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**Cultured epithelial autografts in the management of burn  
injuries: a review of the literature**

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## **Cultured epithelial autografts in the management of burn injuries: a review of the literature**

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## ABSTRACT

**Introduction.** The management of large burn victims has significantly improved in the last decades. Specifically autologous cultured keratinocytes (CEA) overcame the problem of limited donor sites in severely burned patients. Several studies testing CEA's in their burn centers give mixed results on the general outcomes of burn patients.

**Methods.** A review of publications with a minimum of 15 patients per study using CEA for the management of severe burn injury from 1989 until 2011 were recruited by using an online database including Medline, Pub Med and the archives of the medical library of the CHUV in Lausanne.

**Results.** 18 studies with a total of 977 patients were included into this review. Most of the studies did not specify if CEA's were grafted alone or in combination with split thickness skin grafts (STSG) although most of the patients seemed to have received both methodologies in reviewed studies. The mean TBSA per study ranged from 33% to 78% in patients that were grafted with CEA's. Here no common minimum TBSA making a patient eligible for CEA grafting could be found. The definition of the "take rate" is not standardized and varied largely from 26% to 73%. Mortality and hospitalization time could not be shown to correlate with CEA use in all of the studies. As late complications, some authors described the fragility of the CEA regenerated skin.

**Conclusion.** Since the healing of large burn victims demands for a variety of different surgical and non-surgical treatment strategies and the final outcome mainly depends on the burned surface as well as the general health condition of the patient, no definitive conclusion could be drawn from the use of CEA's of reviewed studies. From our own experience, we know that selected patients significantly profit from CEA grafts although cost efficiency or the reduction of mortality cannot be demonstrated on this particular cases.

**Key words.** Cultured epithelial autografts, burn wounds, take rate, TBSA, split-thickness skin grafts.

## INTRODUCTION

Over the last decades, the management of severe burn wounds has been significantly improved. Especially intensive care medicine including the resuscitation standards, nutrition and the management of wound infection that increased the survival of patients with extensive burns [1] urged for the development of novel methods to cover large wounds. The first Xenograft was based on a bovine tendon. Derived collagen matrix was introduced in 1980 by Yannas et al [2] to regenerate their dermal layer. Later, pig and cadaveric dermis were introduced for immediate but temporally wound cover. The wound cover with xenografts initially showed good results of integration in early stages of immunosuppression in large burn victims but were complicated by immediate to late rejections of the xenograft [3]. Immunosuppressive therapies were limited by the risk of a neutropenic state in these patients [4].

The practice of early excision of nonviable tissue followed by immediate skin grafting has resulted in a further improvement in survival [5]. Split thickness largely meshed skin grafting remains the gold standard to cover large wounds in burn victims. Severely burned patients presenting deep burns over 50% of their total body surface area (TBSA) lack healthy skin for skin grafting. One of the major innovations overcoming the limitation covering large wounds with only limited healthy donor sites in extensively burned patients was the innovation of autologous keratinocyte culture [9].

The discovery and application of cultured epithelial autografts (CEA) was invented by Rheinwald and Green [6] in the early 1980s. CEAs resulted in great interest for the treatment of severely burned patients. The CEAs are obtained by a small biopsy of healthy skin of the patient and in the laboratory extracted keratinocytes are then put into culture. Within a delay of 2 to 3 weeks, expansion sheets of keratinocytes are obtained and are available to be grafted on the burn wounds. Despite initial optimism, high cost, complex application and sensitivity to infections were criticized later [7]. However, many burn centers in developed countries can offer CEAs for the treatment of severely burned patients and are the only method to achieve wound coverage in large burn victims [8]. Even though the time to obtain keratinocytes sheets has been significantly reduced to 2-3 weeks throughout the

last decades, the gold standard for burn wounds cover remains the split-thickness skin grafts (STSG) [28], [29], [30]. Since published data on clinical results of CEA treatment are largely miscellaneous, we here reviewed the literature of the last decade of burn centers presenting their results on this subject.

