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# Organization of Hannover Skin Bank: Sterile culture and procurement protocols for viable cryopreserved allogeneic skin grafts of living donors

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## Abstract

Preserved allogeneic donor skin still represents one of the gold standard therapies in temporary wound coverage in severely burned patients or chronic wounds. Allogeneic skin grafts are currently commercially available as cryo- or glycerol-preserved allografts through skin tissue banks all over the world. Most of the skin tissue banks rely on human cadaveric skin donations. Due to the chronic shortage of human allogeneic transplants, such as skin, and increasing costs in the procurement of allografts from other skin tissue banks, Hannover Medical School has been building up its own skin tissue bank based on allogeneic skin grafts from living donors who underwent surgical treatment (i.e., body-contouring procedures, such as abdominioplasties). This article presents procedures and protocols for the procurement and processing of allogeneic skin grafts according to national legislation and European regulations and guidelines. Beside protocols, initial microbiological data regarding the sterility of the harvested grafts are presented. The results currently form the basis for further investigations as well as clinical applications. In summary, a microbiological testing and acceptance procedure is presented that ensures adequate patient safety and skin viability.

**KEYWORDS**

allografts, skin, skin transplantation, tissue banks, tissue donors

## Key Messages

- Vital donor skin was successfully used to establish a departmental skin tissue bank for the care of severely burned patients.

## 1 | INTRODUCTION

Plastic reconstructive surgeons are faced with various problems when treating patients who suffer from severe skin defects, with a high percentage of skin surface loss due to burn injuries or chronic wounds.[1] In severely burned patients, life-threatening conditions can occur due to defect size, local and systemic inflammation or fluid and thermal losses.[2] In addition to complex and comprehensive intensive care in specialized burn centres, the surgical debridement plays a significant role in the multimodal treatment concept to enable sufficient defect coverage.[1,3] Skin grafting has been practised by European surgeons for a little more than 200 years and can be considered as a standardized procedure nowadays.[4] Despite intensive research efforts, autologous split-thickness skin grafting still represents one of the surgical gold standards in the treatment of burn injuries, as no skin substitute or transplant has yet succeeded in sufficiently replacing the function of original skin.[5] Autologous skin grafting shares the main disadvantage of limited donor sites, especially in severely burned patients.[6] Therefore, particularly in large-area burns, xenogeneic materials, such as porcine skin, are used for temporary defect coverage.[1,7]

Allogeneic donor skin still represents another therapeutic option in temporary wound coverage in severely burned patients or chronic wounds.[8,9] Allogeneic skin grafts act as mechanical and biological barrier to reduce fluid and protein loss and decrease the risk of infection.[10] Furthermore, decreased wound pain as well as low frequency of dressing changes have been described in literature.[11] As published earlier, most allogeneic skin grafts are usually obtained from cadaveric donors sharing all of the disadvantages of allogeneic transplants such as disease transmission, immunogenicity, rejection and secondary infection.[7,12,13] Skin allografts are currently commercially available through skin and tissue banks all over the world to enable a better availability and to ensure patients' safety and high product quality.[8,14]

The history of skin tissue banking dates back to the 1990s.[15–17] Harvesting and culture procedures aim to ensure that the largest part of the donated skin is transferred into viable skin allografts for transplantation purposes.[18] It is essential to establish largely sterile harvesting and culture conditions to ensure pathogen-free transplants for further clinical use.[18] There are currently a number of protocols to preserve allogeneic skin tissue such as cool storage (+2 to +8°C), cryopreservation (−20 to −196°C), deep freezing, freeze drying or dehydration using high-concentration solutions such as glycerol.[18–20] Based on the different preservation protocols, the properties of allogeneic skin transplants are influenced to different degrees regarding immunogenicity, skin viability, integrity and microbiological contamination.[19,21,22] Glycerol-preservation can reduce antigenic properties of allogeneic skin grafts, but results in non-viable tissue.[23,24] As published elsewhere, skin allografts cryopreserved with dimethylsulfoxide retained higher viability than glycerol cryopreserved skin allografts.[19] Nevertheless, glycerol-preserved skin is successfully used in burn surgery.[1,25] Cryopreservation procedures are considered to be the superior methods for long-term skin preservation as the allogeneic skin grafts retain a certain level of viability.[18,26] As described elsewhere, the microbiological contamination of donor skin is significantly affected by the type of processing with the highest levels of microbiological and fungal contamination found in cryopreserved donor skin which was not further processed.[27]

Human skin is naturally colonized with a mixture of commensal microorganisms, most of which are harmless or even beneficial to their host.[28] As published by the Lille Tissue Bank, out of 104 deceased donors with in total 433 cryopreserved skin allografts 42 (40.5%) had at least one sampling zone with a positive microbiological test resulting in 106 (24%) contaminated skin samples. However, the contamination rate did not vary according to the harvested zone or type of donor.[29] The use of antiseptic alcohol-containing solutions prior to skin harvesting can reduce the skin microbiome, but a complete elimination is not feasible.[30] Therefore, harvested donor skin is often stored in medium containing antibiotics for transportation and cultivation.[18] These must be cleared, washed away or neutralized prior to microbiological or fungal screening before the skin tissue sample can be approved for transplantation.[26,29] Comparing disinfection protocols, it was determined that the greatest reduction in skin allograft contamination rates could be achieved by utilizing 0.1% peracetic acid or 25 kGy of gamma irradiation at lower temperatures.[30,31] Ideally, the microbiological screening strategy for cryopreserved skin allografts should ensure a total absence of relevant pathogenic microbiological or fungal microorganisms as well as the absence of substantial bio burdens of inherent skin commensals.[26] However, skin tissue allografts still can be accepted for further clinical use without decontamination when microbiological and fungal cultures only reveal low bioburdens of inherent inhabitants of the residential skin microbiome.[18,26] The harvesting and culture procedures of skin allografts as well as their microbiological and fungal screening are much more complex than for sterile products. Therefore, it is difficult to establish realistic and ethically acceptable guidelines that meet the applicable legal framework of the European Union and the implementing countries, such as Germany.[26,32] As published elsewhere, various protocols and harvesting procedures are applied by different skin tissue banks.[33] In 1985, the Brussels military skin bank (Brussels, Belgium) was

established as one of the first skin banks to supply cryopreserved and viable allogeneic human skin for therapeutic purposes. Over the years, the Brussels military skin bank established detailed microbiological and fungal screening protocols and approval procedures to ensure acceptable microbiological colonization.[26] As published elsewhere by a tissue bank in Southern Brazil, there is a significant association of a lower average donor age and the presence of microbial colonization of harvested skin allografts.[34] Furthermore, they observed that Gram-negative bacteria were associated with male gender, source hospital and an over 7-day stay in an intensive care unit prior to skin procurement.[34] Besides the microbial burden, the viability of the skin grafts is a crucial issue as well. The skin bank of Verona (Verona, Italy) observed a mean percentage viability of 45.1 ± 20.1% in cryopreserved allogeneic skin grafts, which is similar to results obtained in other skin tissue banks.[35] It was noted that viability decreased with increasing age of the donor.[36]

