A randomized trial comparing ReCell® system of epidermal cells delivery versus classic skin grafts for the treatment of deep partial thickness burns

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ABSTRACT

Background: Our purpose was to directly compare results obtained with the ReCell system and the classic skin grafting for epidermal replacement in deep partial thickness burns.

Materials and methods: We recruited all patients with deep partial thickness burns admitted at the Burn Centre of S. Eugenio Hospital in Rome over 2 years. Enrollment was conducted with a controlled strategy – sampling chart – that allowed homogeneous groups (ReCell and skin grafting) for age, gender, type of burns and total burn surface area (TBSA). We evaluated as primary endpoints of the study the (i) time for complete epithelization (both treated area and biopsy site) and (ii) aesthetic and functional quality of the epithelization (color, joint contractures). Secondary endpoints were the assessment of infections, inflammations or any adverse effects of the ReCell procedure, particular medications assumed, postoperative pain.

Results: Eighty-two patients were analyzed in two homogeneous groups. All of them received adequate epidermal replacement, but skin grafting was faster than ReCell (p < 0.05). On the contrary, ReCell biopsy areas and postoperative pain were smaller than classic grafting (p < 0.05). The aesthetic and functional outcomes were similar between procedures.

Conclusions: ReCell is a feasible, simple and safe technique. It gives similar results to skin grafting but, harvesting minor areas, can open possible future applications in the management of large-burns patients.

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1. Introduction

Epidermal replacement is an important step in the management of burned patients to restore the pool of keratinocytes, Langerhans cells and melanocytes usually lost during severe thermal injuries. Skin grafts, both meshed and non-meshed, are actually the gold standard and, during the years, proved to be an effective and easy technique. Results have clearly showed that they hasten burn healing, give good aesthetic results, and prevent contractures to establish them as the most accepted method for treating epidermal loss [1,2]. However, limitations still exist: in large burned areas the need for coverage, dictated by the high risk of infections, is counteracted by the difficulty to find non involved donor area.

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In these cases, temporary coverage with cadaveric skin or dermal substitutes allow time for tissue engineers to produce large amounts of keratinocytes, fibroblasts or both for the definitive grafting [3–7].

Recently, keratinocyte cell sprays have been investigated on in vitro and animal models as new methods for the epidermal cell delivery for the treatment of acute burns and wounds [8–11]. In 2005, for the first time, one device was introduced into clinical practice, the ReCell system. This allows immediate processing of a small split-thickness biopsy with Trypsin to produce a complete population of cells including keratinocytes, melanocytes, Langerhans cells and fibroblasts. The biopsy is digested and cells are diluted in a Lactate solution. The final suspension can be applied with a ratio of 1:80 (1 cm² of biopsy to cover up to 80 cm² of damaged area). In our burn center we prospectively examined the ReCell procedure and a signed consent was obtained from each of them.

Informed about the potential risks and benefits of the procedure and a signed consent was obtained from each of them. Inclusion/exclusion criteria were met, patients were suspended from the dermal layers and epidermal cells further divided with the scalpel’s blade (Fig. 1). Cells were further suspended in a Lactate solution, aspirated with a 5 ml syringe and sprayed over the area to be treated (Fig. 1) and the biopsy site. Medication was performed with non-adhering dressings (Adaptic—Johnson and Johnson Wound Management, Ethicon) and gauzes on both areas.

2. Materials and methods

We received approval of the local ethical committee for this study. We recruited all patients admitted at the Burn Centre of S. Eugenio Hospital in Rome over 2 years, that was the period of time in which we evaluated the ReCell device. Subject enrollment was conducted with a controlled strategy – sampling chart – that allowed homogeneous groups (ReCell and skin grafting) for age, gender, type of burns and total burn surface area (TBSA). A previous pilot evaluation, whose results were also examined by the institutional ethical commission, established the following necessary criteria necessary to end the study within 2 years and without excessive costs: to recruit at least 25 patients for each group (ReCell and skin grafting), comprehensive of 5 patients in excess to balance an estimated patient loss – drop-out – of 25% during the study and/or follow-up (unpublished data). The resulting test power was 80% (significance level = 0.05).

