Chapter

Banks of Cryopreserved Skin from Live Donors and Total Skin Allografts in the Surgery of Major Burnt Patients

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Abstract

Scarectomy and prompt coverage are some of the main cornerstones of the actual treatment of major burnt patients. This coverage can be definitive using autologous tissues or temporary with allografts, xenografts, and/or biosynthetic products. Skin allografts (SAs) are the gold standard therapeutic alternative among temporary coverages, since they mimic skin functions. However, cadaveric skin donation and procurement, a common SA source, are infrequent. On the other hand, there is a significant number of patients that, given their health condition, large amounts of skin must be resected for their clinical recovery, including patients submitted to corporal contouring surgeries with esthetic and/or reconstructive motives, usually eliminating the redundant skin as biological waste. This study describes a skin bank model from live donors and cryopreserved total skin cutaneous allografts (CTSCAs), a new type of SA resulting from a particular skin processing.

Keywords: skin allograft, skin bank, burns, cryopreserved total skin cutaneous allografts

1. Introduction

Since the XX century and due to Janzecovich's contributions, scarectomy and prompt coverage of major burnt patients have become one of the mainstays of the surgical treatment [1–3].

This coverage can be definitive, using autologous tissues, or temporary. The lack of availability of donor areas, infected or doubtful vitality beddings, or graft procurement associated with a morbimortality increase are conditions where temporary coverages are preferred. The latter is done using allografts, xenografts, and/or biosynthetic products, which mimic the skin functions and provide a physiological coverage that permits hydro electrolytic loss control, pain and infection risk reduction, and improvement of the local conditions of the bedding.

Skin allografts (SAs) are the gold standard therapeutic alternative among temporary coverages. Their natural evolution consists of its rejection between days 8 and 10, being retarded in major burnt patients, due to the immune system depression between days 15 and 30 [4–7].

In burnt patients, the use of SA began in 1881, when Girdner treated a patient with severe burns with cadaveric SA [8]. Subsequently, Brown et al. popularized SA as biologic grafts in extensive burns [9–10]. The growing need for SA for managing these patients was responsible for the creation of establishments capable of storing skin during the 50th decade. These centers are usually located inside or near hospitals or burnt centers to satisfy the burnt patient's needs and, on the other hand, promote skin donation with high-quality standards [11–14].

The SA necessity resulted in the emergence of facilities for skin storage during the 1950s. Most of them were located inside or near hospitals or burnt centers, permitting, on the one hand, satisfying the burnt patient's needs and, on the other hand, encouraging skin donation with high-security standards [8, 9].

SAs are usually obtained from cadaveric donors in the context of multiorgan donation. They are obtained with a dermatome as partial skin grafts, preserved with high concentrated glycerol, resulting in cellular death and not viable tissue [15].

The relative shortness of donors encouraged the search and use of other SA sources, as live donors submitted to surgeries resulting in skin redundancy and the need for its resection for reconstructive and/or esthetic motives [16–19].

This study aims to describe the clinical features of SAs, particularly cryopreserved total skin cutaneous allografts (CTSCAs) and a model of skin banks from live donors.

2. Cryopreserved total skin cutaneous allografts (CTSCAs)

CTSCAs emerge from the need and search for coverage for burnt patients and complex wounds associated with a low organ and tissue donation rate. Compared to the classical SAs, CTSCAs have three distinctive features: a) derived from live donors, b) total thickness skin, and c) cryopreserved [20, 21].

2.1 Live donors

It is crucial to emphasize that the skin donation request is done in the context of elective surgery and happiness due to the primary esthetic and functional expected results and not in an environment of familiar sadness of skin procurement in cadaveric donors, where the donor remains with a social altruism sensation secondary to the donation of a tissue that would otherwise be a surgical waste. The extensive inclusion and exclusion criteria for skin donation in cadaveric donors (**Table 1**) in order to guarantee the microbiological safety of the tissues are left aside in live donors, since it is understood that patients submitted to elective body contour surgeries do not have contraindications for the performance of such surgeries and the consequently tissue donation.