Over the years, there has been an increase in the demand for skin allografts worldwide.[37–39] Since the foundation of the Donor Tissue Bank of Victoria (Melbourne, Australia) in 1994, the demand for skin allografts for the treatment of burns in Australia has steadily increased and demand always exceeded supply.[40] Being Australia's only operational skin bank for processing cryopreserved human cadaveric skin for the treatment of burns, there is a need to develop strategies to enable increased provision of skin allografts on a national level.[40] Furthermore, there are many other issues to be faced in the day-to-day running of a skin tissue bank such as logistical difficulties, shortage of staff and financial resources as well as a relative lack of public awareness of skin donation.[41] The costs of production as well as processing are also not to be neglected and result in correspondingly high costs for the external procurement of allogeneic skin grafts.[42,43]

The Department of Plastic, Aesthetic, Hand and Reconstructive Surgery at Hannover Medical School (Hannover, Germany) covers the entire spectrum of reconstructive and plastic surgery and represents as the Lower Saxony Burn Center, a cross-regional provider of burn care. Therefore, there is a local need for allogeneic skin grafts in daily clinical practice for the treatment of extensive burns and chronic wounds. In the light of the chronic shortage of human allogeneic skin grafts and increasing costs in the purchase of allogeneic skin grafts from other skin tissue banks,[10,44] Hannover Medical School has been building up its own skin bank for several years now. The aim was the establishment of a regional skin bank for meeting local demand for viable allogeneic skin transplants. Different from other skin tissue banks, Hannover Skin Bank operates on tissue donations from living donors. Body-contouring operations, such as abdominoplasties or thigh lifts, are performed regularly as part of the clinic's surgical business. The resected tissue in the process has been discarded to date, so that reuse also contributes as a decisive aspect to the issue of sustainability. The present study summarizes the results of the approach to establish a skin bank on a regional (in-house) level. In addition to outlining the procurement and processing protocols, initial microbiological data regarding the sterility of the harvested grafts is presented.

## 2 | METHODS

### 2.1 | European and German laws and directives

In the present study, all procedures and protocols for the procurement and processing of allogeneic skin grafts followed national and European regulations and guidelines such as the European Human Cell and Tissue Directives 2004/23/CE, 2006/17/CE and 2006/86/CE, the German Medicines Law (AMG) and the German Transplantation Law (TPG). The safety of skin allografts and in particular the protection of the recipient against infections were of primary importance. All procedures were performed in accordance with the Declaration of Helsinki and the protocols were approved by the Ethics Committee of Hannover Medical School (Ethics Approval Number: 9101_BO_K_2020, Date of Approval: 25 May 2020). In addition, each patient gave his or her written consent, which could be withdrawn immediately before the procedure without following consequences and without giving reasons. The release procedures for skin grafts were defined in such a way that compliance with the necessary quality and safety specifications for collection, testing, storage and distribution to manufacturers and processors was ensured throughout the entire workflow in accordance with §13 and §20c of AMG.

### 2.2 | Donor population and exclusion criteria

Hannover Skin Bank uses allogeneic skin grafts from living donors who undergo surgical treatment at the Department of Plastic, Aesthetic, Hand and Reconstructive Surgery at Hannover Medical School (Hannover, Germany). All patients between the age of 18 and 80, who underwent body-contouring surgery, were screened as potential donors. The exclusion criteria were defined in accordance with §8 of TPG. Table 1 gives an overview of all in-house exclusion criteria for skin donations to Hannover Skin Bank. Prior to the skin donation,

**TABLE 1** Exclusion criteria for skin donations to Hannover Skin Bank.

| |
|---|
| Existing injuries in the tissue area intended for donation (scars/lesions/open wounds) |
| Burns in the tissue area intended for donation |
| Dermatitis/Skin rashes |
| Local infections |
| Ectoparasites |
| Deformities |
| Tattoos |
| Cancers of the skin |
| Autoimmune dermatoses |
| Connective tissue diseases |
| Systemic infections |
| Diabetes |
| Dermal mucinosis |

suitable donors underwent a medical assessment and examination as well as blood tests for serological donor screening for pathogens, such as cytomegalovirus, hepatitis B and C virus, human immunodeficiency virus (HIV) and *Treponema pallidum*. In addition, all donors received a detailed pre-treatment consultation and gave his or her verbal and written consent. Overall, skin donations from 24 donors have been included in the current proof-of-concept study. All skin donations originate from resected skin excess after abdominoplasties.

## 2.3 | Local processing facilities and construction requirements

A laboratory room of the department, which was initially equipped for use on safety level 1 (according to German Genetic Engineering Act), was adjusted to clean room level D with a laminar air flow cabinet with clean room level A function to obtain the necessary manufacturing authorization by the local trade supervisory authority. Clean room qualification in operating and resting condition was certified according to EN ISO 14644-1/2 and inspections are regularly carried out every 6 months. All processing procedures were performed in the laminar flow cabinet. Prior to commencing operations as a tissue bank, Hannover Skin Bank was audited and certified by the local trade supervisory authority. The room and its equipment are cleaned and disinfected once weekly as well as before and after each skin processing. All Hannover Skin Bank staff work under strict hygiene guidelines according to good manufacturing practice (GMP) criteria and internal standardized operating procedures.

## 2.4 | Skin procurement procedures

A patient number was assigned to each patient and sample. Date and time of donation as well as anatomical region of the donor site were recorded so that the tissue could be traced or identified at any time. The skin donations were obtained under sterile conditions in the operating room by an experienced surgeon of the department and all procedures followed good clinical practice according to standardized operating protocols. The donors received an adequate triple pre-operative scrubbing and disinfection of the operating area including the donor site with octeniderm® (Schülke & Mayr, Norderstedt, Germany). Local sterile field was established using sterile drapes according to internal standardized operating protocols to effectively prevent microbial contamination. After resection of the skin excess during body-contouring operations, the resected skin with attached subcutaneous adipose tissue was transferred to a separate sterile instrument table. Another double disinfection procedure with octeniderm® (Schülke & Mayr) was performed for 3–5 min and it was waited for a complete evaporation of the disinfectant in order to minimize the microbial contamination. Three surgeons operating under sterile conditions performed the skin procurement. The donor area was covered with sterile paraffin oil and split-thickness skin grafts were cut by a battery-operated dermatome (Aesculap, B.Braun, Melsungen, Germany) in thicknesses of 400–600 μm. It was ensured that the skin was removed as a uniform and coherent stripe. Each skin stripe was placed on sterile gauze (Fuhrmann, Much, Germany) and rolled up. The wrapped skin was transferred to a sterile absorbent bag (Mölnlycke Health Care, Düsseldorf, Germany) that was tightly sealed. Every skin stripe was placed in a separate absorbent bag to reduce cross-contamination of the procured tissue. Immediately after skin procurement and packaging, the skin samples were placed in a transport box (Transmed, Regensburg, Germany) and were picked up by a member of the Hannover Skin Bank. The samples were transported directly (max. transportation time 30 min without cooling) to Hannover Skin Bank. The exact transportation time as well as compliance with the collection instructions were documented with all deviations in the skin donation protocol. Upon reaching Hannover Skin Bank, the samples were further processed without delay.

