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# Procurement and Use of Cryopreserved Total Skin Allograft in Complex Wounds

*Marcelo Fonseca, Aldo Cañete, Dino Ibaceta, Catalina Buchroithner, Florencia Disi and Juan Olivares*

## Abstract

Cryopreserved total skin allografts are a new therapeutic alternative for the management of complex wounds. Their properties allow them to be classified as a temporary coverage for some patients and as definitive in others. And they can be an alternative option to the use of dermal regeneration templates.

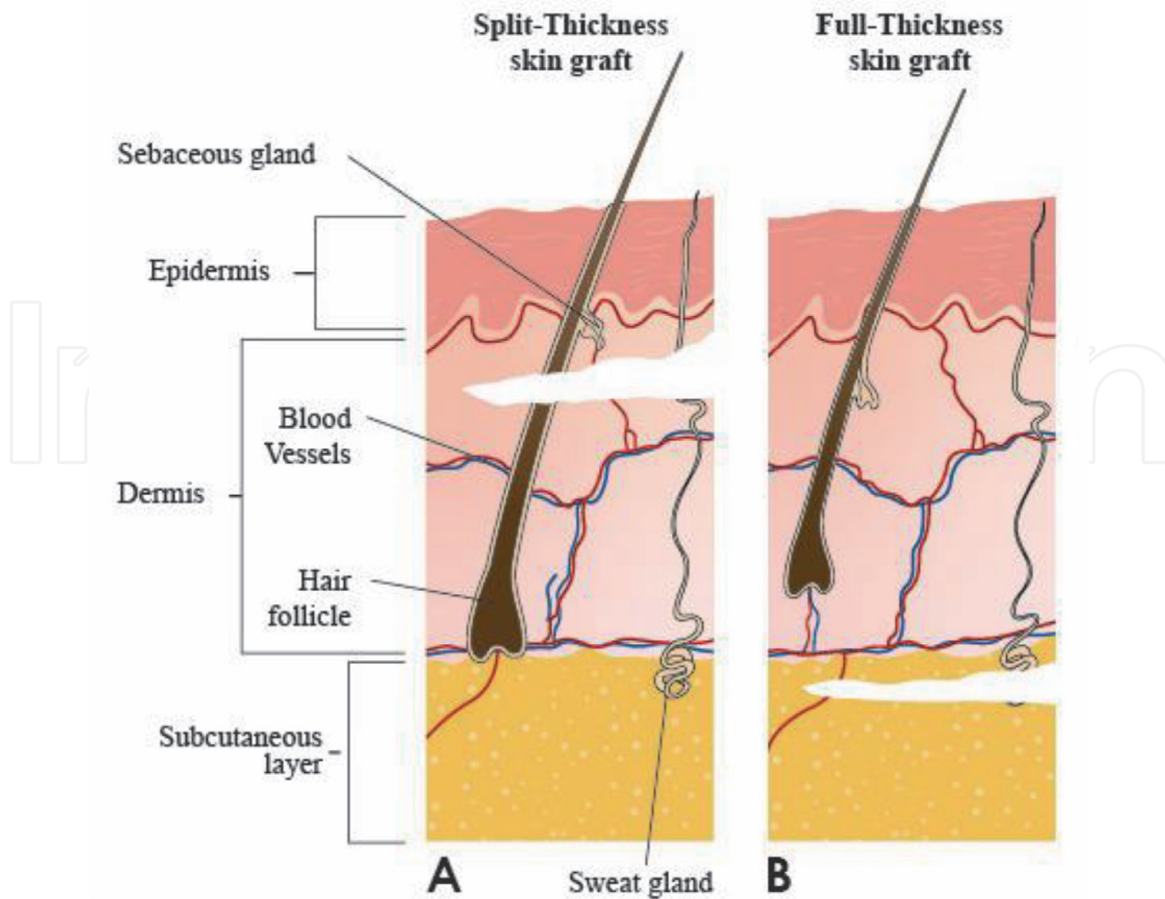
**Keywords:** skin allograft, biological dressings, wound healing, skin substitutes, dermal regenerators

## 1. Introduction

Historically, choosing the therapeutic options for the management of complex wounds was based on the concept of the “reconstructive ladder”, in which reconstructive methods were ranked by complexity and the simplest options capable of solving the problem had to be chosen. The latter has evolved over time, with the emergence of the “reconstructive elevator concept”, where a surgeon can ascend directly to the appropriate level in order to obtain the best qualified reconstruction, and due to the need of incorporating new advances in wound healing management into the “reconstructive ladder” [1, 2].