Patients presented to the hospital for emergency admission and, during the recovery, underwent routine preoperative investigations and a final revision of surgical indications. Eligibility criteria for the study consisted in adult patients affected by deep partial thickness burns, that required surgical debridement and epidermal replacement, and with areas involved less than to 320 cm². Exclusion criteria consisted in the presence of pre-existing local and systemic bacterial infections, pre-existing medical conditions that would interfere with wound healing (i.e. diabetes, malignancy, autoimmune disease), renal failure (glomerular filtration rate inferior to 60 ml/min), medications that could interfere with wound healing (i.e. corticosteroids), antibiotics for more than 48 h prior to grafting for other than prophylactic reasons, hypersensitivity to Trypsin or Hartmann’s solution, a high anesthesiology risk that necessarily postponed surgery. When all conditions were satisfied, and the inclusion/exclusion criteria were met, patients were informed about the potential risks and benefits of the procedure and a signed consent was obtained from each of them.

From admission all patients received Ranitidine for ulcer prevention, Piperacillin for infections prophylaxis and were medicated with nanocrystalline silver (Acticoat—Smith and Nephew) on deep partial thickness areas. Surgery was conducted on the 3rd–5th day following burn, according to principles of early escharectomy. All procedures were performed in the operating room and under general anesthesia. Preoperative sterilization of burned surfaces was conducted with chlorhexidine–cetrimide solution. All escharectomies were performed with tangential excisions.

2.1. ReCell procedure

A thin split-thickness cutaneous biopsy (0.2–0.3 mm) was harvested from an uninvolved area (the inguinal region whenever possible) using a Zimmer dermatome (Zimmer, Indiana, USA). According to the manufacturer’s instruction, as the cellular spread rate is 1:80, the biopsy area was 1 cm² when the recipient area was 80 cm² and 4 cm² when the recipient area was 320 cm². The epidermis was put in 4.5 ml of trypsin solution for 20 min at 37 °C to begin the intercellular detachment. While the biopsy was processed, classic escharectomy was applied on (1) eschars of full thickness burns and (2) deep partial thickness areas with thick fibrin slague. After digestion with trypsin was completed, the epidermis was separated from the dermal layers and epidermal cells further divided with the scalpel’s blade (Fig. 1). Cells were further suspended in a Lactate solution, aspirated with a 5 ml syringe and sprayed over the area to be treated (Fig. 1) and the biopsy site. Medication was performed with non-adhering dressings (Adaptic—Johnson and Johnson Wound Management, Ethicon) and gauzes on both areas.

2.2. Skin grafting

Classic skin grafting was performed with the Zimmer dermatome using a thin split-thickness depth and meshing all grafts (1:2 ratio) except those destined over the face, hands, feet, genital areas. Medication was completed with non-adhering dressings and gauzes on both areas.

Postoperative follow-up consisted of four visits during the first month – one for each week – and two additional visits at the third and sixth month. During this visits, to test the effectiveness of the ReCell system and to compare it with the current gold standard, skin grafting, we evaluated as primary endpoints of the study the (i) time for complete epithelization (both treated area and biopsy site), (ii) aesthetic and functional quality of the epithelization (color, joint contractures). The aesthetic appearance of the epithelization was evaluated with the help of one plastic surgeon unaware of the procedure (A.A.), according to a simplified version of the Vancouver scar scale that analyzed only the scar pigmentation and vascularity [12]. The patient’s active and passive range of motion in a single plane was evaluated with the help of a physical therapist at 1 and 6 months from surgery. Secondary endpoints were the assessment of infections, inflammations or any adverse effects of the ReCell procedure, particular medications assumed, postoperative pain (evaluated with the visual-analogic scale—VAS).

2.3. Statistical analysis

The analysis of data was performed using the Statistical Package for the Social Sciences Windows version 13.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics for quantitative continuous variables were the mean and standard deviation after confirmation of normal distribution. Normality assumptions have been demonstrated with histograms, Q–Q plots, Skewness and Kurtosis, Kolmogorov/Smirnov and Shapiro Wilk testings. Descriptive statistics for qualitative categorical variables was performed with frequencies.

Student’s t-test was used to compare continuous variables among groups, except for the area harvested in which the Mann–Whitney test was used (variances are not equal in this case). $\chi^2$-Test and Fisher’s exact test were used to compare nominal variables. All $p$ values were considered significant if inferior to 0.05.

3. Results

From September 2004 to September 2006 we enrolled 100 patients for the clinical trial; of these, 82 were recruited to receive the allocated treatment and completed follow-up after 6 months while 18 were excluded because not homogeneous for age (8 of them were inferior to 30 years, 10 over 65). Forty-two patients were treated with the ReCell system, 40 with the traditional skin grafting. Demographics, clinical characteristics and outcome for both groups are summarized in Table 1.