## 2.5 | Skin processing and sampling

The skin tissue was processed in the clean room's laminar air flow cabinet immediately after delivery. Only one skin sample was processed at a time. If several skin

samples from one donor were available, intermediate cleaning and disinfection of the work area was performed after processing each sample. All procedures were carried out at room temperature. During processing, all instruments, solutions and materials as well as all surfaces that came into contact with the skin tissue were sterile. Staff was equipped with sterile scubs, surgical masks, hair nets, overshoes or clean room shoes and sterile gloves. The skin donation was removed from the transport container, freed from the wrapping material and transferred to a sterile surgical drape. The donor tissue was macroscopically examined and qualitatively evaluated. Approximately 1 $cm^2$ of skin was removed from each skin stripe for microbiological testing. Microbiological samples were transferred to 50 mL sterile sample vials (Landgraf Laborsysteme, Langenhagen, Germany) and stored in a refrigerator at 4°C until transport to the Department of Microbiology at Hannover Medical School. Subsequently, the skin was cut into rectangular pieces, measuring approximately 4 × 15 cm, with sterile disposable scissors. The length of the grafts procured varied as the length of the grafts varied according to the size of the donor side. Each graft was placed in a 50 mL sterile sample vial (Landgraf Laborsysteme) and filled up with sterile phosphate buffered saline (PBS) (GMP grade; Thermo Fisher Scientific, Waltham, MA). The samples were washed for 3 min with a swinging motion and afterwards transferred to a fresh sample vial. The previously described washing procedure was performed five times. After washing, another 1 $cm^2$ of skin was removed for microbiological testing as described above. The cut skin grafts were covered from both sides with sterile gauze measuring 4 × 20 cm (Fuhrmann). The final graft size was measured with a sterile ruler. Skin grafts covered this way were furled and transferred into 5 mL freezing tubes (Biobank Tube Matrix; Landgraf) that were used for final storage in the nitrogen gas phase. The freezing tubes were filled with 3 mL cryopreservation solution (Stem-Cellbanker DMSO Free-GMP Grade freezing medium; amsbio, Abingdon, UK), pre-cooled to +4 to +8°C with a Perfusor® syringe (B.Braun SE). Each tube was sealed and labelled appropriately. Prior to transport to Hannover Unified Biobank for final storage, the skin samples were stored in a refrigerator at +4 to +8°C.

## 2.6 | Microbiological and fungal screening and cultures

Forty-eight biopsies of donated skin tissue were harvested at different points of time throughout the workflow described above. All samples were pseudonymized and received individual bar codes, so that identification of donors was only possible for the staff of Hannover Skin Bank. Samples were placed in a transport box (Transmed) and transferred to the Department of Microbiology at Hannover Medical School within 20 min time of transportation. Sample integrity was documented in a form sheet. Following the guide for quality and safety of tissue and cells for human applications published by the European Directorate for the Quality of Medicines & HealthCare (EDCM) in 2017 and national guidelines and directives, detection of numerous microorganisms and potential pathogens in tissue allografts should preclude the use of these grafts. Table 2 lists those microorganisms. However, the list is non-limiting and could be updated in the near future by European directives.

**TABLE 2** Non-limiting list of microorganisms and pathogens that should result in tissue discard.

| Name of pathogen | Name of pathogen |
|---|---|
| *Acinetobacter baumanii* | Mucor |
| *Actinomyes* | *Mycobacterium tuberculosis* |
| *Bacillus anthracis* | *Neisseria meningitides* or *gonorrhoeae* |
| *Bacteroides* spp. | *Nocardia* spp. |
| *Burkholderia cepacia complex* | *Pseudomonas aeruginosa* |
| Carbapenem-resistant Enterobacteriaceae | *Salmonella typhi* or *paratyphi* |
| *Clostridium* spp. (*Clostridium perfringens* or *Clostridium tetani*) | *Shigella* spp. |
| *Corynebacterium diphteriae* | *Stenotrophomonas maltophilia* |
| *Erysipelotyhrix rhusiopathiae* | *Streptobacillus moniliformis* |
| *Fusobacterium* | *Streptomyes* spp. |
| *Listeria monocytogenes* | *Vibrio cholera* |
| Methicillin-resistant *Staphylococcus epidermidis* | *Yersinia pestis* or *pseudotuberculosis* |

*Note*: All pathogens are listed in alphabetical order.

The submitted skin samples were minced using an ULTA-TURRAX® high performance dispenser (IKA, Staufen, Germany) in ProbeAX tubes (Axonlab, Reichenbach/Stuttgart, Germany) with sodium chloride/disinhibitor broth (Axonlab). The resulting tissue solution was inoculated on solid and liquid media for culturing. Approximately 2 mL of the minced tissue solution were each transferred to BD BACTEC™ Plus aerobic and anaerobic medium bottles (Becton, Dickinson and Company, Franklin Lakes, NJ), incubated at 36°C for 14 days in BD BACTEC™ FX Blood Culture System incubators

(Becton, Dickinson and Company). Approximately 50 µL of tissue solution each were inoculated on solid media: Columbia blood agar (Becton Dickinson, Heidelberg, Germany), incubated aerobically at 36°C for 2 days, chocolate agar (Becton Dickinson), incubated at 36°C in 5% $CO_2$ athmosphere and on Schaedler agar (Becton Dickinson) for anaerobic conditions at 36°C for 4 days. Approximately 500 µL minced tissue solution were transferred into 10 mL thioglycolate broth (Thermo Scientific Oxoid, Wesel, Germany) and incubated at 20–22°C for 14 days. Thioglycolate broth cultures were checked for turbidity every 2 days. In addition, 50 µL of tissue solution was applied to yeast peptone agar (Thermo Scientific Oxoid) and cultured at 36°C for 2 days and another 8 days at 30°C.