The use of skin allografts (SA) for wound coverage began during the 19th century. Currently occupy a predominant place within the temporary coverage techniques, especially in burn patients, with little residual skin capital, and with extensive clinical experience in other pathologies [3–7]. SA provide a physiological lining to control the hydro electrolyte losses, reduce the pain and infection risk and improve local bed conditions by promoting angiogenesis and the maturation of the underlying granulation tissue [8, 9]. However, due to immunological processes, they are usually rejected after 10–12 days and may eventually be a vector for infectious diseases [10, 11].

Most of the SA come from cadaveric donor; are harvested as partial skin grafts (**Figure 1A**) with a dermatome and are preserved using glycerol at high concentrations, causing cellular death with the obtention of non-viable tissues [12, 13].



**Figure 1.**  
A. Split-thickness skin graft B. Total skin allografts.

## 2. Cryopreserved total skin allografts

### 2.1 Background

The low rate of human organ and tissue donation and the cultural and religious restrictions for the use of cadaveric tissues in certain countries, have opened the door to the pursuit of other sources of SA, particularly in patients who underwent body contouring surgeries (abdominoplasty, reductive mammoplasty), either for esthetic or reconstructive reasons. This allows the entire resected flap to be procured, allowing the obtention of total skin allografts (TSA) (**Figure 1B**) [ 14–17].

### 2.2 Tissue production

The process of obtaining TSA is initiated with the invitation of patients who are candidates for body contouring surgeries to participate in the donation of the redundant fatty skin flap, which would otherwise be a waste product. This represents the first difference with SA obtained from corpses, the TSA are obtained from living donors. Donors are submitted under a health survey (**Table 1**), the absence of exclusion criteria is verified (**Table 2**) and routine examinations for tissue donation are performed (**Table 3**). All the above is assessed to guarantee the microbiological safety of the tissues, which is mainly based on the selection of the donor, permitting the rejection of infections or any biological agent that could cause diseases in the recipient [18].

	YES	NO
Are you in good health today?		
Are you in generally good health?		
Did you sleep at least 5 hours?		
Have you drunk alcohol in the last 12 hours?		
Have you donated blood in the last year?		
Have you been told by a health professional that you cannot donate blood?		
Have you been to another doctor's office in the last 6 months?		
Have you had a dental procedure over the last week?		
Have you been hospitalized or operated during the last 6 months?		
Have you received any transfusion in the last 12 months?		
Have you had any blood tests in the last 12 months?		
Do you have or have had any heart, lung, kidney or thyroid disease, hypertension, diabetes mellitus, bleeding tendency or other illness?		
Are you taking any medication on a regular basis in the last 6 months?		
Have you taken aspirin or anti-inflammatory drugs in the last 3 days?		
Have you had any injections or vaccinations in the last 4 days?		
Have you had diarrhea in the last 7 days?		
Have you ever had cancer?		
Do you have a history of epilepsy, seizures or fainting?		
Do you have or had hepatitis or have you turned yellow?		
Have you had any tissue transplants or grafts?		
Have you received growth hormone treatment before 1985?		
Do you have a family history of Creutzfeldt-Jakob disease?		
Do you or your family have a history of dementia, Alzheimer's, Parkinson's, Multiple Sclerosis or another degenerative disease?		
Do you or your family have a history of Chagas disease?		
Have you had Malaria, Dengue fever or unexplained fever during or after a trip outside the country?		
Have you traveled outside the country in the last 3 years; if yes, where?		
Have you had any tattoo, piercing, acupuncture or accidental needle stick or bloody syringe in the last 6 months?		
Have you tried any type of drug such as marijuana, cocaine, cocaine paste or other; if yes, which ones and when?		
Have you used illegal injectable or inhalant drugs over the last 12 months?		
Have you had sexual intercourse with anyone who has ever injected drugs?		
Have you had sexually transmitted diseases such as Syphilis, Gonorrhea or other in the last 12 months?		
Have you had more than one sexual partner in the last 12 months?		
Have you had sexual intercourse with a new partner in the last 6 months?		
Have you had sexual contact with persons with Hepatitis, HIV or HTLV in the last 12 months?		
Have you paid or received money, drugs, or anything else for sex in the last 12 months?		
Have you been exposed to the risk of becoming infected with the AIDS virus?		

