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Use of an autologous bioengineered composite skin in extensive burns: Clinical and functional outcomes. A multicentric study

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ABSTRACT

Objective: We report clinical and functional outcomes obtained after application of an autologous bioengineered composite skin (ABCS) produced in a single Spanish tissue-engineering unit.

Materials/methods: Twenty-five burned patients treated with ABCS from 1999 to 2007 in five burn centres were included in the study. Mean age was 29 years (SD 11), with mean total body surface area (TBSA) burned being 74% (SD 17) and mean full-thickness injury of 61% (SD 19) of TBSA.

Results: The mean area initially engrafted with ABCS was 24% (SD 13) of TBSA, with a final take of 49% (SD 30, range 0–100%). ABCS achieved permanent coverage of a mean of 11% (SD 8) of TBSA. In subset analyses, lack of pre- and post-application wound bed infection and lack of serious acute systemic complications at the time of engraftment were significantly associated with better ABCS take.

Conclusions: Final take obtained with ABCS could be improved with the use of non-cytotoxic topical antibiotics following engraftment. The use of plasma to prepare ABCS reduces production costs: cost-effectiveness ratio is not a limitation for its use. In terms of patient satisfaction, cosmetic/functional outcomes (general appearance, texture, flexibility, sensitivity and colour) of ABCS and split-thickness autografts are not different statistically.

1. Introduction

Currently, the overall mortality rates of patients with burns have been reported between 5% and 7% (5.3% in the United States [1] and 6.9% in the Rotterdam Burn Centre [2]). Multisystem organ failure has been identified as the most frequent cause of death, with systemic inflammatory response syndrome and infection often associated with mortal-

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ity [2,3]. In patients with extensive full-thickness burns, skin wounds are a common site of infection and persistence of eschar perpetuates systemic inflammatory response [4]. Therefore, early excision of burns and rapid permanent wound closure remains a limiting factor for reducing morbidity and mortality among these patients, and has been shown to be effective in patients without inhalation injury [5]. Split-thickness skin autografts remain the gold standard for permanent closure of excised burns [6], but they have limited availability in extensive burns. Bioengineered skin substitutes (biosynthetic skin substitutes and autologous cultured/non-cultured skin engineering products) are particularly useful for extensively burnt patients, in the absence of sufficient autograft donor sites. However, most of the products currently available have not sufficiently proved as effective as autografts. For the management of full-thickness burns, the efficacy of bioengineered skin substitutes cannot be determined based on the available evidence [7].

Among the bioengineered skin substitutes, the discovery and application in the early 1980 s of cultured epithelial autografts (CEA) resulted in a great interest for the treatment of seriously burned patients, as they allow to obtain, from a small biopsy of healthy skin and in a short period of time, a large epithelium surface enough to cover the needs of a patient bearing a great surface of the body damage [8]. However, the initial optimism has been tempered by subsequent reports of CEA limitations: delay of 2–5 weeks for culture of the autologous sheets of keratinocytes; mechanical fragility and difficult graft handling; need for an appropriate wound bed; unpredictable take; poor long-term durability with loss of the epithelialisation in treated areas and formation of blistering lesions; vulnerability to infection; and high cost [9–16]. In view of these drawbacks, the exclusive use of CEA in the treatment of burns has been questioned, although they have clearly formed the basis for the development of many of the current bioengineered skin substitutes used *in vivo*. Attempts to improve CEA include incorporating fibroblasts and a dermal layer as an essential element for improving wound regeneration and functionality of the substitutes [17,18].

For the first time in Spain in 1999, an autologous bioengineered composite skin (ABCS) was used for the treatment of a burn patient. This bioengineered skin substitute was developed at the Tissue Engineering Unit of the Centro Comunitario de Sangre y Tejidos del Principado de Asturias (CCST, Spain) and the Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT, Spain), based on the use of both autologous fibroblasts and keratinocytes obtained from a single biopsy, and the use of clotted human plasma as a three-dimensional dermal scaffold in which fibroblasts were embedded [19–21].