In case of growth on any of the primary media, except yeast peptone, subcultures were performed on Columbia agar for aerobic culture at 36°C for 2 days, on Schaedler agar at 36°C for 4 days in an anaerobic environment and on chocolate agar, incubated at 36°C and 5% $CO_2$ for 2 days. For blood culture bottles the time to positivity was documented, for other media the first day when growth was detected.

Cultured microorganisms were identified to the species level by matrix-assisted laser desorption time-of-flight mass spectrometry. Susceptibility testing was not performed. The results were transmitted to the Hannover Skin Bank. In case of detection of any microorganisms listed in Table 2, the allograft was discarded. The evaluation and graphical presentation of the results shown here were performed using Microsoft Excel for Mac 2011 software (version 14.6.9) (Microsoft Corporation, Redmond, WA).

## 2.7 | Acceptance procedures and long-term storage

The long-term storage of the allogeneic skin grafts was performed by Hannover Unified Biobank (HUB) according to standardized operating protocols, as Hannover Skin Bank has no own cryopreservation facility yet. The tissue samples packed in the freezing tubes were frozen in an automatic freezer (Jutta Ohst german-cryo, Jüchen, Germany) for final storage at −180°C (nitrogen gas phase) in two stages in a controlled manner. For this purpose, the following program was run in the automatic freezer: −1°C/min to −60°C; −5°C/min to −120°C; −5°C/min to −150°C. After the samples had reached the desired temperature of −150°C, they were transferred to bag cassettes and stored in nitrogen tanks in labelled racks. After completion of the microbiological examinations after 14 days, the examined skin was marked as 'released for transplantation' by the responsible staff of Hannover Skin Bank and HUB. Release would only occur when the review of all necessary documentation and results confirmed that there was compliance of the skin tissues with their specifications, including final packaging. Sample management was performed with CentraXX software (Kairos, Bochum, Germany).

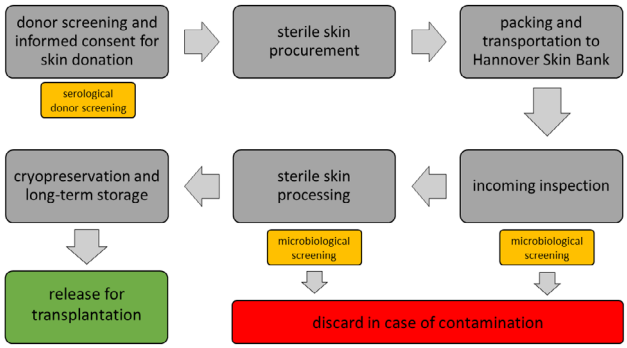


**FIGURE 1** Overview of the general workflow at Hannover Skin Bank.

## 2.8 | Viability assay after cryopreservation

To evaluate cell viability after defrosting of cryopreserved skin allografts, a LIVE/DEAD® Viability/Cytotoxicity kit for mammalian cells (Life Technologies, Darmstadt, Germany) was used according to the manufacturer's instructions. To reduce nonspecific background staining, tissue pieces were washed with PBS (Gibco, Thermo Fisher Scientific) prior to fluorescent microscopy using a Zeiss Axiovert 200M microscope and associated software (Carl Zeiss, Oberkochen, Germany). Viability assays were performed after 2, 7, 14 and 21 days of cryo storage. Images were assorted to plates for this publication with Adobe Photoshop CS5 (Adobe Systems, Dublin, Ireland).

# 3 | RESULTS

## 3.1 | Establishment of the local processing facilities and standardized aseptic work protocols

A laboratory room of the department, which was initially equipped for use on safety level 1 (according to German Genetic Engineering Act), was adjusted to clean room level D with a sterile cabinet with clean room level A function. In parallel, standardized work processes were established and structured in standardized operating protocols. Figure 1 shows an overview of the workflow of the established and certified work steps.

The department's own patient population was screened with regard to the donor criteria mentioned under Methods during the period of establishment of the skin bank. Patients meeting criteria listed in Table 1 were excluded. At least 24 donors were included in the present proof-of-concept study to validate and verify the established processes. The serological donor screening for pathogens such as cytomegalovirus, hepatitis B and C virus, HIV and *T. pallidum* remained negative for all included donors. All skin donations were obtained from resected skin excess after abdominoplasties. The skin donations were performed under sterile conditions in the operating room by an experienced surgeon of the department and all procedures followed good clinical practice according to standardized operating protocols as described above. Figure 2 shows the procurement procedure in the operating room.

Donor skin was harvested on a separate operating table with sterile and unused instruments. The required instruments can be seen in Figure 2A. The resected skin tissue was disinfected multiple times (Figure 2B) prior to harvesting of the skin grafts using a battery-operated dermatome (Figure 2C,D). All skin donations included in this study were taken at a thickness of 400 µm. Figure 2E shows a donor tissue piece. A total of three split-thickness skin sheets could be removed from the donor tissue shown in Figure 2E. The tissue donations were packed on gauze (Figure 2F), rolled up (Figure 2G) and sterile packed for further transport (Figure 2H). All skin donations were transferred directly to Hannover Skin Bank. During transport, no abnormalities or peculiarities occurred that had to be noted in the standardized documentation. Upon arrival at the skin bank, the standardized incoming inspection and the skin processing was performed as detailed in the Methods section. During the subsequent processing, samples were taken at two different time points for microbiological screening (see Figure 1). In short, skin donations were processed under sterile conditions and cut into defined sizes. At the same time samples for microbiological screening as process