	YES	NO
Are you donating tissue so that you can be tested for AIDS?		
Have you been offered money to donate your tissue?		

*HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus; AIDS: Acquired immunodeficiency syndrome.*

**Table 1.**  
*Living tissue donor questionnaire.*

It is important to mention that patients submitted to bariatric surgery with a secondary weight loss, have cutaneous structure and histological changes. Including a lower fibroblast and elastin concentration and collagen fiber organization, sites of chronic inflammation, sebaceous glands infections and metalloproteinases levels similar to the skin of patients with cancer and burns. Therefore, it is expected that the quality of TSAs from this group of patients will be lower [19, 20]. After the donor selection, the process is divided into 10 stages: 1) surgical procedure/body contouring surgery, 2) procurement itself, 3) packaging and identification, 4) storage, 5) transport to the processing center, 6) processing, 7) cryopreservation/quarantine, 8) validation, release, records, 9) transfer and 10) clinical use. All of the above must be within the framework of an organ and tissue donation program that permits the generation and guarantee products of high: biological quality, sanitary safety and therapeutic value [21].

### *2.2.1 Surgical procedure/body contouring surgery*

The skin processing is performed in the operating room, at the same time as the body contouring surgery, with all asepsis and antisepsis measures, under general anesthesia and by the same surgical team. After the redundant skin marking and the adipose skin flap dissection, the resection of the flap is performed. Subsequently, two teams are formed: one finishes the body contouring surgery and the other, on a separate surgical table, performs the skin procurement.

### *2.2.2 Skin treatment*

The dissected adipose skin flap is placed on a separate operating table. Total skin procurement (including dermis) is performed, removing the fat from the deeper dermis with scissors (**Figure 2**). Tissue samples (3) are also taken for current (aerobic), anaerobic and fungal culture. The procured skin is placed in a sterile container with 500 cc of physiological saline solution with 1 g of Cloxacillin and 80 mg of Gentamicin, hermetically closed, making sure the skin is completely submerged. This process of the allograft obtention gives the second distinctive characteristic to the TSAs: they are total skin allografts.

### *2.2.3 Packaging and skin identification*

The vials containing the skin are stored in double sterile bags of at least 90 microns. Each vial is hermetically sealed, removing as much air as possible, and labeled with the tissue code and the date and time of processing.

### *2.2.4 Skin storage*

The skin is transferred from the operating room to a temporary storage place in a refrigeration unit, in order to maintain the temperature between 2 and 8°C.

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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
    - a. Any type or manifestation of Dementia.
    - b. Alzheimer's disease.
    - c. Parkinson's disease.
    - d. Multiple sclerosis.
    - e. Creutzfeldt-Jakob disease.
  - Infectious diseases
    - a. Bacterial encephalitis.
    - b. Viral, fungal or parasitic meningitis.
    - c. Bacterial meningitis.
    - d. Progressive multifocal leukoencephalopathy.
    - e. Subacute sclerosing panencephalitis.
    - f. Active viral encephalitis or encephalitis of unknown cause.
    - g. 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).

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

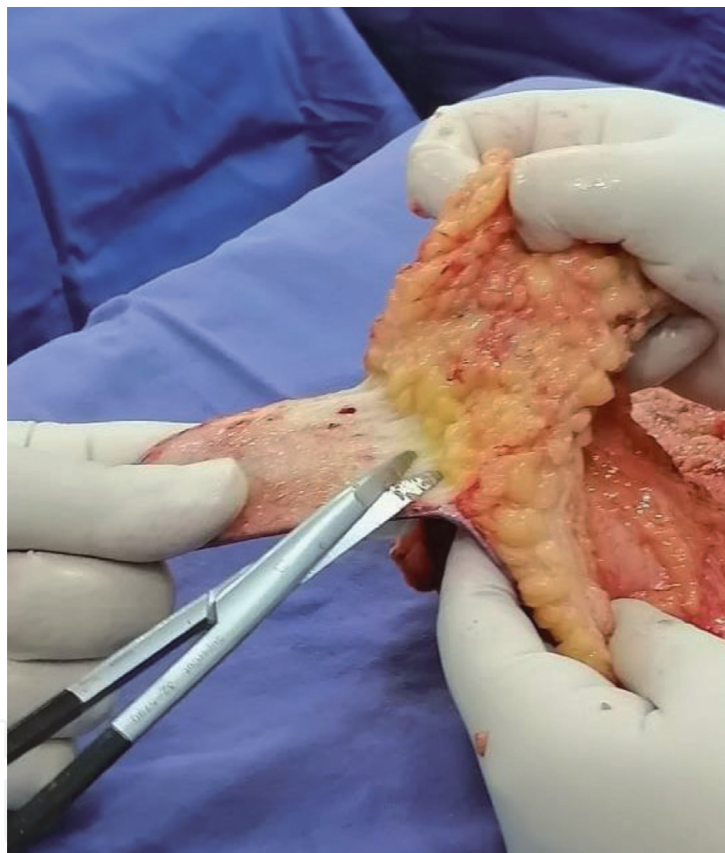
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**Table 2.**  
*Exclusion criteria for skin donation.*