To obtain objective data of the clinical outcomes after ABCS engraftment in burn patients, a multicentre retrospective study was undertaken. In addition, patient satisfaction was assessed by comparing cosmetic and functional outcomes with the use of ABCS vs. those obtained with the use of conventional split-thickness meshed autografts (control area). We report the results obtained with the use of ABCS in 25 patients with severe burns.

2. Materials and methods

This was a multicentre retrospective observational cohort study.

Eligibility for inclusion into the study was all patients with burns treated with ABCS from 1999 to 2007 inclusive in five Spanish burn units. Participation in the study was voluntary and participants were requested to sign an informed consent to permit use of the data obtained. Before starting the study, ethic approval was obtained from the ethical committee. A total of 69 patients treated with ABCS were identified and initially considered for inclusion. Finally, data from 25 patients were included in the analysis. The information from the other patients was discarded due to lack of informed consent or loss of follow-up.

Twenty-five patients treated with ABCS from 1999 to 2007 inclusive were therefore included in the study. The mean age was 29 (SD 11) years with a mean total body surface area (TBSA) burned being 74% (SD 17) and a mean total full-thickness injury of 61% (SD 19) of TBSA.

Five Spanish burn units participated in the study: Hospital Universitario de Getafe (Madrid), Hospital de Cruces (Bilbao), Hospital Universitario La Fe (Valencia), Hospital Vall D'Hebrón (Barcelona) and Hospital Virgen del Rocio (Sevilla).

The ABCS used in all patients was produced in similar conditions in a single Tissue Engineering Unit in the CCST in Asturias, Spain, using a small skin biopsy (range 4–8 cm^2) from the patient. In all cases, the ABCSs were transported using conventional land-based means (coach-train). Data collection included demographic and clinical characteristics, treatment and outcomes. Data were compiled from medical records, patient examination and a questionnaire on patient satisfaction with outcomes. In this questionnaire, patients were asked to compare the qualitative outcomes of paired sites of excised full-thickness burns treated with either ABCS or 3:1 split-thickness autografts (control area). These comparative sites were assessed by ordinal scoring for satisfaction with respect to colour, texture, flexibility, sensitivity and general appearance. The assessment used a five-point visual analogue scale in which '1' corresponded to 'very unsatisfied', '2' to 'unsatisfied', '3' to 'neutral', '4' to 'satisfied' and '5' to 'very satisfied'. We converted these data into a new dichotomous variable 'satisfaction' with a positive/negative scoring, considering 'positive' when the response was ''very satisfied/satisfied'' and 'negative' when the response was ''neutral/unsatisfied/very unsatisfied.'' The questionnaire also included some open-ended questions so that the patient could express problems related to the areas investigated. Before completing the questionnaire, a health-care professional indicated the body areas to be compared in each case and explained the assessment scale and the questions.

For long-term medical examination and for assessment of patient satisfaction, data from 18 cases were used. Seven patients were excluded because of ABCS coverage $\leq$5% of TBSA (five cases: patients 8, 17, 21, 22 and 23), which meant that they did not have a sufficient area for evaluating the cosmetic outcomes of ABCS and because they could not answer the questionnaire. The other two patients were excluded because of incomplete questionnaires due to lack

of answers about the control area (one case: patient 9) and death (one case: patient 11).

Given the resulting sample size, most of the results were treated descriptively. Thus, for numeric values, the number of assessable observations, mean, standard deviation, range (minimum and maximum) and 95% confidence interval for mean were tabulated, whereas categoric variables were described by frequency and percentage. Retrospective statistical analyses were performed using Statistical Package for Social Sciences (SPSS®) version 16.0 (SPSS Inc., Chicago, IL, USA). The relationship between final ABCS take and possible prognostic factors was examined by univariate analysis. Subgroups of interest were compared with the Mann–Whitney *U*-test (two independent groups) and the Kruskal–Wallis *H*-test (K independent groups). The Spearman correlation coefficient was calculated for continuous variables. The relationship between satisfaction (positive/negative) and the type of treatment (ABCS/autografts) was assessed for colour, texture, flexibility, sensitivity and general appearance, using a McNemar test. A multivariate analysis was not possible given the small sample size.

