Case report

Second-degree burns with six etiologies treated with autologous noncultured cell-spray grafting

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A B S T R A C T

Partial and deep partial-thickness burn wounds present a difficult diagnosis and prognosis that makes the planning for a conservative treatment versus mesh grafting problematic. A non-invasive treatment strategy avoiding mesh grafting is often chosen by practitioners based on their clinical and empirical evidence. However, a delayed re-epithelialization after conservative treatment may extend the patient’s hospitalization period, increase the risk of infection, and lead to poor functional and aesthetic outcome. Early spray grafting, using non-cultured autologous cells, is under discussion for partial and deep partial-thickness wounds to accelerate the re-epithelialization process, reducing the healing time in the hospital, and minimizing complications. To address planning for future clinical studies on this technology, suitable indications will be interesting. We present case information on severe second-degree injuries after gas, chemical, electrical, gasoline, hot water, and tar scalding burns showing one patient per indication. The treatment results with autologous non-cultured cells, support rapid, uncomplicated re-epithelialization with aesthetically and functionally satisfying outcomes. Hospital stays averaged 7.6 ± 1.6 days. Early autologous cell-spray grafting does not preclude or prevent simultaneous or subsequent traditional mesh autografting when indicated on defined areas of full-thickness injury.

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1. **Introduction**

The initial clinical diagnosis of a burn wound depth usually determines the treatment, however, intermediate partial thickness burns can be difficult to classify accurately with an early evaluation [1]. Deeper partial-thickness injuries may need to undergo surgical treatment, including excision and optional split-skin mesh grafting [1–3]. Conservative treatment of extensive, deep partial-thickness wounds avoids early mesh grafting at the risk of a delay in wound closure, which may result in infection and poor aesthetic and functional outcomes. Possible complications of this therapeutic approach include hypertrophic scarring, contracture, and poor functional and aesthetic outcomes that could result in a reduced range of motion and unsatisfactory psychosocial adjustment [4]. Thus, in this borderline indication, an early autologous cell-spray grafting of extensive, deep partial-thickness wounds could be an interesting therapeutic option [5]. In addition, an enlargement of the donor-to-graft-area ratio from a routine 3:1 has its typical clinical limitation at 6:1, while using cell-spray grafting the ratio is between 20:1 and 80:1 [6,7].

Skin regeneration is a dynamic process that involves different cell lineages and cell signaling, which leads progenitors cells to restore the tissue structure and function [8]. Epidermal burn-wound regeneration starts from the edge of the wound where the epidermal structures remain. The adjacent epidermis contains all different functional structures including the hair follicle (HF), interfollicular epidermis (IFE), and the sebaceous glands that are involved in the healing process [9–11]. Epidermal homeostasis and regeneration are enabled by quiescent epidermal stem cells (Fig. 1A), some of which can be activated and proliferate as transient amplified keratinocytes in the Stratum basale [12,13]. Through post-mitotic differentiation and migration, cells from the basal layer can regenerate the entirely stratified epidermis [10,14–17]. In deep partial-thickness burn wounds, mesh and cell-spray grafting aim to distribute these cells over the center of the wound and speed up central re-epithelialization.

Various cell-spray grafting methods have been introduced and are thought to provide a fast re-epithelialization and then reduce the healing time and minimize complications [5,18,19]. This innovative technique is still under clinical evaluation and to address planned future clinical studies, suitable indications are of interest. Since 2008, skin-cell-spray grafting, using isolated non-cultured autologous keratinocytes (Fig. 1B), has been used at our center as a treatment option for 45 partial-thickness burn patients. Our chosen regulatory Innovative Practice Institutional Review Board (IRB) approach precludes a study with controls. At times, the procedure has been used in combination with mesh grafting for patients with combined second- and third-degree burn wounds. Here, we present six second-degree burn patients and their treatments, showing different burn etiologies: gas, chemical, electrical, gasoline, hot water, and tar (Table 1). In all indications, the results, after early autologous cell-spray grafting shows a fast re-epithelialization and an aesthetic and functionally satisfying outcome with no major complications. We suggest considering these indications for future clinical studies in this new field.