Exclusion criteria:
a. For infectious diseases
Patients with history or carriers of HIV/AIDS.
Patients with a history of Hepatitis B or C.
Patients with a history of active Tuberculosis.
Patients diagnosed with Syphilis or positive VDRL.
Patients diagnosed with HTLV I and II.
Patients diagnosed with Chagas disease.
Patients with diagnosis of Rabies, Congenital Rubella and Malaria.
Patients diagnosed with untreated bacterial or fungal endocarditis.
b. For central nervous system diseases
- Degenerative diseases
- Any type or manifestation of Dementia.
- Alzheimer's disease.
- Parkinson's disease.
- Multiple sclerosis.
- Creutzfeldt-Jakob disease.

Infectious diseases
- Bacterial encephalitis.
- Viral, fungal or parasitic meningitis.
- Bacterial meningitis.
- Progressive multifocal leukoencephalopathy.
- Subacute sclerosing panencephalitis.
- Active viral encephalitis or encephalitis of unknown cause.
- Fungal or parasitic encephalitis.

c. For presence of cancer and/or tumors
- History of neoplasia except for cervical uterine cancer in situ.
- Lymphadenopathy for more than one month.
- Lymphomas, lymphosarcomas
- Leukemias.
- Metastasis of primary or secondary malignant tumors (lung, breast, cervical, colon, prostate, squamous cell, melanomas, lymphomas, leukemias, central nervous system, among others).

d. Other pathologies
- Patients who have been treated with growth hormone.
- Patients with Hemophilia.
- Patients carriers of autoimmune diseases or Mesenchymopathies such as Rheumatoid Arthritis, Systemic Lupus Erythematosus.
- Patients who have been treated with prolonged corticosteroid therapy.
- Any suspicious skin alteration.

e. Behavioral:
- Unsafe sexual behavior.
- Drug abuse (including intravenous, intramuscular and subcutaneous).
- Commercial sex workers.
- Inmates.
- Individuals with tattoos, (or) body piercing performed in the last 6 months.
- Individuals from whom no history of sexual behavior can be collected.

f. Specific skin criteria:
- Skin contaminated by toxins.
- Pyoderma.
- Any skin lesion: infectious, traumatic or vascular.
- Psoriasis.
- Epidermolysis bullosa.
- Loxocelism.
- Structurally damaged skin (due to autoimmune or collagen diseases).

HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; AIDS, Acquired immunodeficiency syndrome; VDRL, Venereal Disease Research Laboratory.

Table 1.
Exclusion criteria for skin donation.

Besides, there are multiple myths around the organ and tissue donation process; thus, the skin procurement in live donors permits the breakdown of two important myths: a) cadaveric body disfigurement secondary to the skin extraction, a factor that affects the low skin donation rates in many countries, which becomes a "refinement" obtained after a body contour surgery, and b) poor patients donate their tissues and organs to wealthy patients, since people with higher incomes have more access to body contour surgeries and burns are more common in the poor population [22, 23].

2.2 Total thickness skin

Skin resection in body contour surgeries allows the procurement of a cutaneous-subcutaneous flap, which is defatted with scissors, obtaining total skin cutaneous allografts (TCSAs) (**Figures 1** and **2**) compared to classical SA, which are procured with a dermatome, obtaining only partial skin allografts. The amount of TCSA obtained is variable. In an adult abdominoplasty, 3–4% of the total body surface is resected, and once the skin is processed, the valuable surface is of approximately 250–300 cm^2. The skin surface obtained is more extensive in patients submitted to body contour surgeries post-bariatric surgery. However, histologically, the skin presents chronic inflammation areas, sebaceous gland infections, lower collagen fiber organization, elastin and fibroblast concentration, and metalloproteinase levels similar to oncologic or burnt patients [24, 25].