3. Results

Table 1 summarises demographic and clinical data on the patient population ($n = 25$). Tables 2 and 3 present demographic and clinical data for each patient with their status at the time of the ABCS engraftment.

The mean age was 29 years (SD 11, range 9–58 years). The burns resulted from work-related accidents in 15 patients, domestic accidents in four, recreational incidents in three, self-inflicted injury in two and assault in one patient. The agent of injury was fire in 14 patients, fire with inflammable liquid in nine, contact in one and scalding in one patient. Inhalation injury was the most commonly associated lesion (15 patients (60%)). Relevant medical antecedents were reported in four patients (patient 4: chronic obstructive pulmonary disease (COPD) and emphysema; patient 12: suprarenal failure; patient 17: major psychiatric illness; and patient 5: drug abuse).

Patients needed a mean of nine (SD 3) surgical procedures. The number of packed red blood cell units administered to the patients while in hospital for a month (15 days prior to and 15 after engraftment) was 29 (SD 21, range: 0–66 units).

In all patients, burns were excised tangentially or to fascia as early as possible after admission. Excised areas awaiting ABCS were temporarily covered by allografts in 20 patients (80% of the cases), Biobrane® (Smith & Nephew S.A., Barcelona, Spain) in three cases (12%), Integra® (Integra Life Science Corporation, Plainsboro, NJ, USA) in one case (4%) and conventional dressing in one case (4%).

A mean of 4329 cm^2 (SD 2795) of ABCS was used per patient, engrafted in 32 surgical procedures. One procedure was performed in 21 patients, two procedures in three patients and five procedures in one patient. ABCS was engrafted onto a mean of 24% (SD 13) of TBSA, with the anterior aspect of the trunk and the legs being the most frequent sites for engraftment. The recipient wound bed of ABCS engraftment was granulation tissue in which the investigators did not detect the presence of either autologous or heterologous residual dermal tissue in 12 patients (48%); heterologous residual dermis from allografts in nine patients (36%); and autologous residual dermis partially covering the recipient bed in four patients (16%). Before ABCS engraftment, common antiseptics were used for dressing burns, most frequently povidone iodine, chlorhexidine and silver sulfadiazine with 1% cerium nitrate. Since the moment of engraftment, ABCS was covered in all cases only with Vaseline gauzes and occlusive dressing with sterile compresses. Neither antiseptics nor antibiotic solutions were used in the immediate postoperative period.

At the time of ABCS engraftment, wound infections, serious systemic complications, intravenous antibiotic therapy for infectious complications and administration of intravenous noradrenaline for haemodynamic instability were assessed with the following results: (1) six patients (24%) had wound infection diagnosed in the 7 days prior to engraftment;

Table 1 – Demographic and clinical data of the patient population.

Parameter	Mean ± SD	Range
Age (years)	29 ± 11	9–58
Male/female	23/2	
TBSA burns (%)	74 ± 17	35–100
TBSA of full thickness injury (%)	61 ± 19	25–90
Delay between burn and first graft (days)	34 ± 12	20–67
Delay between biopsy and graft (days)	23 ± 5	12–28
ABCS grafted (cm^2)	4329 ± 2795	1500–12,750
ABCS grafted (% of TBSA)	24 ± 13	7–60
ABCS take (%)	49 ± 30	0–100
% of TBSA definitively covered with ABCS	11 ± 8	0–36
Total number of operations	9 ± 3	3–16
Number of pre-engraftment operations	5 ± 2	2–12
Number of post-engraftment operations	2.5 ± 2	0–9
Length of hospital stay (days)	132 ± 69	40–269
Long term follow-up (months)	45 ± 27	2–91

TBSA: total body surface area; ABCS: autologous bioengineered composite skin; SD: standard deviation.