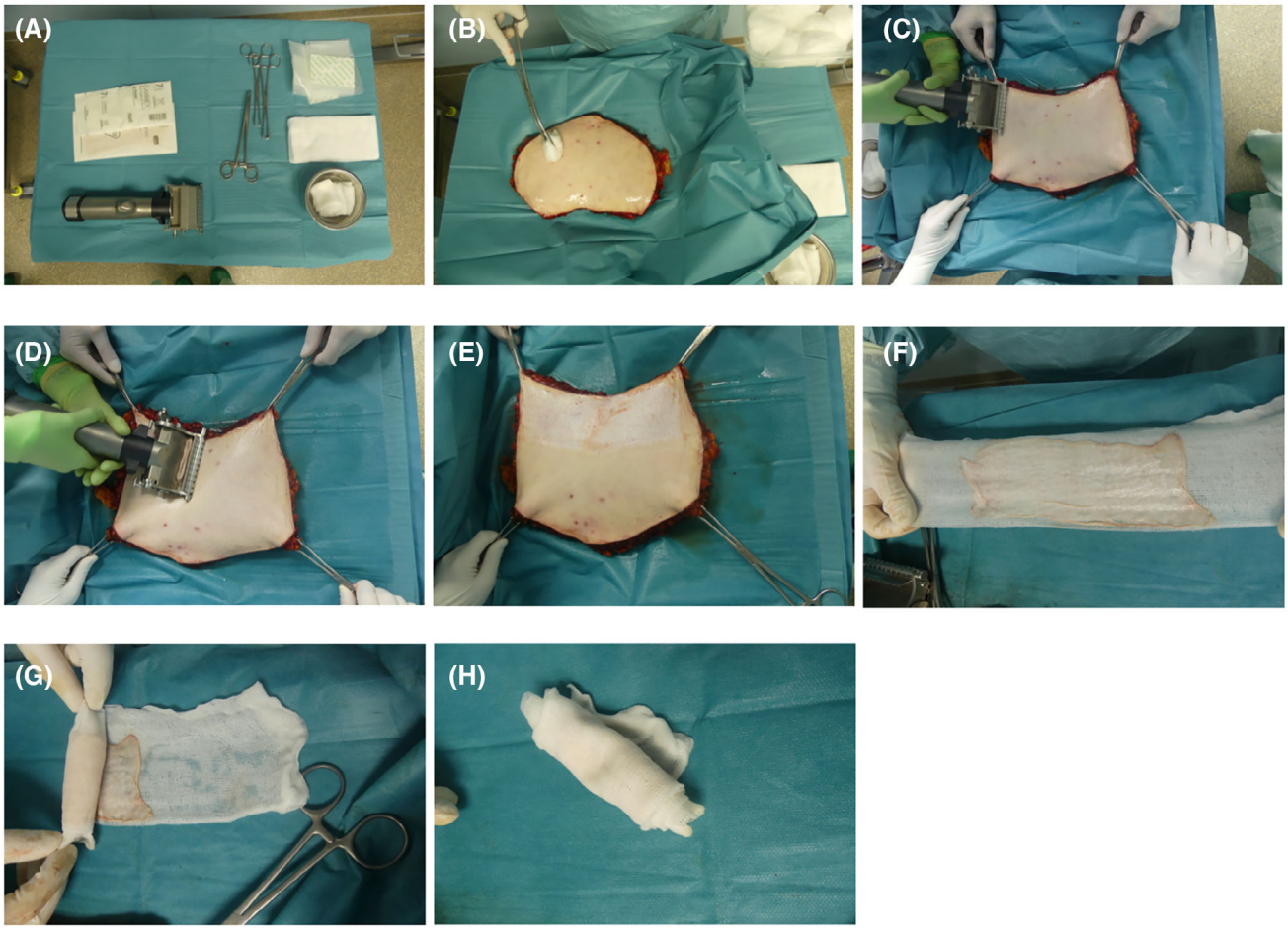


**FIGURE 2** Procurement procedure in the operating room. (A) Separate operating table with the required instruments. (B) The resected tissue is disinfected several times. (C, D) The tissue donation is harvested with a thickness of 400 µm using a battery-operated dermatome (Aesculap, B.Braun), with two assistants appropriately stretching the tissue. (E) Tissue after removal of the skin donation. (F) The removed skin donation on a sterile gauze. (G, H) The gauze with the skin donation is rolled up for further transport and taken directly to Hannover Skin Bank for further processing.


**FIGURE 3** Overview of skin processing at Hannover Skin Bank. (A) Skin preparation, cutting and washing. (B) Transfer of gauze wrapped allograft into biobank tube. (C) Allograft in biobank tube with cryo medium.

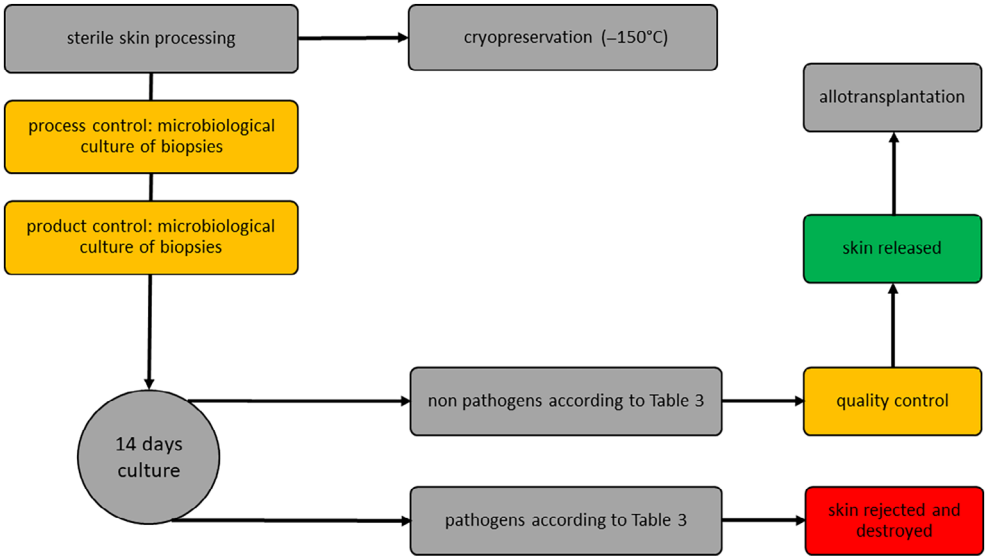


**FIGURE 4** Workflow for processing skin donations and microbiological and fungal screening resulting in acceptance decisions. Skin samples measuring 1 $cm^2$ were collected for microbiological analysis as process and product control. After 14 days of sterile and standardized culture, samples were evaluated and either released for transplantation or rejected and discarded.

control were collected (Figure 3A). After multiple washing steps, skin donations were wrapped in sterile gauze and transferred to biobank tubes (Figure 3B). Tubes were filled bubble free with cryo medium (Figure 3C). Each allograft was clearly labelled and further processed for cryopreservation and long-term storage.

Skin samples of 1 $cm^2$ were collected for microbiological analysis as process and product control upon incoming inspection and after skin processing (Figure 4).

A decision regarding acceptance or discarding of the prepared allogeneic skin graft was made after 14 days of microbiological cultivation based on the results (Figure 4, quality control). Table 3 lists the pathogens leading to discarding of the allograft and is based on the specifications and guidelines of the regulatory oversight agencies.

## 3.2 | Microbiological screening showed no pathogens apart from skin flora

All 24 skin donations were included in the microbiological evaluation, resulting in a total of 48 samples with two samples per donation. The samples were incubated for 14 days. Culture conditions were appropriate to enable growth of bacteria and fungi listed in Table 2. Figure 5 provides an overview of the protocols, the details are described in the Methods section.