Even if the skin surface obtained compared to a cadaveric skin procurement is smaller, its potential is associated with the number of patients submitted to these

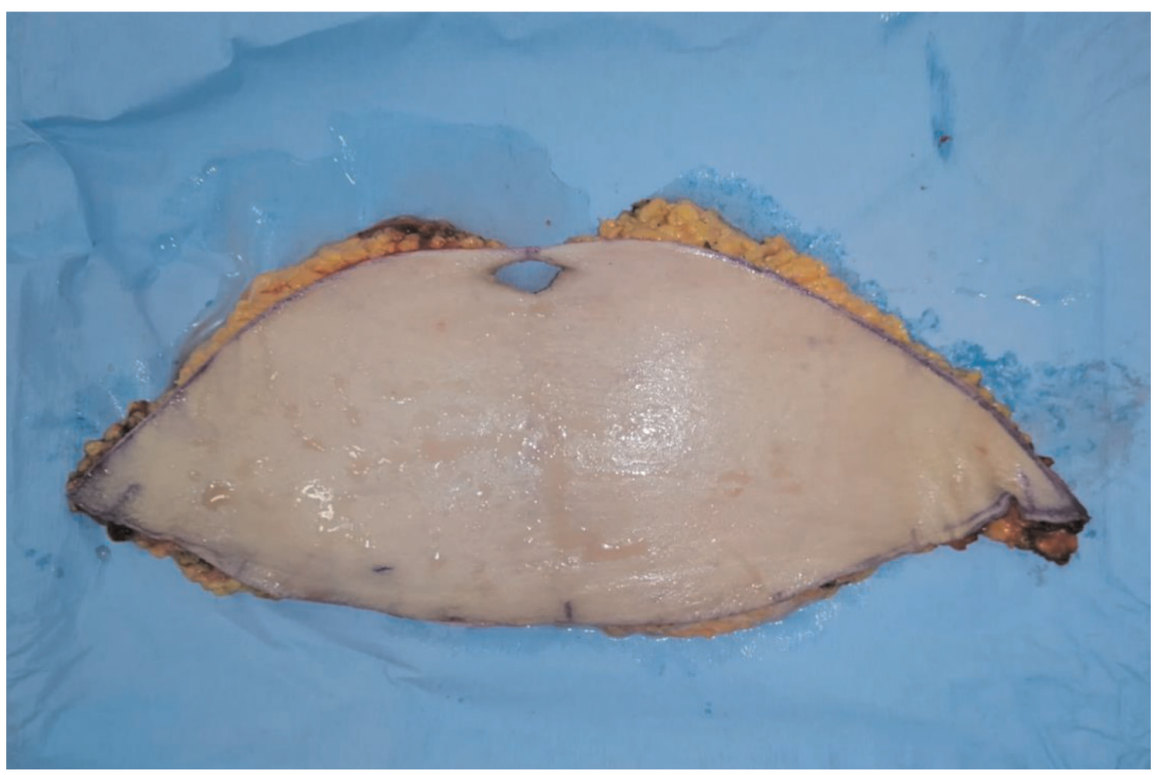

Figure 1.
Abdominal cutaneous-subcutaneous flap.