## **MATERIAL AND METHODS**

Using a databank research in refer to cultured epithelial autograft and keratinocyte transplantation on Medline, Pub Med and the archives of the medical library of the CHUV in Lausanne, we included studies between 1989 and 2011, that have been using CEA's only or in association with STSG's for burn wounds coverage in large burn victims. Included studies presented at least a number of 15 patients in a single or multi center study and were either prospective or retrospective studies. Publications were reviewed emphasizing on:

- Number of patients
- Sex
- Age
- TBSA % of burn
- Standard burn wound care
- Definite wound coverage (CEA and skin graft only or in combination)
- CEA culture
- Take rate
- Time of hospitalization
- Mortality

Results were compiled in a table and data were analyzed extensively in each study included.

## RESULTS

**Included studies.** 18 studies have been included in this review. 15 of them were single-center studies whereas 3 were multi-center studies. The majority of the studies were retrospective and there was only one prospective study.

**Patient population.** The total number of patients in all the studies included is 977 patients with an average of 54.22 patients per study. The smallest collective was 15 patients and the tallest collective was 240 patients. The mean age of the patients was 28 years (+/- 9.3 years) (Table 1). The cut-off between pediatric and adult age was not found in every study but was at 15 years in the Carsin & al study [11]. Studies that provided data about the sex of the patients all included more men than women into their studies. The lowest ratio of women was 8% in the Gómez & al study whereas the highest ratio was 43.75% in the Foyatier & al study (Table 1).

**TBSA.** Only 14 studies provided the TBSA% and 11 studies the TBSA% of full-thickness burn (Table 1). The mean TBSA% of included patients was 48% (+/-23%) whereas the mean TBSA% of full-thickness burn was 38.79% (+/-20.17) (Table 1).

**Inhalation injuries.** Studies, which provided the information if an inhalation injury was present or not showed a higher mortality in patients with compared to patients without an inhalation injury. To diagnose the inhalation injury bronchoscopy was commonly performed at the time of admission [11], [12], [16].

**Standard burn wound care.** On the day of the admission of the burn victim into the burn center, authors commonly evaluated the TBSA of burn in the shower and further diagnostics were conducted to detect inhalation or other injuries. Showers were performed commonly every day or at least every 72 hours. Wound dressings mainly included antiseptic topical silver sulfadiazine containing creams such as for example Flammacérium® [11].

Authors followed the gold standard of early excision of the burn wounds as the patient general conditions would allow, usually during the first 48 hours to days after the injury [13]. The maximum excision procedure cut-off varied among studies and was maximally limited at 30% in the Cirodde study [12]. Excised areas were temporally covered with allografts such as

Alloderm® [13], which is an acellular collagen matrix made of cadaveric skin, Integra® [12] [16], which is a bilayer silicone and porous matrix or porcine skin. If CEA was planned to cover wounds, a small biopsy of full-thickness healthy skin of a few square centimeters were taken at the site of unburned skin or preferably in the groin or axillary regions of the patient [13] and were sent to laboratories for culturing of the keratinocytes.

### **Definitive wound coverage**

**STSG.** STSG were preferably taken from the anterior surfaces of the body such as the trunk, anterior surfaces of the lower limbs and of the arms, which are less exposed to trauma and pressure in supine position [12]. Surgical techniques included all kinds of air or electric driven dermatomes. STSGs were commonly meshed with a 1:2 to 1:6 ratio.

