic products. After the quality control assured, including morphological and bacteriological studies, and the cross-match, the graft is ready for use as a temporary biological coating for wounds.

Conclusion

The scientists of the Sklifosovsky Research Institute for Emergency Medicine continue investigating the properties of cadaveric skin, making the preparations of cadaveric skin highly demanded in the treatment of extensive burn wounds, and traumatic skin defects.

The adoption of simple to use standardized protocols for the procurement and storage of skin and other allografts, the development of legislative support will help to treat many patients in need for skin grafting and lacking their native skin resources, as well as the patients with non-healing wounds, in whom the use of allografts has a stimulating effect on healing.

Summarized reports from the manufacturers and consumers of cadaveric allografts suggests that the use of these tissues for extensive skin defects and, in particular, for burns, makes it possible to reduce mortality, expand the possibilities of reconstructive surgery, and greatly contribute to the development of medical science in general.

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