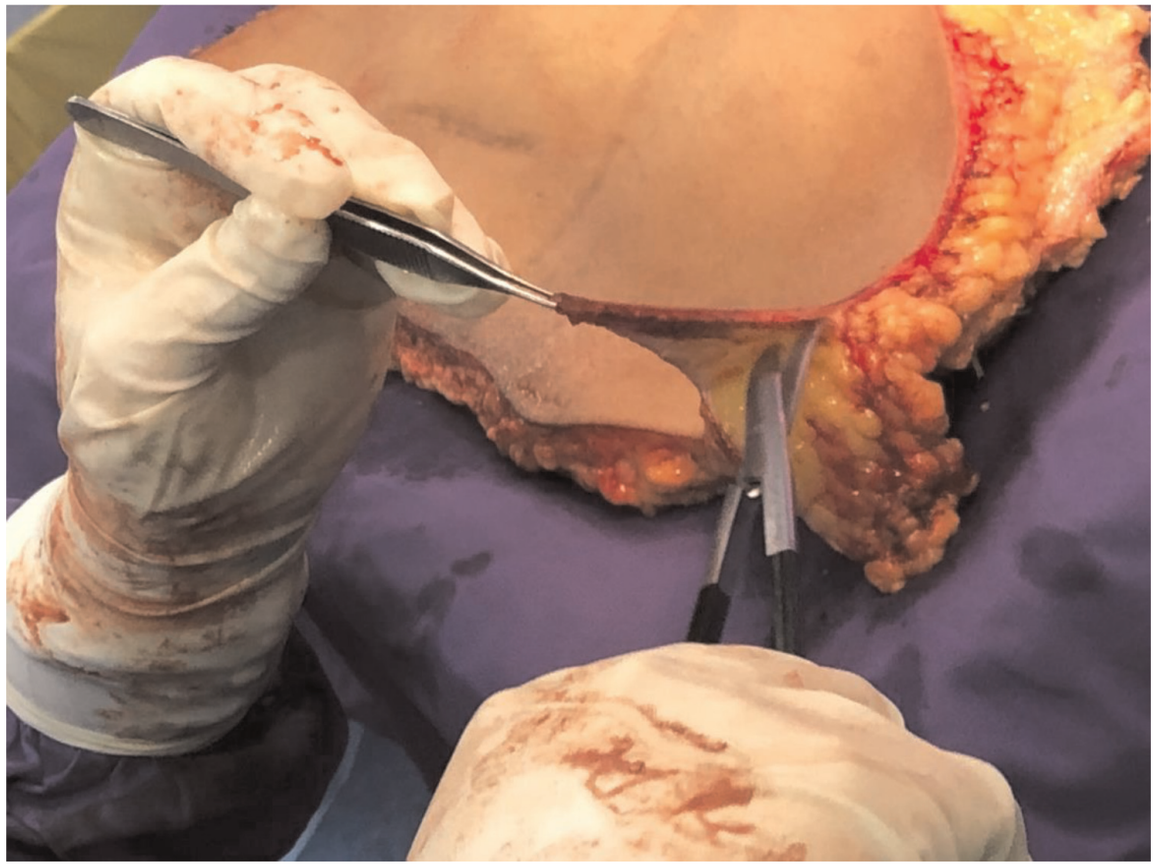

Figure 2.
Procurement of total skin with scissors from redundant adipose skin flap.

types of surgeries and the possibility of obtaining all the skin layers, making it an attractive option [26, 27].

2.3 Cryopreservation

There are multiple forms to preserve tissues: high-concentration glycerol and cryopreservation are the most used techniques in skin banks [28, 29]. The main difference among both techniques is that in the first case, the tissue is nonviable but maintains its structural and mechanical properties, generating a biological dressing. In contrast, the second preserves some cellular viability, and the tissue can be integrated into a wound bedding.

Preservation with high-concentration glycerol is the predominant method in most skin banks since its lower cost and easier storage and distribution. Cryopreservation freezes the TCSA in the presence of a cryoprotectant (glycerol 10%), which prevents the crystallization effects, maintaining the viability of keratinocytes, fibroblasts, endothelial, and Langerhans cells over the time following the freezing. The viability of the obtained tissues is crucial for the clinical results [30–34].

Dimethyl sulfoxide (DMS) is another cryoprotectant frequently used in cryopreservation procedures; however, there are contradictory publications regarding the best alternative for cryopreservation compared to glycerol 10% [35–36].

Precisely, both partial and total skin allografts can be cryopreserved, but the viability is one of the features of CTSCA.

3. Technical aspects of skin banks

Human skin storage started at the beginning of the XX century, following the description of skin transplant after its refrigeration, but modern skin banks began in 1949, after the creation of the Tissue Bank of the United States Marine. However, Tissue Banks arrived in developing countries three decades later than the developed world [37–43].

According to the American Association of Tissue Banks, a Tissue Bank is defined as "an entity that provides or is dedicated to one or more services related with tissues from live or dead persons, with a transplant objective. These services include obtaining authorization and/or informed consent, evaluating donor eligibility, recuperation, harvest, acquisition, processing, storage, labeling, distribution, and dispensing tissues." [41].

Skin donation, a source of SA and CTSCA, is mainly influenced by cultural and religious factors but regulated by specific laws depending on each country and can be divided into seven big stages: 1) donor selection, 2) procurement, 3) processing, 4) storage, 5) radiation, 6) distribution, and 7) clinical use [42–45].

3.1 Donor selection

The appropriate donor selection allows the generation of safe tissues, primarily reducing the risk of disease transmission during the CTSCA transplant [46].