2. **Material and methods**

2.1. **Patient criteria for study inclusion**

The Institutional Review Board (IRB) from UPMC Mercy Hospital, through its Technology and Innovative Practice Assessment Committee, approved the cell-based grafting procedures under an innovative practice approach. Therefore, performing a clinical study with controls was not possible. Patient data collection for this retrospective analysis was performed under an authorization from the Institutional Review Board (IRB# PRO14010023, 23-01). Exclusion criteria for the treatment consisted of age <18 years, pre-existing local and systemic infections, hypersensitivity to trypsin or other enzymatic wound treatments, and the risks associated with anesthesia. For inclusion, the team of surgeons at UPMC Mercy Trauma and Burn Centers (Pittsburgh, PA) decided that the treatments should be limited to deep, relatively extensive partial-thickness burn wound patients. The decision to offer a patient treatment was based on clinical judgment of the surgeon in charge, and consultation with the burn service as a whole. Consideration typically involved the wound appearance after the first debridement, and at 24 and 48 h. Before cell-spray therapy, a consent form was obtained from each patient after the alternative conservative or invasive treatment was explained in detail. The physicians explained to the patient

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Fig. 1 – Single cell-spray therapy proof of concept. (A) The basal layer contains epidermal stem cells and transient amplifying proliferative keratinocytes that we included for spray-grafting on the wound. (B) Three-enzymatic step isolation process. Epidermal–dermal separation is performed by dispase and followed by trypsin digestion of the epidermis and, a collagenase digestion on the dermis. (C) Isolated cells are combined and seeded onto wound through cell-spray deposition.

that their decision would have no impact on potential alternative treatments.

2.2. Cell-isolation process

This process is described in detail in the previously published literature [5,18,20]. Briefly, split-skin biopsies of 0.2 mm thickness were obtained in the hydrotherapy unit of our center by using a sterile manual dermatome (Teleflex, Limerick, PA, USA) or an electric dermatome (Zimmer Inc., Warsaw, IN, USA) without mineral oil. The donor area was disinfected with chlorhexidine preparation (Hibiclens®, Mölnlycke HealthCare US, LLC, Norcross, GA), which was followed by local anesthesia using a subcutaneous injection of 2% lidocaine with epinephrine (Hospira, Inc., Lake Forest, IL). The skin sample was obtained with the dermatome, transferred into a 100 mm disposable sterile Petri-dish (Becton Dickinson, Franklin Lakes, NJ, USA) and then cut into small, connected strips of skin using a surgical scalpel. The specimen was placed in a tube with 37 °C pre-warmed dispase-II solution (Roche, Indianapolis, IN, USA) for 40 min to break down the dermis–epidermis connections. The epidermis was mechanically separated from dermis using two 16G ½ (Becton Dickinson, Franklin Lakes, NJ, USA) needles. The epidermis was then placed into a 37 °C pre-warmed trypsin/EDTA-solution (0.05%/0.02%) (Gibco, Life Science Technologies, Grand Island, NY, USA) for 15 min with intermittent shaking. Keratinocytes were sieved with a 40 mm strainer (CORNING, Corning, NY, USA) and then washed twice with Ringer’s lactate (Baxter, Deerfield, IL) and centrifuged at 200 g for 5 min. Dermal tissue was digested using collagenase (SERVA Electrophoresis GmbH, Heidelberg, Germany) for 40 min at 37 °C, periodically shaken, then centrifuged, and washed in the same manner.

2.3. Debridement and excision

Prior to cell-spray grafting, the wounds were debrided or excised, depending on the depth of the burn wound, while using a Norsen blade (Teleflex, Limerick, PA, USA), Versajet scalpel (Smith & Nephew, London, UK), or Weck blade (Teleflex, Limerick, PA, USA).

2.4. Cell-spray process

A physician performed the cell-spray grafting in the operating room after wound debridement, excision, and hemostasis (epinephrine-soaked pads). The process involves an electronically controlled spray device combined with a cell solution, pushed out of a Luer-lock syringe 30G needle into an air stream (Skin Gun, RenovaCare, Inc., NY), and forming liquid droplets in a gentle manner without jet streaming the cells through a small sprayer orifice. The device generates 3185 ml/min of airflow that surrounds a 2.2 ml/min liquid flow produced by a linear force applied to the plunger of a sterile, Luer-lock slip tip, and 10 ml syringe (Becton Dickinson) containing the cell solution. The cells were distributed continuously using an alternating movement with the spray head approximately 20 cm from the wound surface (Fig. 2).