![ReCell procedure](image)

**Fig. 1** – ReCell procedure. Digestion with trypsin (left top), separation of the epidermal layer from the dermis (right top), suspension in Lactate solution (left bottom) and spray over the recipient area (right bottom).

**Table 1 – Subject demographics, clinical characteristics and outcomes**

<table>
<thead>
<tr>
<th></th>
<th>ReCell ($n = 42$)</th>
<th>Skin grafting ($n = 40$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 9</td>
<td>53 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (males)</td>
<td>24 (57.1%)</td>
<td>26 (65%)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 9</td>
<td>172 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73 ± 12</td>
<td>70 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>25 ± 3</td>
<td>24 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Area to be treated (cm$^2$)</td>
<td>186 ± 96</td>
<td>180 ± 100</td>
<td>NS</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td>14 (33.3%)</td>
<td>16 (40%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hands</td>
<td>10 (23.8%)</td>
<td>8 (20%)</td>
<td>NS</td>
</tr>
<tr>
<td>Legs</td>
<td>12 (28.6%)</td>
<td>10 (25%)</td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>6 (14.3%)</td>
<td>6 (15%)</td>
<td></td>
</tr>
<tr>
<td>Post-escharectomy hemostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenaline gauzes</td>
<td>21 (50%)</td>
<td>20 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Normal gazes</td>
<td>10 (23.8%)</td>
<td>14 (35%)</td>
<td></td>
</tr>
<tr>
<td>Diathermy</td>
<td>11 (26.2%)</td>
<td>8 (20%)</td>
<td></td>
</tr>
<tr>
<td>Area harvested (cm$^2$)</td>
<td>2.2 ± 1</td>
<td>110 ± 50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area effectively treated (cm$^2$)</td>
<td>176 ± 84*</td>
<td>180 ± 100</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of the procedure (min)</td>
<td>59 ± 4</td>
<td>20 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postoperative pain</td>
<td>3.3 ± 1.6</td>
<td>6.8 ± 1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Complete healing (days)</td>
<td>13 ± 2</td>
<td>12 ± 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

* In the ReCell group the area effectively treated was slightly minor than the area to be treated because seven patients also received meshed skin graft and this zones were not included in the analysis.

Associated diseases were chronic obstructive pulmonary disease in three patients in the ReCell group (7.1%) and two patients in the grafting group (5%), surgical hypothyroidism (thyroidectomy) in two patients (4.8%) and three, respectively (7.5%), hypercholesterolemia in five (11.9%) and three (7.5%), respectively, Hepatitis B in three (7.1%) and one (2.5%) respectively, Hepatitis C in two patients (5%) of the grafting group. These differences were not significant ($p > 0.05$).

In all patients ($n = 82$) an adequately debrided wound bed was achieved with the tangential escharectomy. In the ReCell group, 33 patients (78.6%) had their cutaneous biopsy harvested from the inguinal region, 9 patient (21.4%) from the forearm. Seven patients (16.7%) also received meshed skin grafts in different and distant areas from the ReCell application. This was done initially to directly compare ReCell and skin grafts.

### 3.1. Statistical analysis

Significant differences were present between ReCell group and skin grafting for the duration of the procedure (longer for
ReCell; $p < 0.001$), the area harvested (greater for the classic grafting; $p < 0.001$) and the postoperative pain ($p = 0.03$).

Healing was complete in approximately 13 days for the ReCell and 12 days for the classic grafting group ($p > 0.05$). Sixty-nine patients needed only one operative procedure (84.1%), 13 required two steps (16.9%) to complete few remaining areas that did not heal, and no significant differences were observed between groups for patients requiring two procedures (7 in the ReCell group versus 6 in the grafting group). No significant differences in the aesthetic quality of healing was observed, according to the judgment of both plastic surgeons: pigmentation and vascularity were similar between ReCell and unmeshed grafts and slightly superior between ReCell and meshed grafts ($p > 0.05$). Results with the ReCell system are presented in Figs. 2–5; part of these figures include a direct comparison with skin grafts, as the initial seven patients of the ReCell group received both ReCell and grafting. Of the 82 study patients, 27 (33%) developed at least one contracture at hospital discharge after one month from surgery. Of these, 12 were after ReCell and 15 after skin grafting (chi-square test: not significant between groups). None of the 82 patients reported intraoperative or postoperative adverse effects.