When deciding the body contour surgery, mainly abdominoplasty, patients are invited to be donors of redundant skin flaps, which would otherwise be a surgical waste. A health survey, verification of exclusion criteria absence, and routine laboratory tests related to organ and tissue donation (hepatitis B surface antigen, hepatitis C antibodies, HIV antibodies, VDRL, HTLV I and II, Chagas, and cytomegalovirus) (**Table 2**) are done. As previously mentioned, most exclusion criteria for organ and tissue donation are usually inexistent in the live donor submitted to elective surgery.

3.2 Procurement

The skin procurement takes place in the surgical ward, with the same surgical team and time of the body contour surgery, respecting all the asepsis and antisepsis

1. Hepatitis B surface antigen.
2. Antibodies against Hepatitis C.
3. Antibodies against HIV.
4. VDRL
5. HTLV I and II.
6. Chagas.
7. Citomegalovirus

HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; VDRL, Venereal Disease Research Laboratory.

Table 2.
Laboratory tests.

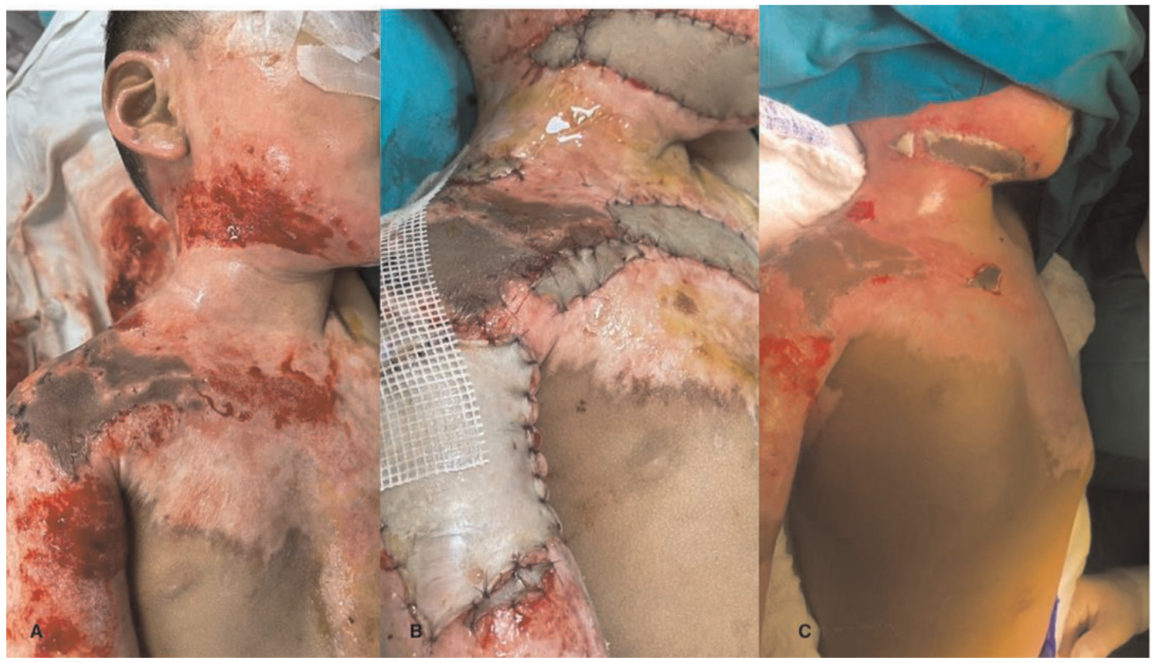

Figure 3.
A. Patient with intermediate and deep burns. B. Burnt patient following a CTSCA use, immediate postoperative. C. Burnt patient, 14 days postoperative with CTSCA coverage.

measures. In the particular case of abdominoplasty (the most frequent CTSCA source), the redundant skin is demarcated in a transverse-infra umbilical-ellipse form, followed by dissection and resection of the skin flap. The abdominoplasty is developed independently, and the skin procurement is done in a separate surgical table.

The subcutaneous component of the cutaneous-subcutaneous flap is resected using scissors, exposing the deeper dermis (**Figures 3** and **4**). Three tissue samples are taken for current (aerobic), anaerobic, and fungal cultures. Procured skin is placed in a sterile recipient with 500 cc of physiological serum, Cloxacillin 1 g, and Gentamicin 80 mg, hermetically closed, promoting the complete skin submersion. The same receipt is saved in a double sterile bag and stored at 2 and 8°C until processing. All the information needed to guarantee the traceability and biosecurity of the tissues is consigned.