1. Hepatitis B surface antigen.
2. Antibodies against Hepatitis C.
3. Antibodies against HIV.
4. VDRL
5. HTLV I and II.
6. Chagas.
7. Cultures intraoperative
  - Standard culture (aerobic)
  - Anaerobic culture
  - Mycotic culture.

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

**Table 3.**  
*Laboratory tests.*



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

### *2.2.5 Skin transportation*

The skin is sent for processing, in our particular case, to the National Tissue Bank (NTB), always within 36 hours from harvesting, assuring the container maintains a temperature between 2 and 8°C.

### *2.2.6 Processing*

The TSAs are prepared in the NBT as implants, following two stages: In the first one, the measurement of the flap is conducted, performing cuts in segments of

different sizes according to the request; for example, in an average abdominoplasty, approximately 300 cm<sup>2</sup> of useful surface are obtained.

Subsequently, a revision of the shaving, washing cycles (to reduce the microbial load), obtention of sample number 2 for cultures (the first one was intraoperative) and immersion in cryopreservative solution for 1 hour are performed. In the second stage, the preparation, cutting, measurement, packaging and labeling of each obtained flap is conducted. Samples number 3 and 4 are taken for culture, which are performed during the packaging stage. Finally, a 5th sample is taken, also for culture, as a backup.

#### *2.2.7 Cryopreservation and quarantine*

Cryopreservation freezes the TSAs in the presence of a cryoprotectant (10% glycerol), which prevents the crystallization effects while maintaining viability over time after freezing. Viability being understood as the capacity of a biological unit to remain alive. The cryopreserved total skin allograft (CTSA) is kept frozen at  $-80^{\circ}\text{C}$  awaiting the results of serial cultures until irradiation. The latter is performed with a dose of 25 to 28 kGy, in dry ice to maintain the cold chain, being a complementary procedure for tissue sterilization. The viability of the tissues obtained gives the third distinctive characteristic to CTSA, which is fundamental for the clinical results [22, 23].

#### *2.2.8 Validation, release and records*

An adequate quality management system requires having a dossier or record of each tissue processed, which allows the evaluation of each of the steps from the generation to the implantation of the CTSA in the recipient, permitting the monitorization and traceability. Once the donation, procurement and processing procedures have been reviewed and the microbiological results have been verified, the tissues are released for clinical use.

#### *2.2.9 Transportation*

Following the tissue release, they are transferred for clinical use on dry ice, maintaining the cold chain ( $-80^{\circ}\text{C}$ ).

#### *2.2.10 Clinical use*

In the preoperative period, the size of the defect to be covered should be calculated in order to choose the CTSA. The CTSA should be washed 3 times with warm saline (no more than  $40^{\circ}\text{C}$ ) to remove cryoprotectants. The wound bed is prepared by resection of necrotic, devitalized tissues and areas with granulation disorder. The CTSA is fixed with stitches and/or brackets associated with negative pressure therapy [24].

### **3. Clinic**

The CTSA has an initial attachment similar to an autologous skin graft, to later evolve towards rejection, at 21 days average, which is clinically evidenced by coloration change and the formation of a superficial necrotic eschar. When the latter is removed, it exposes vital tissue adhered to the host (**Figure 3**).