4. Discussion

Cultures of keratinocytes have been used for over 20 years in the treatment of burns [13–14] and initial techniques involved the grafting of confluent sheets on sterile backing materials. Results obtained with these procedures were positive, especially in patients where large burned areas rendered not possible classic grafting, but were often biased by poor attachments in regions associated with mechanical forces (e.g., joints) or complex topography (face and hands), and a not complete aesthetic restoration of the original skin due to the lack of some epidermal elements (e.g., melanocytes) [14,15]. For all these reasons, new techniques of keratinocytes delivery were investigated and, among them, the aerosol spraying recently obtained promising results [8–11,16–24]. In the present study we evaluated a new device for the spray of keratinocytes, the ReCell system, that enzymatically digests intercellular bonds and deliver cells with a high donor/receiving ratio (1:80).

Aesthetic results obtained with the ReCell system were very similar to those of skin grafts (Figs. 4 and 5). As the aim of our study was to evaluate the effects of ReCell on healing and re-epithelization processes, this was accomplished with a strict protocol, similar for all patients, in order to avoid possible confounding factors. We selected, after that initial patients had their biopsy harvested from the forearm, the inguinal area—far from burn sites and without any possible influence of heat on donor cells. Although this choice could have introduced differences in aesthetic outcomes (color matches between the donor and the receiving site), results obtained seemed to exclude this possibility. A possible theoretical explanation could be that ReCell created a suspension of cells that, when dispersed and subsequently spread over the affected surface, attenuated any eventual aesthetic difference. However, this hypothesis needs further clarification in future work.

An important difference between the ReCell system and skin grafting consisted in the smaller harvested area (1–4 cm²) obtained with the former, derived from the ability to spread cells with a high ratio (1:80). Given the experimental nature of the study, the clinical protocol was initially discussed between the manufacturer and surgeons and subsequently proposed to the attention of the local ethics committee. The committee indicated to treat in this study only small areas and apply the device on larger burns in future work, after confirmation of its feasibility and safety. For all these reasons we selected patients with small burned surface area (up to 320 cm²) but we believe that the results obtained could open future important applications also in patients with major burns. In these patients, in fact, too often the amount of uninvolved skin is not sufficient (the extension of skin grafts, even when meshed, largely depends on the amount of normal skin available for harvesting) and dermal substitutes (cadaveric skin, bio-artificial products) are often required to temporarily

![Fig. 4 – Seventy years old female with fire deep partial thickness over her breast (left). The superior area was treated with ReCell, the inferior with skin grafts (middle). Results after 1 week (right).](image-url)
cover exposed surfaces while waiting for tissue engineering for the definitive treatment. In this context, the ReCell system could prove to be more indicated and useful.

The ReCell procedure took longer than skin grafts, mainly due to the time required by Trypsin to digest intercellular bonds (20 min). We tried to optimize operating times beginning with the biopsy and, during digestion, preparing the receiving areas. However, times were still longer than skin grafting and this probably produced greater surgical stresses for the patient and additional costs for the operating room, consisting in those for the ReCell kit and those for the longer operation time, without real benefits concerning outcomes (function and aesthetic) over classic treatments. In this context, patients with greater burns would probably benefit more with this procedure. In fact, in large burns (e.g. 60% TBSA, 30% deep dermal, 30% full thickness burns) the factor “additional costs” will or might be reduced due to a shorter hospitalisation time. Using cells would reduce the donor sites necessary to cover deep dermal injured areas and the rest of the donor sites could be used to cover full thickness injured areas.

ReCell was generally well tolerated, even in the immediate postoperative period. All patients received the same postoperative analgesics and with the same doses, but those that underwent skin grafts complained of an additional painful site (the area of harvesting). The ReCell biopsy site, on the contrary, were only 1–4 cm² and produced little if no pain at all. However, both issues (the patient’s surgical stress and the increased economic aspects) need to be verified in specific studies and in patients with larger burns.

5. Conclusions

Our experience lead us to believe that the ReCell system does not alter the basic surgical indications and principles of epidermal replacement in burn patients but gives similar results and is less invasive in removing small quantities of skin to cover the exposed areas. These characteristics do not substitute, in the immediate future, the more experienced skin graft technique, but could favour in the long term, the ReCell system in the treatment of particular situations as in example patients with large burned areas, in which the available amount of uninvolved skin is not enough to cover the affected areas.

REFERENCES