**CEA culture.** Studies mostly provided only a brief protocol for CEA culturing. The protocols included small biopsies of full-thickness skin of a few square centimeters unburned skin [12] [13] and sent to laboratories for culturing of the keratinocytes. Each laboratory had their own protocol, but consisted usually in a first step of many washes to remove contaminants. The samples were then placed with trypsin and EDTA to isolate the keratinocytes. Trypsinization was ended with the use of a trypsin inhibitor and the products were seeded into a fetal bovine serum (FBS)-free and cultured at a 37°C temperature in a 5% to 10% CO<sub>2</sub> incubator until the keratinocytes grew to confluence [13], [15]. Once ready the cultured keratinocytes were sent back to burn centers prepared as sheets in Petri dishes consisting of stratified layers of keratinocytes fixed on a petrolatum gauze [12]. Culturing of the CEAs ranged from two to four weeks depending on the study.

**STSG & CEA.** Most authors used wide meshed STSG in combination with CEA, authors did not specify which ratio indicated a switch from only STSG to STSG plus CEA. The exception was the Carsin & al study [11] where they used the cut-offs of 70% of TBSA and 60% of TBSA of full-thickness as these represented their general institutional target indication for CEA application. Authors state that CEA allowed a much wider mesh of STSG than when STSG was used alone [14]. Authors did not mention a maximum cut-off to mesh STSG in

combination with CEA. Most of the publications used STSG in association with CEA when the TBSA of full-thickness burns were over 50% [11], [12], [16]. In the study by Wood et al [14] only 3 patients out of the 62 patients received CEA sheets alone, whereas the 59 other patients were grafted with a combination of STSG and CEA. STSG were preferred for the back regions of the body as they could prepare the wounds to receive CEA a few weeks later to help reepithelialization, indeed CEA alone in those regions are very weak and sensitive to any friction.

**Take rate.** Most authors report a take rate over 30% without details about if the wound was covered with CEA alone or in combination with STSG. The take rates globally ranged from 26% to 73% with a mean value of 47.7% (+/-26.8%) (Table 1).

The correlation of take rate and other factors were not described in all studies. Carsin & al reported that they could not find any statistical relationship between take rate and TBSA as well as they could not show a significant relationship between the take and the timing of CEA culture, but they significantly showed that the only factor associated with a better take rate was a younger age ( $p < 0.04$ ) [11].

**Time of hospitalization.** The mean time of hospitalization was 63.9 days (+/- 34.2 days). Hospitalization times ranged from 43 days [15] to 132 and 275 days [16], [17]. Wood & al found that only the number of operative procedures correlated with the hospitalization time ( $p < 0.001$ ) [14].

**Mortality.** The overall mortality rate reported in the reviewed studies was 14% (+/- 5.13%). The highest mortality rate was reported by older studies conducted in 1980's and 1990's with mortality rates ranging from 10-20% [20] (Table 1), while the lower rates were reported in more recent studies (4-16%) [5], [11], [12], [16].

Investigators	Burn unit	N° of patients	Sex (M/F)	Age (years)	TBSA of burns (%)	TBSA of 3° burns (%)	Graft	Take rate (%)	Hospitalization time (days)	Mortality (%)
Carsin & al (1999)	Clamart (France)	30	19/11		78±10	65±16	CEA	69±23	114±30	10
Wood & al (2006)	Perth (Australia)	62			62.3±14.8		CEA		95±70	16.1
Gómez & al (2010)	5 burn units of Spain	25	23/2	29±11	74±17	61±19	ABCS	49±30	132±69	4
Yim & al (2011)	Seoul (Republic of Korea)	29	26/3	42 (30-49)	55 (44-60)	40 (30-46.5)	CEA	96 week 2, 100 week 4		
Cirodde & al (2011)	Clamart (France)	63	45/18	29 (17-41.5)	81±10	69±14	CEA	26.1±15.5	114 (93-149)	16
Sood & al (2010)	Indianapolis (USA)	88		29.4 (0.5-73)	58.5 (28-98)	51.2 (0-94)	CEA	72.7		9
Archer & al (1998)	Cincinnati (USA)	100	71/29		56.9	50.5	CEA (n=18)		58.3	22
Clugston & al (1991)	Vancouver (Canada)	18	16/2		49	38	CEA	33.8		
De Luca & al (1989)	Genoa (Italy)	40			53.1	37.75	CEA	37	43.1	
Donati & al (1992)	Milano (Italy)	26			33	28.91	CEA	32.5		
Foyatier & al (1989)	Lyon (France)	16	9/7	34 (6-88)	66 (30-92)		CEA	9 pat>50, 3 pat<50, 4 pat=0		18.75
Mühlbauer (1995)	München (Germany)	141					CEA	39.5		
Munster & al (1996)	Baltimore (USA)	22	19/3	31.8±3.1	71.8±2.5	43.7±4.8	CEA		96.4±15.2	14
Odessey & al (1992)	Cambridge (USA)	240		28			CEA	60		
Rue & al (1993)	Birmingham (USA)	16		29.7 (10-56)	68.2 (42-85)		CEA	46.7	132 (50-275)	12.5
Still & al (1994)	Augusta (USA)	15					CEA	71.5		13.33
Tamisani & al (1992)	Genoa (Italy)	17					CEA (n=7)	57.1		
Williamson & al (1995)	Vancouver (Canada)	28	23/5	35.3 (13-81)	52.2 (15-84)	42.4 (12-80)	CEA	26.9	90.4	