2.5. Wound care management

Standard wound dressings were used after the cell-spray process. The donor-site wound dressings that were used included Mepilex Ag (Mölnlycke, Norcross, GA) or Xeroform (Covidien, Mansfield, MA). The sprayed areas were covered with Adaptic® (Systagenix, Gatwick, UK) or N-Terface® (Winfield Labs, Dallas, TX), in combination with Bandnet (Western Medical LTD., Walnut, CA), Intersorb, Kerlix (Covidien, Mansfield, MA), Kling Johnson & Johnson, New Brunswick, NJ). Triple-antibiotic ointment (Taro Pharmaceuticals, Hawthorne, NY, USA), Sulfamylon (Mylan labs, Canonsburg, PA), Mercuricure (Humco Holding), Povidone-iodine microbicide (Betadine, Stamford, CT), Silver Sulfadiazine (Silvadene Dr. Reddy laboratories Inc., Bridgewater, NJ, US), Aquaphor (Beiersdorf, Hamburg, Germany), Santyl (Smith & Nephew, London, UK), and Polysporin (Johnson & Johnson, New Brunswick, NJ) were used at various stages of the healing process. Eucerin (Beiersdorf, Hamburg, Germany) was applied to the re-epithelialized areas once the wounds were dry, and no further wound dressing was used.

3. Results

Here, we present the individual clinical treatment of six patients, each with a different burn etiology, treated with cell-spray grafting:

3.1. C1: Gas explosion burn

The patient was a 43-year-old male (BMI 32.18 kg/m²) with a history of gastric bypass surgery, who obtained a deep partial-thickness injury comprising 13% TBSA on his upper right extremity and right flank, and a superficial burn on his right face and neck caused by a paint can explosion while painting the inside of a tank. The patient also had an ulnar styloid fracture and right 3rd and 4th phalangeal fractures due to the
Fig. 2 – Wound healing process for five patients with different etiology. Case 1: a gas explosion burn, Case 2: a chemical explosion burn, Case 3: an electrical burn, Case 4: a gasoline flame burn, Case 5: a hot water scalding burn, and Case 6: a hot tar scalding burn.
explosion. The right upper extremity was initially treated with Silvadene, and the right face was treated with Triple Antibiotics with Sulfamylon to the right ear. The patient was prepared for wound debridement and cell isolation on HD#3. The donor skin sample area was about 32.2 cm², with a 0.2 mm thickness obtained with an electric dermatome, and covered with Mepiplex Ag. The treated areas were the face, right hand, right forearm, right arm, and the right flank, with a total sprayed area of 2646 cm². After the cell isolation, 20 million cells were obtained to cover the burned surface with a density of 7559 cells/cm². The spray area was covered with Adaptic® combined with triple-antibiotic ointment and wrapped with N-Terface®, Kerlix, and Bandnet. The dressings were changed on POD#4 and then daily. Small re-epithelialized areas were observed on POD#4 on the wound edges of the right flank, anterior forearm, and hand. The wound became dry on POD#6 on the right flank, forearm, arm, and hand and the patient were discharged that day. The patient had to undergo multiple orthopedic surgeries after discharge for his wrist injury and chronic shoulder problems. On his follow-up visit, complete epithelialization and some hypopigmentation were noted on POD#14. A full range of motion in the right hand with some limitations in wrist motion was noted on POD#30. At his 5-month follow-up, minimal hyperpigmentation on the right side of his face and very slight hypopigmentation on the right shoulder and upper extremity were noted. There was no evidence of hypertrophic scarring throughout the prior burn area, and his only functional impairment, at this point, was due to his wrist injury.