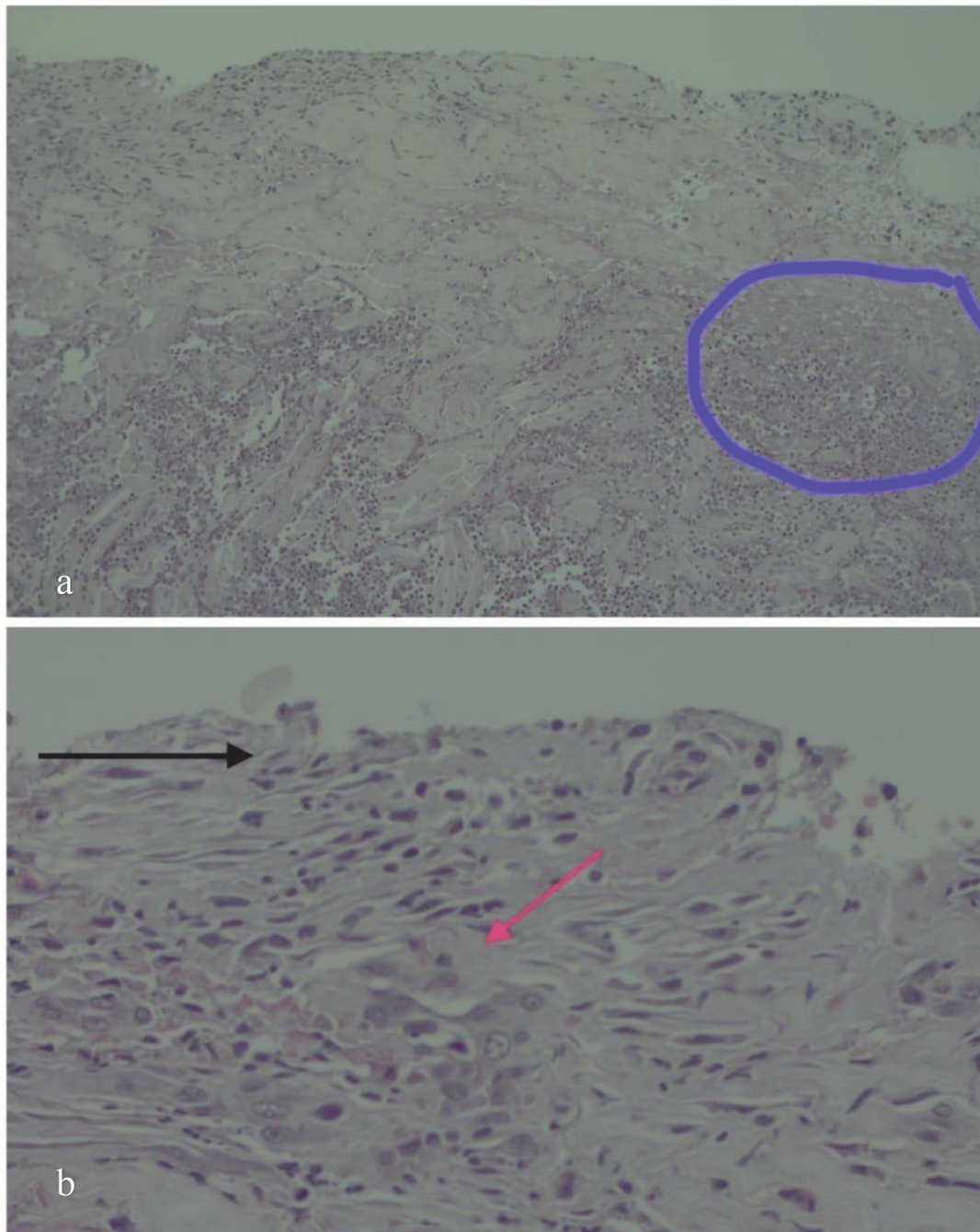
**Figure 3.**

*Diabetic patient, 68 years old, with flap necrosis after transmetatarsal amputation. a. Coverage deficit with bone exposure associated with disordered granulatory tissue. b. Coverage with CTASs. c. Evolution CTASs after 28 days. d. Receiving bed after escharectomy of superficial part of CTASs. e. Partial skin autograft, result at day 7, which was subsequently lost in its entirety and managed with advanced healing. f. Result: 4 months after initial surgery.*

This is evidenced histologically in the CTASs by necrosis foci with infiltration mainly of nuclear polymorphs (**Figure 4a**) and in the recipient bed by the presence of an interface rich in fibroblasts and neoformation vessels (**Figure 4b**).