**Table 1: Summary of reviewed papers.**

## DISCUSSION

The management of extensive burn victims is a complex combination of operative and non operative treatment approaches. The development in intensive care and the concept of early debridement and skin grafting significantly increased the survival of extensive burn [30]. This manuscript particularly reviews the outcome of studies using CEA that were published in the last 11 years. In summary, we found that published studies presenting the outcome of burn patients treated with CEA lack of standardized method and detailed but essential information as TBSA, comorbidities and wound coverage. As former metaanalysis on clinical results of the use of CEA no conclusion concerning the effectiveness of patient hospitalization, take rate or mortality could be made [7], [19].

The discovery of CEA has evolved from a revolutionary technology to a criticized method because of high costs, complex application and sensitivity to infections [7]. The question of the indications for the use of CEA is doubtless the main question all authors wanted to answer in their papers. Sood & al conclude that CEA should strongly be used in the management of patients with burn wounds over 50% TBSA and with patients with limited donor site availability [5].

First evaluation of the CEA coverage was evaluated at the first dressing change after skin coverage that was usually performed after 6 to 14 days [11], [12], [13], [16], [23]. Carsin & al report that the evaluation of graft take was complicated by the tendency of large areas that showed a patchy pattern of developed epidermis and open wounds [11].

Studies showed a large range of TBSA's and take rates from 26% to 73%. Only Odessey et al [22] gave detailed description on how they calculated the take rate. The take rate was defined as  $1 - (\text{area requiring regrafting} / \text{area grafted with CEA}) \times 100\%$ . Other authors usually defined areas of take as the areas that did not have the need for a regraftment at the end of the treatments [11].

Different parameters were discussed to correlate with take rates. Authors commonly described a lower take rate when patients presented an inhalation injury, but unlikely the Carsin & al study showed the opposite [11]. Sood & al [5] assess alongside with De Luca & al

[15] that the main causes of graft failure were bleeding and infection. Two main elements seem to have an important contribution to the take rate of CEA, first the prevention of infection [11] with the use of topical antibiotics, and second, which is also considered as a key component, the early excision of all the burn wounds areas [5], [22], [23] followed by the placement of Alloderm® or Integra® on the wound beds and eventually the placement of CEA on the partially excised allodermis structure. This good evolution with this protocol is explained by the ability of allografts to form a biologic barrier and the promotion of the vascularization on the wound granulation tissue [22]. This assessment also appear in the Donati & al study [24] where they compared two groups of patients, one of them being treated with an old protocol consisting in spontaneous debridement and achieving a take rate of 25.8% and a second group with a protocol consisting in immediate excision and temporary cover by allografts and or other skin substitutes and achieving a significantly higher take rate of 37.4%. The study from Odessey [22] also compared the average final take of CEA between a group that undergone early excision (<10 days after burn injury) and had a final take of 78%. The group that underwent late excision had a 40% final take rate. Early excision seemed to prevent from wound infection as 13% of the wounds that were excised early developed signs of infection compared to a 48% rate of infection in wounds that underwent late excision [22].