### 3.2. C2: Chemical explosion burn

The patient was a 37-year-old male (BMI 24.07 kg/m²) who presented with 12.5% TBSA deep partial-thickness injury on his bilateral upper extremities and hands caused by a potassium nitrate explosion. The burn wounds were initially covered with Mepiplex and the patient underwent daily hydrotherapy. On HD#3, the patient was prepared for debridement, and a 14 cm² skin donor skin sample of 0.2 mm thickness was obtained using a manual dermatome from the left hip. The donor site was covered with Mepiplex Ag, and 9.4 million cells were isolated and used to spray the entire burned surface with a density of 6356 cells/cm² on the debrided burn wound. The treated areas were the left arm, dorsum of the left hand, and all left-hand digits with a total left upper extremity area of 331.25 cm²; also, the right forearm, dorsum of the right hand, and all the right-hand digits with a total right upper extremity area of 1148 cm². The total spray area (1479.25 cm²) was covered with Xeroform, triple-antibiotic ointment Intersorb, Kerlix, and Bandnet. The dressings were changed on POD#3, and it was noted that the wounds were still open. On POD#5, all wound areas were mostly dry except for some open areas, especially on the right hand where no sign of re-epithelialization was detected. On POD#7, the patient underwent an additional split-thickness skin grafting on the right hand, right middle finger, right ring finger, right small finger, and right lateral wrist. The patient was discharged on POD#9, and during his follow-up visit on POD#14, it was noted that there were some small open areas primarily on the left forearm (<5 mm in maximal dimension) and they were treated with Mercuroclear (Humco Holding Group, Texarkana, TX) multiple times a day. The difficulty with full flexion of R fingers was also noted on POD#14; however, on POD#25 full range of motion was observed. On POD#55, minimal hypertrophic scarring on a few of his right fingers was noted. On POD#607, the areas of autografts were noted to be almost indiscernible with the normal skin and no hypertrophic scarring or contractures were noted. The patient maintained a full range of motion in all extremities without restriction.

### 3.3. C3: Electrical burn

The patient was a 35-year-old male (BMI 22.72 kg/m²) with a 20-year history of cigarette smoking, narcotics, and recreational drug abuse who presented with 36.5% TBSA electrical burn after grabbing a live wire. The patient had a deep partial-thickness burn injury on the head, chest (more superficial in nature), abdomen, bilateral upper extremities, and back (the left back was more superficial in nature), and full-thickness injuries on the right hand and foot. Silver Sulfadiazine was initially used for his wounds. The patient was prepared on HD#4 for wound debridement, tangential excision of deep wounds on forearms and hands with a Weck blade and excision of more superficial wounds on the left back and chest with a Norsen blade before spray grafting. A 30-cm² donor skin sample was taken from the right anterior thigh using a 0.2 mm manual Weck blade at the hydrotherapy unit and it was covered with Mepiplex Ag. The 23.8 million cells taken were isolated and used to spray the entire partial-thickness surface with a density of 4162 cells/cm² on the excised and debrided burn wounds. The cell-sprayed areas included the right hand, right forearm, right arm, the left hand, left forearm, the left arm, chest, abdomen, and left back with a total area of 5719 cm². Concurrently, split-thickness autografts were isolated from the donor skin sample with a 0.3 mm Zimmer blade from the right anterior thigh and then “pie-crusted” and applied on the right hand and foot. The treated wounds were covered with Adaptic®, triple-antibiotic ointment, Intersorb, Kerlix, and Bandnet. On POD#4, re-epithelialization of the chest and the arms were observed with few open areas. On POD#5, the arms were noted as “healed”, and hands were still noted as open. Silver Sulfadiazene and topical emollients, such as Aquaphor and Mercuroclear, were applied to the open areas and Santyl/Polyborin was used to lift an eschar. On POD#6, the right hand and foot were still open and Adaptic was applied, but the left hand was healed. On POD#13, an eschar was noted on the right arm, and it was treated with Santyl/ Polyborin, and the left foot was treated with wet-to-dry dressing changes. On POD#20, all of the areas treated with cell-spray grafting were noted as completely healed and re-epithelialized. There was no evidence of hypertrophic scars or wound contracture, and the patient had a functional range of motion in all extremities.

### 3.4. C4: Gasoline flame burn

The patient was an 18-year-old male (BMI 30.91 kg/m²) with a history of poor wound healing in the right foot with split-thickness skin grafting about 3 months prior to presentation.
This prior wound had been healing well by the time the patient presented with a new 14% TBSA gasoline flame burn injury. He had superficial partial-thickness injury to bilateral upper extremities and deep partial thickness injury to lower extremities. His wounds were initially treated with Sulfamylon, and his left arm wound was placed in Mepilex. The patient was prepared on HD#5 for wound debridement with a Norsen debrider. Approximately 36 cm² of skin with 0.2 mm thickness was obtained with a manual dermatome Weck blade from left upper thigh and the donor skin sample area was covered with Xeroform. Forty-five million cells were obtained and used to spray the entire burn wound surface with a density of 15,198 cells/cm². The treated area included right hand, right forearm, right upper arm, right lower leg, left forearm, and left lower leg. The total 2961 cm² sprayed area was covered with N-terface®, Sulfamylon, Kerlix, and Bandnet. Wound areas started showing signs of re-epithelialization on POD#3 and dressings were changed to Adaptic dry dressing for healing areas (bilateral lower extremities), and only Eucerin for healed areas (bilateral upper extremities). The majority of the wounds were healed by POD#6. The donor site developed an eschar that resolved with Santyl® Polysporin. Wounds were completely healed by POD#13 and there was no evidence of hypertrophic scarring or contractions, and the patient demonstrated a full range of motion in all extremities.