3.3 Processing

The skin processing is subdivided into three stages: 1) cutting, b) cleaning, and c) packing/labeling, all of them take place in a clean room, with rigorous aseptic technique and a biosecurity cabinet.

3.3.1 Cutting

Once the skin flap is measured (length, width, and thickness), cuts using a scalpel and scissors are done according to the requested or standardized measures (**Figure 2**). Standard measures are 10 x 10 cm, 10 x 5 cm, and 5 x 5 cm. According to the redundant skin, other dimensions cuts are done, and the smallest size accepted is 2 x 2 cm.

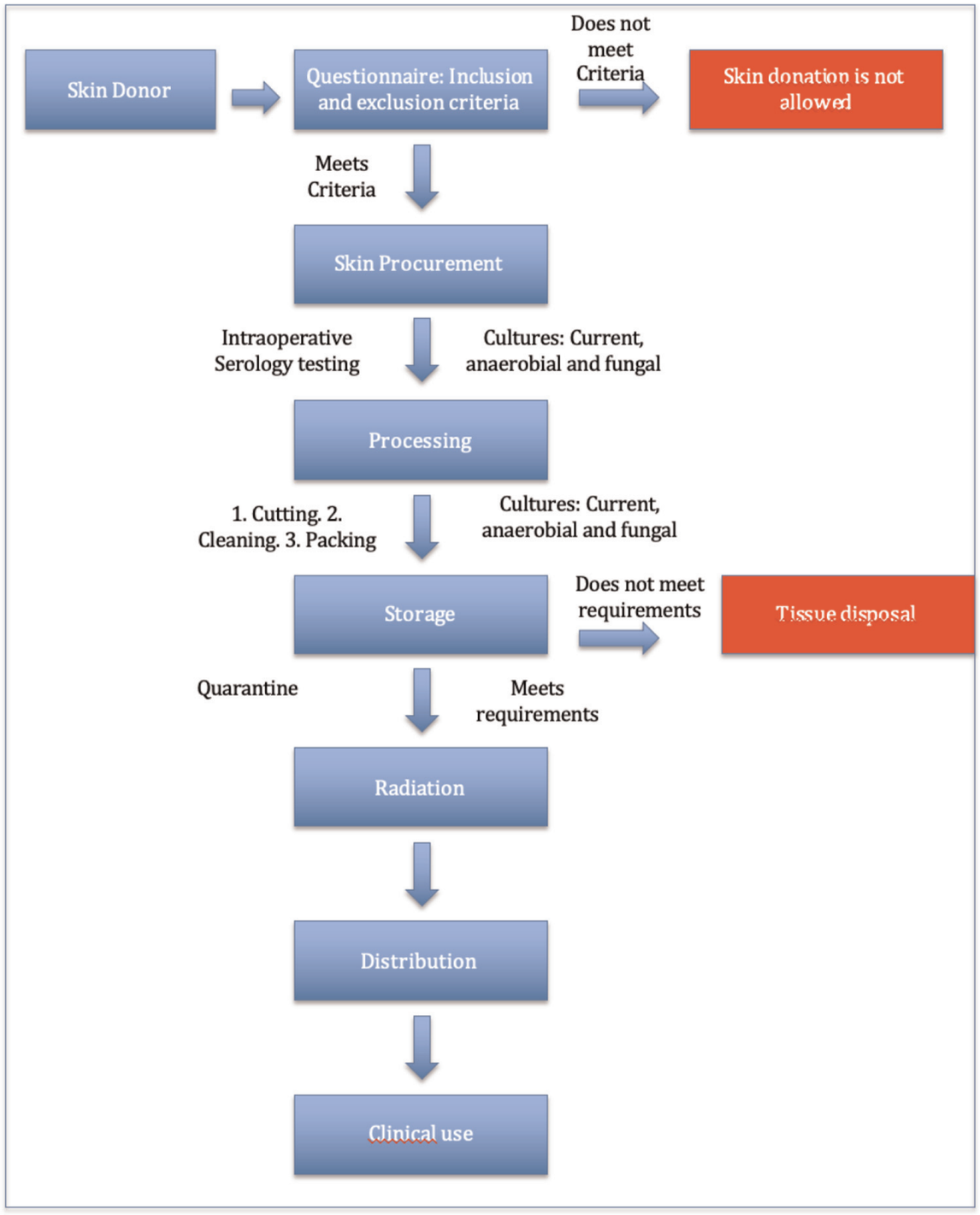

Figure 4.
CTSCA processing flow chart.