Overall, 47.9% of the skin samples showed bacterial and fungal contaminants (23 out of 48 skin samples) with 11 out of 23 samples (47.8%) having a mixed bacterial flora. Despite multiple disinfection steps, only 25 of 48 samples (52.1%) were found sterile. A total of 40 microorganisms of 11 different species were detected. Table 3 outlines a detailed overview of detected pathogens and their prevalence. The most common microorganisms were *Staphylococcus* species. None of the microorganisms detected were among the listed pathogens in Table 2 that should lead to immediate discarding of the skin allograft. All microorganisms detected are part of the normal skin microbiome. Therefore, no skin allografts were discarded. Figure 6 shows the determined times to positivity. Ten out of 23 samples showed positive results after 1 day. After 2 days, seven more samples showed positive microbiological results and one further sample after 3 days. Only one

**TABLE 3** Overview of detected pathogens and their prevalence.

| Pathogens according to Table 2 | Prevalence | Non-pathogens according to Table 2 | Prevalence |
|---|---|---|---|
| None | | Cutibacterium sp. | 1 |
| | | Cutibacterium acnes | 4 |
| | | Cutibacterium granulosum | 2 |
| | | Staphylococcus epidermidis | 7 |
| | | Staphylococcus lugdunensis | 11 |
| | | Staphylococcus capitis | 6 |
| | | Staphylococcus caprae | 5 |
| | | Staphylococcus saccharolyticus | 1 |
| | | Staphylococcus warneri | 1 |
| | | Gram-positive coryneform rod | 1 |
| | | Candida parapsilosis | 1 |

*Note*: A total of 48 skin samples of 24 donors were included in the microbial analysis. Twenty-three samples showed bacterial contaminants, with 11 samples showing mixed bacterial flora. A total of 40 pathogens from 11 different species were detected.

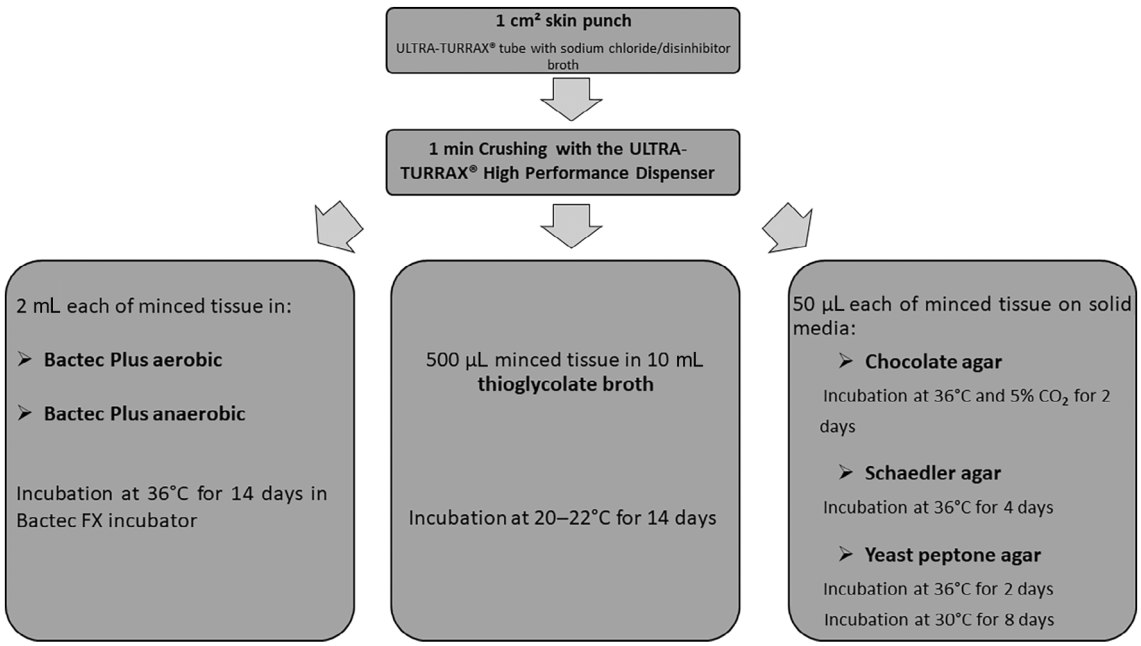


**FIGURE 5** Workflow for the microbiological screening of skin donations at the Department of Microbiology at Hannover Medical School.

sample was positive after 11 days. Unfortunately, there was no time to positivity available for four samples. However, it can be assumed that a 3-day culture is sufficient to detect most of the pathogens present in skin allografts processed for skin tissue banking.

Impression tests of working surfaces remained negative all the time. To sum up, a sterile working process with reliable microbiological results could be established to ensure skin allograft safety.

## 3.3 | Long-term cryopreservation had no influence on skin allograft viability

The present proof-of-concept study also aimed to investigate graft viability after processing and freezing. For this purpose, cryopreserved skin allografts were defrosted and viability assays were performed at different points of time over a period of 21 days. Assays were performed after 2 (Figure 7A), 7 (Figure 7B), 14 (Figure 7C) and 21 days

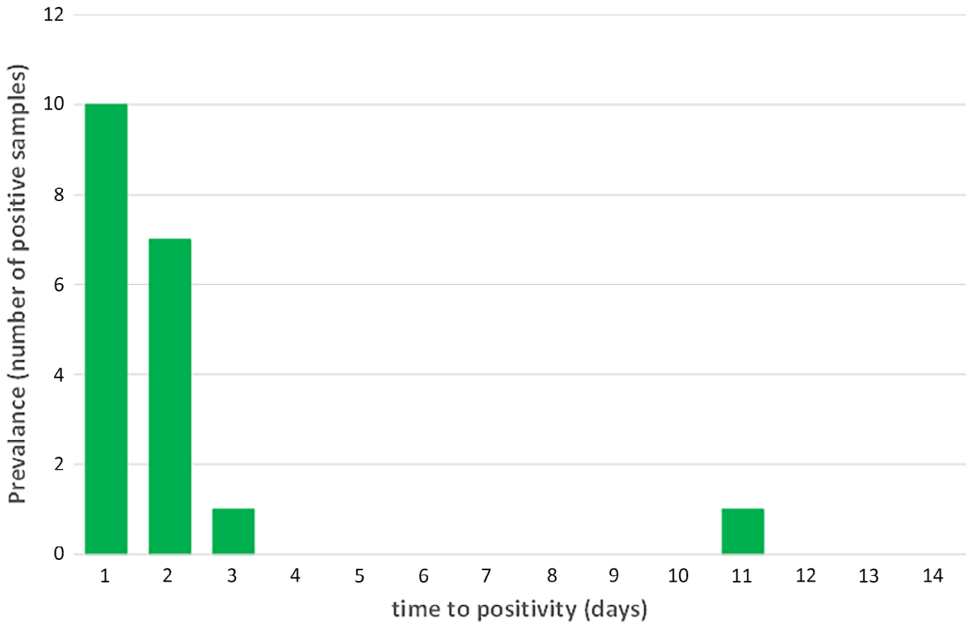


**FIGURE 6** Time to positivity in relation to prevalence of sample with pathogens. Overall 23 skin samples showed positive microbiological results. There was no time to positivity available for four samples.