3.5. C5: Hot water scalding

The patient was a 43-year-old male (BMI 28.53 kg/m²) who presented with a 10% TBSA burn wound caused by a hot water scalding. He was initially treated at an outside facility with Bactracin and presented to our center the day after when his wounds appeared worse. The patient had a partial-thickness burn on the left upper extremity, shoulder, and back. The wounds were dressed in Sulfamylon first and the patient underwent daily dressing changes at hydrotherapy. The patient was prepared on HD#5 for wound debridement with Versajet before cell spraying. Approximately, 30 cm² of skin with 0.2 mm thickness was obtained with a manual dermatome Weck blade from the left thigh. The donor area was covered with Xeroform, triple-antibiotic ointment, Kerlix, and Bandnet. Seventeen million epidermal cells were sprayed on the wound surface with a density of 8695 cells/cm² on the excised burn wounds. The area treated with the cell-spray grafting included the left shoulder, arm, forearm, and back with a total sprayed area of 1955 cm². The cell-sprayed wounds were covered with Adaptic® and triple-antibiotic ointment and were wrapped with Intersorb, Kerlix, Kling, and Bandnet. The dressings were changed on POD#3, and the areas of cell-spray grafting were noted to have good re-epithelialization with only small scattered open areas. Adaptic and dry dressing were applied and the wound was rechecked on POD#6. A 100% re-epithelialization was noted and the patient was discharged that day with instructions to apply Eucerin moisturizer to the wound. On POD#6, the patient was also noted to have a full range of motion in his extremities. On POD#136, a very small area of hypertrophic scarring was noted on the left lateral humerus just inferior to the deltoïd. However, the area was very small and was not causing functional deficits. He was noted to have an excellent aesthetic outcome.

3.6. C6: Hot tar scalding

The patient was a 43-year-old male (BMI 28.53 kg/m²) who presented with 15% TBSA burn wounds caused by hot tar scalding. The patient had a partial-thickness burn on the right arm, right hand, and anterior trunk. At hydrotherapy, the affected areas were cleaned and blisters were debrided. On HD#5, the burn areas were tangentially excised with #10 Weck blade, a Norsen blade, and a Versajet (Smith & Nephew) before cell spraying. Approximately 42 cm² of skin with 0.2 mm thickness was obtained with a manual dermatome Weck blade from the right thigh. The donor area was covered with Xeroform, triple-antibiotic ointment, Kerlix, and Bandnet. Twenty-seven million epidermal and dermal cells were sprayed on the wound surface with a density of 15,607 cells/cm² on the excised burn wounds. The area treated with cell-spray grafting included the right arm and hand, and anterior trunk with a total sprayed area of 1730 cm². The cell-sprayed wounds were covered with Adaptic®, and triple-antibiotic ointment and wrapped with Intersorb, Kerlix, Kling, and Bandnet. The dressings were changed on POD#3, and patient’s right arm and hand were noted to be nearly healed. The anterior trunk was healing with some spotty open areas. The wound areas were placed back in Adaptic and dry dressing and rechecked on POD#5 when re-epithelialization was noted in all areas except for small areas to the central chest and abdomen, which remained minimally open. With physiotherapy and Occupational therapy, the patient was noted to have a full range of motion without restrictions due to his burn wounds at this point. By POD#7, all areas were noted to be healed and re-epithelialized and the patient was discharged. On POD#14, he was noted to have complete re-epithelialization with hyperpigmentation, but without hypertrophic scarring. On POD#66, his skin pigmentation appeared to be returning to normal and he was still without any hypertrophic scarring or contractions.