3.3.2 Cleaning

The cleaning takes place by depositing the skin in sterile jars with 10% glycerol, followed by a manual agitation for 5 minutes. The process is repeated five times in different jars. During this procedure, jars change from a tainted red color to transparent. Concomitant, new samples are taken for culture. Finally, the last jar with skin sheets in a cryopreserving solution (glycerol 10%) is carefully transferred to refrigeration (between 2 and 8°) for at least 1 hour.

3.3.3 Final packing and labeling

This phase includes measuring, packing, and labeling each piece of the skin set. It is done once the tissue has been immersed in the cryopreserved solution (glycerol 10%) for at least 1 hour.

Samples for culture (current, anaerobic, and fungal) are taken at the beginning and end of the packing. The packing used to pack the skin is completely transparent, allowing the tissue visualization with its features and conditions. The obtained tissue is then labeled according to internal codes, with the information needed for their traceability. It is important to emphasize that in contrast to organ transplant, where a donor usually benefits one receptor, tissue transplants from one donor may benefit many receptors.

3.4 Storage

The processed skin is kept in quarantine in an ultra freezer at −80°C until the skin cultures, and donor serology results are obtained. Following the entrance of the samples, the clinical laboratory usually informs the results of the aerobic cultures in 72 hours and the fungal cultures in 15 to 18 days. If any culture develops any microorganism (positive result), the table of microorganisms allowed in the skin for radiation (**Table 3**) is consulted to determine if the skin set will complete the final phase of radiation or will be discarded.

3.5 Radiation

The objective is the sterilization of the CTSCA and bacterial contamination risk reduction, using gamma radiation (25 to 30 kGy). Dry ice must be used to keep the cold chain during the skin transportation from the skin bank to the radiation center.

Twenty-five kilograms of gamma radiation to an ultra-frozen skin with low glycerol concentration sterilize the tissue with no histological, cytotoxic, or physical alteration, in contrast to normal cryopreserved skin [47, 48]. Only tissues with a negative serology from the patient and negative cultures or accepted microorganisms for radiation will complete the final radiation process.

3.6 Distribution

Facing the CTSCA requirements, the skin sets must be transferred in dry ice, in a pellet-like presentation, keeping the temperature between −76 and − 80°C from skin banks to the different hospitals for their clinical use.

Microorganisms allowed in skin for radiation	Microorganisms not allowed in skin for radiation
Staphylococcus aureus, beta hemolytic streptococcus, enterococcus, yeast	Aerobic and anaerobic Gram-negative bacilli, Gram-negative cocaceae, clostidium, anthacis bacilli

Table 3.
List of microorganisms allowed in skin for radiation.

3.7 Clinical use

In the preoperative phase, the size of the defect to be covered must be calculated to request the appropriate amount and size of skin sheets. All the information that guarantees tissue traceability and biovigilance from the donor and the final receptors must be consigned.

It is essential to emphasize the elasticity of the CTSCA, which may also expand doing small incisions on its epidermal layer, so the cover surface of the CTSCA is more extensive than its size.

In the preoperative, the size of the defect to be covered must be calculated to request the appropriate amount and size of skin sheets. It is essential to emphasize the elasticity of the CTSCA, which may also expand doing small incisions on its epidermal layer, so the cover surface of the CTSCA is more extensive than its size.

Prior to clinical use, the CTSCAs are washed three times with warm physiological saline (without exceeding 40°C) to remove the cryoprotectants (glycerol 10%).

The receptor bedding is prepared with scarectomy of the necrotic, devitalized, and disorganized granulation tissue; subsequently, the CTSCA is fixed in our case with stitches and/or medical clasps associated with negative pressure therapy [49].