(Figure 7D). Viable cells and tissue are shown with green fluorescent colour, whereas dead cells and tissue appear with red fluorescent colour. As seen in Figure 7, there were no dead cells or tissue observed over the period of 21 days. Furthermore, there was no change in cell and tissue viability over the period of time investigated in all other skin samples. To sum up, skin processing and freezing protocols established in the study at hand preserve cell and tissue survival over a period of 21 days resulting in viable skin allotransplants for further clinical purposes.

## 4 | DISCUSSION

Skin allografts continue to be an important therapeutic tool in the management of severely burned patients and the development of skin banking in individual burn units has given way to the establishment and management of large regional and national skin banks. There are currently high quality standards due to increasing regulations and accreditations to run a skin tissue bank.[45,46] We presented and validated a procurement and processing protocol for allogeneic skin grafts of living donors. Throughout skin tissue banks worldwide, allogeneic skin grafts are mainly obtained from cadaveric donors sharing all of the disadvantages of allogeneic transplants such as disease transmission, graft rejection, immunogenicity and secondary infection.[7,12,13] Furthermore, another disadvantage compared to organ donation could also be a lower willingness to donate, as the relatives of the body donor may be concerned about external disfigurement of the cadaver. Tissue banking standards generally set limits on the maximum warm and cold ischemia times after the death of the donor because circulatory arrest and thereby ischemia lead to progressive cell death.[47] It is known that postmortem bacteria from the gut can be released to the vascular system and thereby to the skin.[47] The procurement protocol outlined here circumvents postmortem ischemia times, as tissue donations are obtained immediately in the operating room and processed directly. Using the established protocol, we were able to demonstrate high cell viability even after processing and cryopreservation by immediately cooling the tissue samples, thus preventing extensive apoptosis. As early as 1903, the successful storage and grafting of human skin autografts at temperatures near 0°C for 14 days was described and later on proved.[48,49] Nowadays in burn units, autologous skin grafts can be stored in a refrigerator for a short period of time if immediate transplantation after harvesting is not feasible. The refrigerator storage is possible for up to 14 days, but the best transplantation kinetics are seen after 7–8 days.[50–52] Compared to autologous skin tissue samples, refrigerator storage of viable skin allografts is not feasible in the long run. A better method for long-term storage of skin allografts is cryopreservation that facilitates the cooling of the skin grafts to ultralow temperatures while protecting the viability of the cells. Therefore, the long-term cryopreservation storage for final storage at −150°C (nitrogen gas phase) of the allogeneic skin grafts takes place in the Hannover Unified Biobank.

The major issue with the use of fresh, viable skin allografts is the risk of disease transmission.[12,18] Therefore, quality control should be performed at various points during tissue donation and subsequent processing to detect contamination and respond accordingly. In the

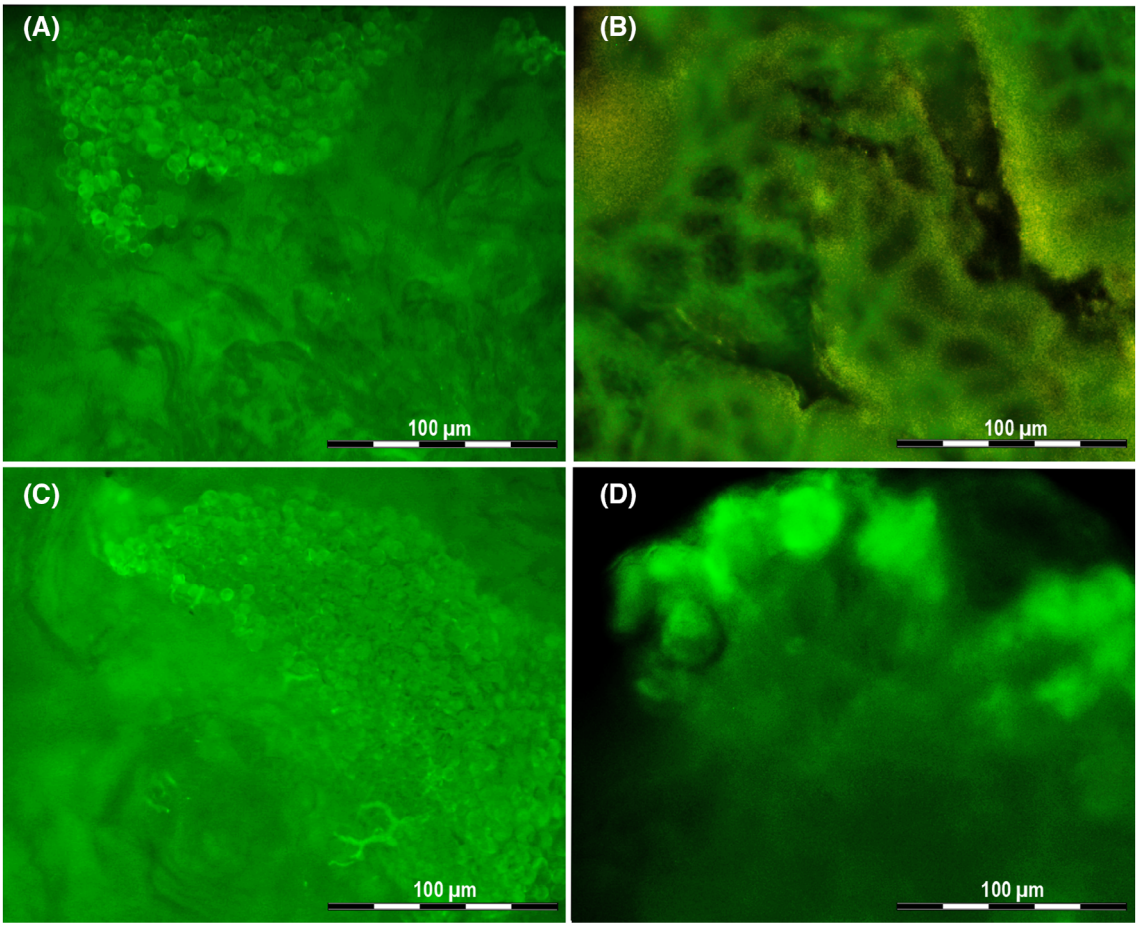


**FIGURE 7** Viability assays of cryopreserved skin allografts. Long-term cell and tissue survival in cryopreservation was observed over the period of 21 days with no differences at the different points of time. (A) Live-dead-assay after 2 days, (B) live-dead-assay after 7 days, (C) live-dead-assay after 14 days, (D) live-dead-assay after 21 days.

present study, an appropriate protocol was established and approved by the trade supervisory authority. In addition to the abovementioned reduction of ischemia time until tissue donation, viral donor screening and microbiological examination of skin grafts are of particular importance. In the present study, all donors showed no abnormalities in serologic screening, so that all tissue donations could be delivered to the skin bank for further processing. It is of interest that a case involving the transmission of HIV from a skin donor to a recipient has been reported in 1987.[53] Since the serological screening tests are very precise nowadays, a further molecular biological examination of the skin donations in the present study by means of polymerase chain reaction could be dispensed in accordance with the trade supervisory office.