4. Discussion

The clinical results of burn trauma, depending on the burn area, are often devastating and may be complicated or fatal [21]. In the USA, more than 1.25 million burns are reported per year. The NIH spends about 6.5 million dollars annually on patients wound healing projects and an excess of 25 billion is spent annually on wound treatment [22,23]. Although cultured epidermal autografts (CEA) have been available in burn therapy for some time, the waiting time associated with the process can be extensive and the results unsatisfactory [24]. We are promoting the use of non-cultured autologous cells [5,18] using a patient’s own wound as a “bioreactor” for cell expansion. Departing from the use of cultured cells [20] we are currently using a three-step enzymatic-cell-isolation process [5,18] that allows a tissue-specific enzymatic digestion. The process starts with a dispase-mediated epidermal/dermal junction separation, and subsequently a trypsin-mediated epidermal cell isolation of the exposed basal layer, together
with collagenase-mediated dermal cell isolation. In comparison to other approaches, like the ReCell® kit (Avita, South Perth, Australia) [19], our specific isolation regime exposes the epidermal basal layer first and then allows the trypsin to liberate the epidermal stem cells and divisionally active keratinocyte progenitors [25]. Our method allows the enzyme elimination after the cell isolation, using a centrifugation step and a Ringer’s Lactate cell re-suspension that dilutes the residual traces of the enzyme. The enzyme elimination step is important to avoid extended protolitic activity on the isolated cells especially during a prolonged waiting time between cell isolation and cell spray process. In contrast, to the use of pre-cultured cell sheets [26,27] homogeneously distributed sprayed cells grow from multiple starting points in a well-distributed pattern until they reach confluence and close the wound by re-epithelialization. However, the ReCell® method and the electronically controlled cell spray device used in our study have a different coverage area. The ReCell® kit allows a maximum wound coverage area of 320 cm², according to the manufacturer’s instructions [19], while our cell isolation method has no maximum wound coverage. In the presented study, the patient’s treated area varies from 1500 to 5700 cm² (see Table 2).

Compared to CEA, non-cultured cell-spray grafting avoids prolonged culture waiting times for patients with open wounds, reduces the amount of in vitro cell manipulation, and, importantly, avoids an early cell differentiation with a loss of progenitor cells and epidermal stem cells as a consequence of in vitro culture expansion [25]. The use of non-cultured cells and suitable spray deposition devices can be performed in an on-site setting of isolation and immediate grafting. Early autologous cell-spray grafting of severe partial-thickness wounds is thought to help provide a fast re-epithelialization while maintaining the possibility of mesh grafting. Cell-spray grafting is also especially suitable for hands and joint areas, where prolonged times to re-epithelialization may significantly impact functionality and esthetic outcome.

Our current innovative practice regulatory approach does not allow for clinical comparative studies. This limitation precludes controls comparing the normal healing time without cell-spray transplantation or with mesh grafting, and our results summarize clinical evaluation data with subjective assessments of the initial burn depth only. In order to plan for controlled clinical studies under an FDA IDE/IRB approach, we described the healing process and the outcomes after six patients were treated with the described methods for partial- and deep-partial-thickness burn injuries from six different etiologies. We conclude that all etiologies were suitable as study inclusion criteria. The patients treated with autologous skin cell spray grafting experienced a visible re-epithelialization by POD#3-6 in general, except on some smaller areas that were more deeply excised or represented deeper burn areas. While the wounds treated became dry on POD#6 on average and were fully healed in two weeks at maximum, the patients’ post-operative hospital stays were between 6 and 10 days (Table 2).

In conclusion, we present acceptable clinical results using autologous epidermal progenitors and basal layer derived stem cells to treat extensive partial-thickness burns of six etiologies by way of an innovative cell-spray grafting method, and we believe that our results provide a foundation for future clinical studies.

Conflict of interest

J.G. and R.E. have a financial interest in the spray-grafting device technologies through payments of RenovaCare, NY.

Author contributions

R.E. coordinated the cell-isolation process and the follow-up, analyzed data, and wrote the manuscript. J.G. and R.E designed and coordinated the manuscript design and revised the manuscript. M.C. compiled the medical records data, provided advice and revised the manuscript. M.Y. and P.O. performed the cell isolations. A.C., J.Z designed the manuscript structure, performed the cell-spray procedure and the patient follow-up. M.Y., M.C., A.C., J.Z. revised the manuscript and provided discussions.

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Table 2 - Information related to cell-spray therapy and healing process.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treated area (cm²)</th>
<th>Donor sample (cm²)</th>
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<th>Pre-op days</th>
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*Treated area is the defined as the burned area that was cell sprayed. Cell density is the number of cells sprayed on the treated area. The Hospital Length of Stay (LOS) includes the preoperative days (Pre-op) and the postoperative days (POD).

* Dermal and epidermal cell combination for cell spray.
REFERENCES