This interface or neo-dermis is also visualized with imaging studies; in magnetic resonance imaging (MRI) CTASs can be seen with a superficial non-catching component and a deep component that is enhanced by the contrast medium, similar to the vascularized dermis (**Figure 5**) [25]. For SA, it had already been described that some elements of the dermis, mainly fibroblasts, could be permanently integrated into the host, with exceptional situations of complete attachment and absence of rejection of the SA. The most mediatic case involved Sir Winston Churchill, who during the Nile War, donated skin and it was grafted to a wounded comrade and “remains there to this day and has lasted well in many ways. Myself, keep the scar as a souvenir.” [26–28].

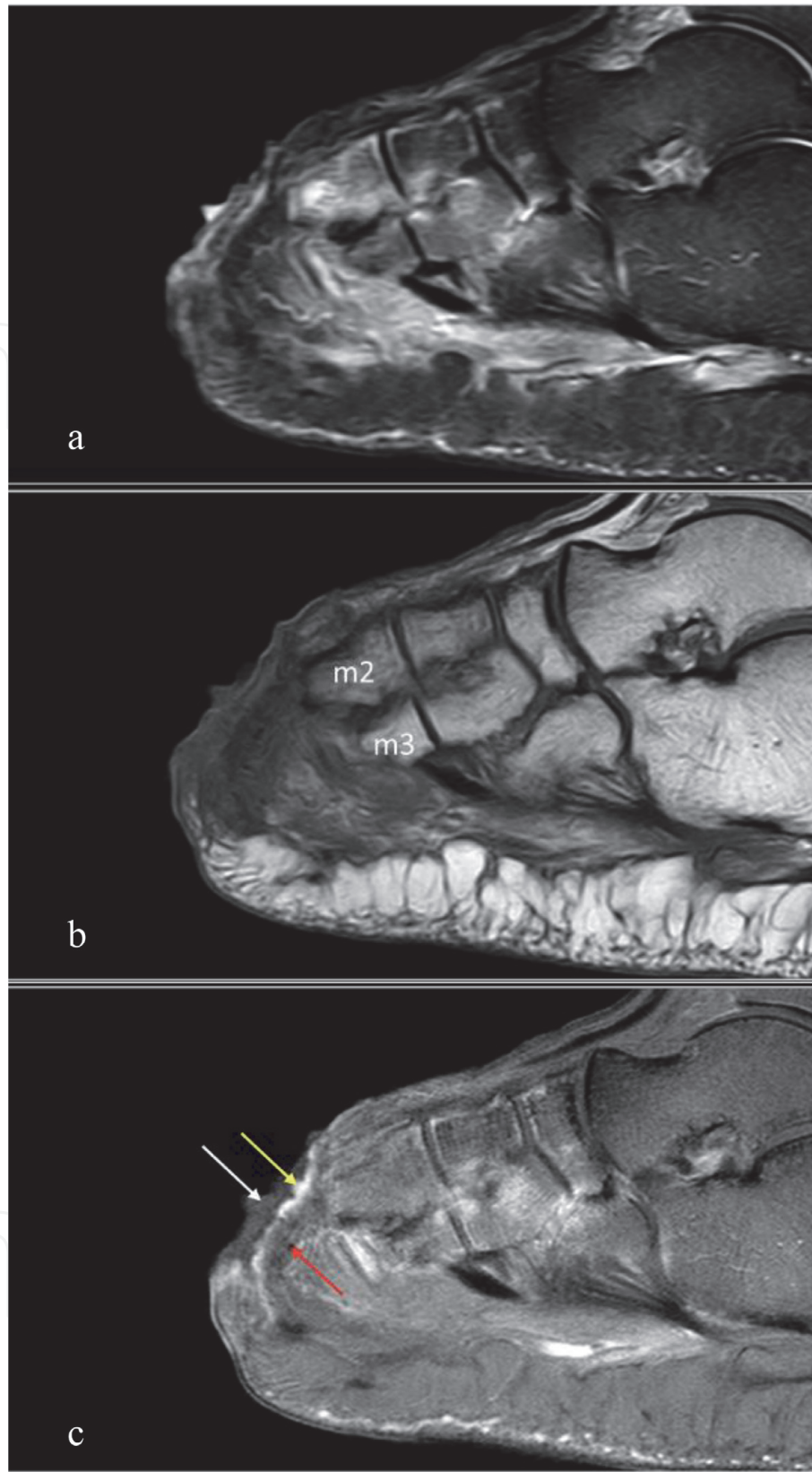
CTASs maintain the entire donor dermis, which participates in the proliferation of a neo-dermis rich in fibroblasts and neoforming vessels, also acting as a scaffold and a biological inducer in the wound bed. CTASs have been shown in xenograft models to promote angiogenesis and type 1 collagen production without eliciting a significant fibrotic response [29]. Rejection of the CTASs should not be interpreted