Unlike other tissues, the human skin is colonized with multiple microorganisms and therefore cannot be considered sterile at the time of tissue donation.[54] The microbiome of the human skin has been investigated intensively, but the published literature of the microbiome of skin allograft procurement mainly focuses on cadaveric donors.[28,30,55] An optimized local disinfection of the donor side before harvesting of the skin donation could minimize or eliminate microbial contamination of the skin prior to processing.[18,56] Disinfection is defined as a process of intervention applied to skin grafts following recovery which reduces bacterial and fungal bioburden.[30] However, bacterial culture and disinfection of allografts are mandatory but the specific practices and methodologies are not regulated by standards.[30] As described elsewhere, rinsing and cleaning of the donor side could be performed with a variety of agents including 7.5% polyvidone-iodine soap, 0.5% chlorhexidine with 70% isopropanol solution and betadine scrub.[30] In the present study, multiple pre- and intraoperative scrubbings and disinfection of the donor side were performed. Thus, the present study joins the published literature, as all solutions used for disinfection were alcoholic in nature.

It was reported elsewhere that 70% of pre-processing cultures from cadaveric skin donations showed bacterial growth with *Escherichia coli*, *Staphylococcus aureus* and *Candida* species being the predominant microorganisms.[33]

Other studies described that the most commonly isolated microorganisms were skin contaminants like coagulase negative *Staphylococci*, especially *Staphylococcus epidermidis*.[27,32,57,58] It is of importance that relevant sampling for microbiological analysis is performed as the distribution of microbiological contamination is heterogenous throughout the sample.[26] In the present study, the collection of two 1 $cm^2$ pieces of skin was considered sufficient for microbiological testing. The results are in line with the published literature as the most commonly isolated microorganisms were *Staphylococci* species, which belong to the normal skin flora. It is possible that not all contamination could be identified and microorganisms can of course survive cryopreservation. Pirnay et al. reviewed their clinical results and did not find any adverse reactions or infections of recipients of cryopreserved skin.[26] These findings correspond to Neely et al. who found no microbes isolated from the recipient matched the microorganisms of the donor graft.[58]

For viable skin grafts, sterilization techniques cannot be applied, however, antibiotics may be used to decontaminate the tissue.[18] The greatest reduction in contamination and allograft discard of cadaveric donors was described using a combination of broad-spectrum antibiotics and antifungal agents, such as penicillin, streptomycin and amphotericin B[26]; penicillin, streptomycin and 85% glycerol[27] or penicillin, streptomycin, kanamycin, gentamycin and nystatin.[58] It was shown that the optimal effect of antibiotic treatment occurs at approximately 37°C.[18] Rooney et al. reported that approximately 22% of skin allograft donations were not reliably decontaminated by antibiotic treatment,[59] whereas Mathur et al. showed that 52.4% of grafts became sterile after first cycles of antibiotic treatments.[33] In agreement with the trade supervisory office, the use of antibiotics to reduce the skin microbiome was dispensed with in the establishment of the skin tissue bank presented. On the one hand, the microbiological samples would have been falsified and the testing procedure would have become considerably more complex. On the other hand, the increasing resistance to broad spectrum antibiotics worldwide should not be disregarded.[60] Especially in the treatment of severely burned patients, a conscious consideration of antibiotic therapy should be made.[61] A possible transfer of antibiotic residues with the transplantation of an allograft from a skin bank should therefore be avoided.

Multiple publications described the antimicrobial effect of glycerol to decontaminate positive cultures of skin allografts in tissue banking.[21,62,63] As an example, the Skin and Keratinocyte Bank at Queen Astrid Military Hospital in Brussels (Brussels, Belgium) successfully described a glycerol-based recovery method for contaminated skin allografts.[54] The described protocol efficiently proved to inactivate colonized bacteria and fungi with the exception of spore-formers. At the same time, glycerolized skin tends to be more rigid than fresh or cryopreserved skin.[54] It is known that glycerol-preserved skin allografts can lead to immunization of the recipient organism and should be avoided.[24,64] Viable donor skin allografts also tend to generate beneficial effects, mainly shown by an improved granulation of the wound bed.[54] The clinically observed positive effects can be most likely attributed to living cells in the allograft that can transfer organizational signals and growth factors to the wound, resulting in the formation of granulation tissue.[65] Gamma irradiation of the donor skin is also not considered to be effective, as this would destroy the living cells.[31] Since the data of the present study are based on tissue donations from living donors and incubation was performed without the addition of broad-spectrum antibiotics or glycerol, the results are not comparable to previous published literature and should be considered as an addition to the existing scientific discourse.

Another point that is critically discussed in the literature is the cultivation time of the microbiological samples to prove sterility or to exclude the growth of pathogenic microorganisms. The results presented in the study at hand are in line with the published literature. For example at the Skin and Keratinocyte Bank at Queen Astrid Military Hospital in Brussels, human donor skin allografts are tested for bacterial and fungal contamination using a protocol based on 14-day microbiological cultures.[54] Culture-positive skin allografts could only be released for clinical use if growth of non-pathogens appeared post 7 days of culture.[54] Furthermore, Pirnay et al. showed 70.3% sterile cultures after 14 days of incubation.[26] Britton-Byrd et al. analysed 735 skin samples in 7-day cultures and observed only one positive culture with a longer incubation time than 3 days. They concluded that 3-day microbiological cultures are as safe as 7-day cultures.[11] However, possible slow growers and their pathogenic character, such as *Candida albicans*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Klebsiella pneumonia*, are only taken into account to a limited extent in the 14-day cultures. With the results of the present study, we could show that a 3-day culture is as efficient as a 14-day culture to detect relevant pathogens.

Taking the chronic shortage of human skin for the treatment of burns into account as well as aspects of sustainability, every effort should be made to ensure that donated skin does not go to waste.[29,41,66] To sum up, the microbiological testing and acceptance procedures presented ensure patient safety, maintain cell viability to contribute to optimal patient outcomes and guarantee an increased availability of skin allografts on a regional level. However, further research is needed to analyse the

benefits as well as clinical performance of our processed skin allografts, especially with regard to increasing workload and costs associated with procurement in the operation theatre and further processing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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