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Cultured autologous keratinocytes in the treatment of large and deep burns: A retrospective study over 15 years☆

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ABSTRACT

Aim: The aim was to review the use and indications of cultured autologous epidermis (CAE) in extensive burns and to evaluate the efficiency of our strategy of burn treatment.
Materials and methods: This retrospective study comprised 15 years (1997–2012). *Inclusion criteria*: all patients who received CAE. *Exclusion criteria*: patients who died before complete healing and patients who received exclusively cultured allogeneic keratinocytes. Evaluation criteria were clinical. Time and success of wound healing after CAE graft were evaluated.
Results: A total of 63 patients were included with severity Baux score of 107 (from 70 to 140) and mean percentage of TBSA of 71% (from 40% to 97%). The CAE were used as Cuono method, in STSG donor sites and deep 2nd degree burns and in combination with large-meshed STSG (1:6–1:12) in extensively burned patients. Cuono method was used in 6 patients. The final take was 16% (0–30) because of the great fragility of the obtained epidermis. Nine patients with deep 2nd degree burns (mean TBSA 81%, from 60 to 97%) were successfully treated with only CAE without skin grafting. Combined technique (STSG meshed at 1:6–1:12 covered with CAE) was used in 27 patients (mean TBSA 69%, from 49% to 96%) with 85% success rate. Finally, donor sites treated with CAE in 49 patients could be harvested several times thanks to rapid epithelialization (time of wound healing was 7 days (from 5 to 10 days)).
Conclusion: The CAE allow rapid healing of STSG donor sites and deep 2nd second degree burns in extensively burned patients.

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1. Introduction

Cultured epidermis were first performed in vitro by Rheinwald and Green in 1975 [1], then clinically used for the treatment of burns [2]. As early excision became the gold standard for the patients suffering extensive burns [3], cultured autologous epidermis (CAE) generated great hopes for prompt reconstruction of the epidermal barrier. For patients suffering extensive burns, prompt skin coverage is not only life-saving, but is also the key for acceptable functional and esthetic outcome.

First used on small surfaces, CAE were progressively used for patients with greater severity of the size and depth of the burns. Further experience showed inconsistent take of CAE, sensibility to infection, mechanic fragility both in the acute phase, and in rehabilitation. It was admitted that graft take is dependent on the metabolic state of the patient [4]. Finally, the cost effectiveness and even the relevance of the method were put into question [5]. Alternative techniques such as artificial dermis [6,7] and intraoperative enzymatic expansion of keratinocytes [8] were developed.

Our burn team works in close cooperation with the Cell and Tissue Bank which is next to our operating room. We have used CAE since 1988 for most patients burned over 60% of total body surface area (TBSA). Generally, 3 weeks are necessary to obtain CAE. Thus, CAE are planned when skin replacement is expected to last more than 3 weeks. Since surgical treatment (burn excision) starts and continues in this period, we use cultured allogenic keratinocytes (CAE being not available yet) at the beginning of the treatment [9] to accelerate epidermization of dermal burns and STSG donor sites. Medical teams, nurses and rehabilitation therapists are trained in the postoperative wound care and follow-up. However, the cost effectiveness of the method is one of our concerns.

This retrospective study over 15 years evaluates patients treated with CAE alone or after cultured allogenic keratinocytes. The demographics and severity of the patients, the techniques of grafting, the results in term of graft take and length of stay (LOS) are reported.

2. Patients and methods

2.1. Patients

This retrospective study was conducted after Institutional Board Approval and comprises 15 years (from January 1997 to December 2011). The files of all patients treated with CAE at the Department of Burns and Plastic Surgery at Edouard Herriot Hospital, Lyon, France were examined. All survivor patients who received only CAE or CAE after allogeneic culture epidermis were included.

Inclusion criteria: all patients burnt over 50% who survived
Exclusion criteria were, as follows:

- Patients who died before complete wound healing. The inclusion of these patients would artificially decrease the length of stay.
- Patients who received only cultured allogeneic epidermis.

Primary endpoint: Definitive epithelialization without re-grafting.

Secondary endpoint:

- *Tolerance*: all adverse events due to epidermal sheets.
- *Data to measure efficacy*: Detailed data included age, percentage of burned TBSA, anatomic regions, Baux index [10], number of CAE applications, indications and number and surface of CAE used per patient and by session, period of wound healing, eventual complications, length of stay (LOS), time from the admission to transfer to rehabilitation center were recorded. Since the aim of this study was to analyze the use of CAE we focused our analysis and evaluation on the application of CAE. Nevertheless, all other data concerning the other surgical techniques used were recorded. Data concerning the studied parameters can be found in Table 1 with median and interquartile range, and minimum and maximum values.

2.2. Technique of culturing of keratinocytes

The CAE were produced at the hospital laboratory, Banque de tissus et cellules des Hospices Civils de Lyon, Lyon France. The CAE are obtained by extracting and culturing keratinocytes, according to a modified method of Rheinwald and Green [1]. Healthy skin biopsy is preferentially harvested on pubis at patient admission when the decision to use CAE is done.

Presently, keratinocytes are extracted by enzymatic treatment using trypsin 0.5 g/l–EDTA 0.2 g/l (In Vitrogen) for 60 min to extract the cells, which were collected every 20 min. A master cell bank then a working cell bank are prepared by keratinocyte amplification on a feeder layer of irradiated human fibroblasts [11,12,10] in keratinocyte medium composed of Dulbecco's modified Eagle's medium (DMEM®), (In Vitrogen®) Ham's–F12 2.78/1 (In Vitrogen®), 10% fetal calf serum (Hyclone®), 0.4 g/ml hydrocortisone (Upjohn®), 0.12 UI/ml insulin (Umuline, Lilly®), 0.4 g/ml isoprenaline hydrochloride (Isuprel, Sterling Winthrop®), 2×10^{-9} M tri-iodothyronine (pharmaceutical preparation) and 24.3 μg/ml Adenine (pharmaceutical preparation), 10 ng/ml epidermal growth factor (Austral Biologicals®), and antibiotics. After microbiological [13], tumorigenicity [14,15], and viral and non-conventional transmissible agents controls according to European Regulation and Pharmacopea n° 1483, EDQM and CFR 11,353, epidermal sheets are prepared by seeding 8000–10 000 cells/cm^2 in peelable flasks (TPP, Dutscher, Lyon, France). Cells are cultured 10–14 days then sheets are detached from its culture support by enzyme treatment for 15 min and transferred onto petrolatum gauzes (Covidien, Mansfield, Ireland). Obtained sheets should be freshly grafted. In case the local or general status of the patient contra-indicates surgery, autologous CK can be cryopreserved at −80 °C in a solution containing 10% DMSO (Braun®) and 20% calf serum (Hyclone®) for later application.

2.3. Surgical technique

Burn wounds were treated with topical antibacterial ointments. Hydrotherapy has not been used in our Department