The Carsin & al study [11] statistically showed that a good initial take rate was only significantly associated with a younger age ( $p < 0.04$ ), even if they had strong co-morbidities such as inhalation injuries. The explanation for this assessment seems to be that the more traumatized patients are also the youngest. Donati & al [24] also observed that patients over 65 years had a systematic lower take rate comparing to younger patients, and explained it by the significantly longer laboratory phase before complete confluence and stratification of the CEA sheets.

In the Cirodde & al study [12], the take rate is not provided as they believed that the percentage of TBSA permanently covered with CEA at the time of discharge from the burn unit was preferable than the percentage of CEA that actually was grafted. In their series, the area grafted with CEA represented  $26.1 \pm 15.5\%$  TBSA.

The mean time of hospitalization was 63.9 days with a great range since depending largely on the initial TBSA. Carsin & al study [11] stated 1.5 days hospitalization per percent of TBSA burned. Wood & al [14] found that the most significant factor influencing hospitalization time was the number of surgical procedures ( $p < 0.001$ ).

Wood & al [14] showed that the risk of death was correlated to an older age, male gender, increased percentage of TBSA wound, and with initial intubation. The risk was by cons decreased with each additional day of hospitalization. In most of the articles, the principal causes of death were infections and multiple organ failure (MOF). The most common cultures that grew on the infection sites were *Pseudomonas*, *Candida* and *Staphylococcus aureus* [6], [11], [12], [23]. Munster & al, as the only prospective study in this review was also the only showing a reduction of the mortality with the use of CEA [25]. The patient collective was split into one group who received CEA and another group with controls. Initial financial limitations allowed this prospective study design. The study permitted to show a reduction of mortality from 48% in the control group to 14% in the CEA group ( $p < 0.007$ ).

Complications of CEA use were commonly separated between early and late complications. The early complications were mainly blistering and shearing. Indeed the Foyatier & al study [18] assess that one of the primordial issue is the adherence of the CEA to the conjunctive tissue underneath and that even if all the components of the basement membrane are present at the 8<sup>th</sup> day after grafting, many month are necessary for the dermo-epidermal junction to mature. This could explain early fragility of grafts until final maturation. For their, late complications were mostly delayed CEA loss and scar contractures [6].

The disadvantages shown in many studies were more or less the same, but as shown in the critical review of the literature from Wood & al [7] those were mainly the time necessary to culture CEA sheets, the reliability of take rate, the vulnerability of grafts, the long-term durability and the cost implications of CEA therapy. Concerning the cost of CEA, Carsin & al [11] concluded that since this treatment option is a life-saving technology that is necessary only in a small patient collective per year, the cost to society is acceptable.

The main advantage on CEA is the possibility to cover large burn victims, even if no advantage in hospitalization time or mortality could be demonstrated yet. One of the

advantages of CEA may be the reduction of the hypergranulation of wounds that is seen in largely meshed STSG by inhibiting connective tissue growth by keratinocyte cover [20]. In the Clugston & al study [23] they conclude and emphasize on the fact that CEA should be used and considered as an adjunct and not as an alternative to the conventional STSG coverage.

## Conclusion

Cultured epithelial cells are a mile stone in the treatment of large burn victims. Since a complex treatment of large burn victims is life saving, most of the studies combine various surgical approaches in a non randomized manner, which makes the conclusion of effectiveness of CEA's difficult. No reduction of hospital stay, number of needed operations or mortality, except one, could be shown with the use of CEA. Further well designed studies are important to better understand the ideal application, indication and effectiveness of CEA treatment in large burn victims.

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