All the skin processing, from donation to clinical use, is resumed in the flow diagram of **Figure 4**.

4. Clinical indications

Scarectomy and prompt coverage have increased the survival of major burnt patients. However, on many occasions, the available skin for autografts is limited, and the lack of donor areas impedes grafting the totality of the excised areas. In the latter conditions, the SA has become the reference cutaneous substitute, which can be used alone or combined with autografts. Other SA clinical indications among burnt patients are bedding with doubtful vitality or infection, or when the autograft procurement significantly increases the morbimortality of the patient.

The current role of the SA in treating burns varies among the burnt units or centers, where most of them lack the access or experience of using this product [50].

4.1 Sole skin allografts use

4.1.1 Lack of donor areas

When donor areas are minimal or lacking, a scarectomy and coverage with SA are done directly on the residual bedding, which is replaced after the healing of intermediate burns, and the donor areas allow the harvesting of new autografts.

It is vital to emphasize that in burnt inpatients, the hospitalization time and economic costs are lower in the group of patients who first receive an SA followed by an autograft than in the group that only receives autografts. The latter could probably be since the autograft obtention generates new bloody areas and that the autograft in a non-completely defined vitality could imply its loss [51].

4.1.2 Engraftment test

When the bedding to cover has a doubtful vitality, the use of SA is preferred, since it permits an engraftment evaluation before using autografts. As autologous skin grafts, CTSCAs suffer revascularization, providing the wound bedding with crucial growth factors and cytokines, promoting cellular chemotaxis and proliferation.

The increased wound bedding vascularization stimulates angiogenesis and favors the bedding preparation for an autologous skin graft.

Another indication for using allografts in burns surgery could be in the context of infected burns when the risk of losing the autograft is considered significant [52, 53].

4.1.3 Unstable patient

Scarectomy of extensive body surfaces, mainly associated with the autografts harvesting in the same surgical time, produces significant bleeding. Using SA, scarectomy may be done alone and the graft harvesting during a second time, reducing the bleeding and hemodynamic instability.

4.2 Use of SA associated with autografts

Alexander et al. described the "In sandwich compound graft." Following the scarectomy of the burnt patient, the bedding was covered with expanded autografts (meshed 1/6 or higher), which were then covered by a SA expanded in smaller meshes (1/1, 5, or 1/3). The latter worked as tutors and avoided the autograft dissection. This technique allows the coverage of extensive body surfaces with high success rates. Besides, Cuono et al. demonstrated that the engraftment of keratinocyte culture grafts improved significantly using dermic bedding provided by allografts [54, 55].

5. Clinical evolution

CTSCAs have initial engraftment, similar to an autologous skin graft, subsequently evolving to rejection. The rejection is clinically manifested as a gradual color change and formation of a necrotic scar in a 21-day average interval, when removed, exposing a vital tissue adhered to the receptor (**Figures 1**–**3**). The latter is histologically evidenced in the CTSCA as necrotis foci with mainly neutrophils and the receptor bedding exposing an interface rich in fibroblasts and neoformation vessels.

CTSCA acts as a scaffold and biological inductor, which becomes colonized by cells from the receptor, creating a neodermis. This model has been verified in xenograft models, where CTSCAs promote angiogenesis and collagen type 1 production without causing a significant fibrotic response.

Secondary to an immunologic phenomenon, the cellular elements of the skin allografts are rejected; however, the dermal components can persist and incorporate into the healing dermis of the receptor. The biologic mechanisms underlying this integration are not fully understood [56, 57].

6. Conclusions

Live donor skin donation is an alternative and readily available source of SA, particularly in countries with a low rate of cutaneous donation. Besides, total skin SA can be obtained, maintaining their vitality after cryopreservation, resulting in CTSCA.

Even if the total surface skin obtained is lower than in cadaveric procurement, this may be compensated by the high number of patients submitted to body contour surgeries. The clinical indications of CTSCA in burnt patients are multiple, including patients with lack of donor areas, infected or with doubtful vitality beddings, or when the graft harvesting process increases the morbimortality of the patient.

Conflict of interest

The authors declare no conflicts of interest.

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