**Figure 4.**  
*a. Histology. Hematoxylin/eosin stain. CTSA at the time of escharectomy (21 days). Circled area shows infiltration of nuclear polymorphs, necrosis areas. b. Post-CTSA receptor bed. Black arrow: Fibroblasts, red arrow: Neoformation vessels.*

as a therapeutic failure, but as a stage of what we have called intermediate coverage. Since part of the CTSA is lost and part adheres to the recipient bed, obtaining an interface of neodermis allows the completion of the definitive coverage with an autograft and a complete healing by second intention. This also produces a secondary contraction of the wound, reducing size of the defect.

The increased survival of seriously burnt patients with severe sequelae, mainly due to the limited contribution of the autologous partial skin graft dermis and second intention scarring of the burnt skin, was the main motive for the dermal matrix development. Currently, available dermal substitutes (biologic, synthetic or biosynthetic origin) are fundamentally used as a scaffolding or biocompatible structural support, that is colonized by the patient's cells in order to form a neodermis, which is practically identical in structure as the recipient's dermis [30–34].



**Figure 5.** Magnetic resonance images of the foot in the sagittal plane enhanced in STIR -short tau inversion recovery- (a), T1 (b) and T1 with fat saturation and contrast medium (c), showing the graft with a superficial non-capturing component (white arrow) and a deep component that enhances with the contrast medium (yellow arrow) similar to the vascularized dermis. Between the latter and the bone tissue, there is low signal material with discrete enhancement (red arrow). (m2: Second metatarsal; m3: Third metatarsal).

It is important to emphasize that dermal regeneration templates share various advantages with CTSA, including: a) immediate wound closure, thus avoiding fluid loss and restoring the functional barrier of the skin, b) coverage of structures

such as bone, cartilage and tendons and c) better scar quality and elasticity. But they differ mainly in the high cost of dermal regeneration templates, which is the main reason for their limited use in clinical practice, and the fact that they necessarily

Patient	Age	Genre	Diagnosis	Evolution
1	68 Y	Masc	Diabetic foot. Transmetatarsal amputation	Surgical debridement / bone resection / CTSA / Scarectomy / Split-thickness skin graft / graft loss / Advanced wound healing / <b>Figure 3.</b>
2	26 Y	Masc	Complex wound of the leg	Surgical debridement / CTSA / Advanced wound healing / Complete healing and closure
3*	53 Y	Fem	Relapsing Dermatofibrosarcoma of the scalp	Tumor resection / Skull Fenestration / CTSA / Advanced wound healing. <b>Figure 6.</b>
4	55 Y	Masc	Diabetic foot. Transmetatarsal amputation	Surgical debridement / CTSA / Scarectomy / Split-thickness skin graft / Loss of follow-up
5	70 Y	Masc	Abdominal sepsis. Open abdomen	Complicated inguinal hernia / abdominal sepsis / multi-organ failure / contained laparostomy, CTSA / clinical improvement/ extubation / Death due to pneumonia
6*	2 M	Masc	Premature. Necrotizing enterocolitis. Contained laparostomy.	Premature 30 weeks / Dysfunctionalisation of intestinal transit / evisceration (2 occasions) / Contained laparostomy / CTSA / Para-ostomal hernia and inguinal hernia. Periostomal and inguinal hernioplasty. <b>Figure 7.</b>
7	60 Y	Masc	Diabetic foot	Surgical debridement / scarectomy / CTSA / Split-thickness skin graft / 90% success / Loss of follow-up
8	49 Y	Masc	Diabetic foot	Surgical debridement / scarectomy / CTSA / Loss of follow-up
9	57 Y	Masc	Skin stripping of the leg	Scarectomy / CTSA / Split-thickness skin graft / 100% success.
10*	3 M	Masc	Premature. Necrotizing enterocolitis. Contained laparostomy.	Premature 30 weeks / CTSA replacement / Abdominal wall continent / Second intention healing. <b>Figure 7.</b>
11*	53 Y	Fem	Relapsing Dermatofibrosarcoma of the scalp	CTSA replacement in the central área / No autologous graft due to absence of neodermis in central area (initial bone wax in this area). <b>Figure 6.</b>
12	75 Y	Masc	Melanoma of the cheek	Melanoma (biopsy confirmed) resection. Local flap reconstruction.
13	83 Y	Masc	Melanoma of the forearm	Melanoma resection. Staging in process.
14	56 Y	Masc	Diabetic foot	Surgical debridement / bone resection / CTSA / Scarectomy / Split-thickness skin graft / Advanced wound healing / complete wound closure
15	31 Y	Fem	Necrotizing fasciitis of the forearm	Surgical debridement / CTSA / Awaiting progress for skin autografting.

Years (Y), Months (M), Feminine (Fem), Masculine (Masc), CTSA: Cryopreserved total skin allograft. \* Patient undergoing two interventions with cryopreserved total skin allograft.

**Table 4.**  
 Clinical experience. Summary.

require a second time for skin autografting. Regarding the latter is that CTSA's can be used as an alternative to dermal regeneration templates [35, 36].

#### 4. Clinical experience

Our clinical experience was initiated on August 1st, 2020 and persists to the date. Mainly following the pursuit of cutaneous coverages for seriously burnt patients, given the lack of dermal regeneration matrix since their high cost and limited availability. The latter is supported by the disposal of concomitant residual abdominal skin grafts following abdominoplasty surgeries. The donor sample was constituted by fourteen female patients, of 31 to 55 years with an average age of 40 years. The receipt sample was composed by 13 patients (two patients underwent two surgical procedures), ages ranging from 2 months to 83 years. With the following diagnosis: Diabetic foot (5), contained laparostomy (2) complex extremities wounds (3), relapsed scalp sarcoma (1) and melanoma (2) [37]. Clinical evolution is summarized in **Table 4**.



**Figure 6.** Female patient, 53 years old. With a history of dermatosarcoma of the scalp. With resection and subsequent reconstruction with local flaps and partial skin grafts. Tumour recurrence. a. Dermatosarcoma of the scalp. b. Defect of coverage post resection. c. Fenestration of the skull. d. CTSA 30 days. e. CTSA 60 days f. CTSA 150 days, completely healed wound by second intention.



**Figure 7.**  
*30-week preterm infant. The patient evolves with necrotizing enterocolitis, requiring intestinal transit defunctionalization and evisceration on two occasions. a. Laparotomy contained. b. Immediate postoperative CTSA on open abdomen. c. Postoperative 21 days. d. Postoperative 35 days. e. Postoperative 60 days, protrusion of paraostomal hernia associated with Valsalva is observed, however the CTSA area shows continent, in addition to smaller size. f. Postoperative 90 days with healing by second intention, without intra-abdominal protrusion. Paraostomal hernias and transit reconstitution, associated with CTSA change performed on day 70.*

## 5. Specifications

The therapeutic uses of CTSA can be numerous. As we previously stated, we emphasize in our experience the diabetic foot, extensive and complex coverage deficits, contained laparotomies and post skin tumor resections, awaiting the biopsy results for an eventual margin enlargement and reconstruction (Figures 6 and 7) [38–42]. CTSA can cover bone, cartilage and tendon exposures, being a simple alternative for the management of complex wounds, becoming an intermediate coverage for some patients and definitive for others.

## 6. Conclusions

The distinctive characteristics of CTSA (from living donor, full-thickness skin, and cryopreserved, therefore viable tissues) permits the obtention of a new type of

coverage, which we have called intermediate coverage, easy to use, more economical than current alternatives and with numerous clinical indications.

## **Acknowledgements**

The present study is an initial clinical experience, not a clinical trial, and was conducted under the supervision of the National Coordination of Organ and Tissue Procurement and Transplantation of the Chilean Ministry of Health.

## **Conflict of interest**

The authors declare no conflict of interest.

## **Appendices and nomenclature**

SA	Skin allograft
TSA	Total skin allograft
CTSA	Cryopreserved total skin allograft
NTB	National Tissue Bank
MRI	Magnetic Resonance Imaging

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