Table 2 – Demographic and clinical data.

Centre no.	Patient no.	Age	TBSA burns (%)	Full-thickness injury (%)	Inhalation injury Y = yes N = no	ABCS grafted (cm^2)	ABCS grafted (%TBSA)	Surface to which ABCS were applied[a]	ABCS take (%)	ABCS final coverage (%TBSA)
1	1	20	97	90	Y	8400	60	A	60	36
	2	33	85	80	N	8175	55	A	45	25
	3	21	60	45	Y	2600	17	G	90	15
	4	58	50	45	Y	1500	10	A	80	8
	5	29	55	50	Y	3000	20	G	65	12
2	6	26	90	80	N	10,000	50	G	15	7
	7	29	95	85	N	12,750	37	A	30	11
	8	22	60	50	Y	1500	7	A	10	1
3	9	9	35	25	N	2250	25	AD	60	15
	10	25	60	35	N	2000	18	G	40	7
	11	29	90	60	N	5000	27	A	40	11
	12	48	80	60	N	3300	30	A	40	12
	13	17	95	90	Y	2500	18	A	50	9
4	14	44	85	40	N	2250	18	AD	80	14
	15	31	55	50	Y	3000	16	G	40	6
	16	26	75	65	Y	4500	27	AD	80	22
	17	23	80	65	Y	4500	24	G	10	2
	18	16	70	60	Y	3400	15	A	90	13
	19	24	85	80	N	3000	22	AD	30	7
	20	30	50	35	N	2520	8	A	100	8
	21	25	70	65	Y	3775	15	G	0	0
	22	33	70	65	Y	4300	20	G	10	2
	23	43	70	40	Y	6500	20	G	15	3
5	24	37	100	84	Y	3750	21	G	88	18
	25	20	80	75	Y	3750	20	G	50	10

TBSA: total body surface area; ABCS: autologous bioengineered composite skin.
[a] Surface to which ABCS was applied: A = allodermis; G = granulation; AD = autologous dermis.

Table 3 – State of the patient at the time of engraftment.

Patient no.	ABCS take (%)	Wound infection		Other complications	I.v. antibiotic therapy	Vasoactive drugs
		Pre-engraftment	Post-engraftment			
1	60	N	N	*Pneumonia*	Y	N
2	45	N	N		Y	N
3	90	N	N		N	N
4	80	N	N	*Pneumonia*	Y	N
5	65	N	Y	*Atelectasia*	N	N
6	15	N	Y	*Sepsis*	N	N
7	30	N	Y	*Sepsis*	Y	Y
8	10	N	U		N	N
9	60	N	Y		Y	N
10	40	N	N		Y	N
11	40	N	Y	*Heart failure*	N	N
12	40	N	Y	*Respiratory failure*	N	N
13	50	N	U		N	N
14	80	Y	Y		N	N
15	40	N	N	*Sepsis*	Y	N
16	80	N	N	*Sepsis*	Y	Y
17	10	Y	Y	*Heart failure*	Y	Y
18	90	N	N		N	N
19	30	Y	Y	*Sepsis*	Y	N
20	100	N	N		N	N
21	0	Y	Y	*Sepsis*	Y	Y
22	10	Y	Y	*Sepsis*	Y	N
23	15	Y	Y	*Sepsis*	Y	Y
24	88	N	U		N	N
25	50	N	Y		Y	N

TBSA: total body surface area; ABCS: autologous bioengineered composite skin; Y = yes; N = no.

Table 4 – Univariate relationship between potential quantitative prognostic factors and final ABCS take.

Prognostic factor	Correlation coefficient Spearman rho	P
Age	−0.19	0.928
Total burn area (%)	−0.143	0.495
Deep burn area (%)	−0.225	0.280
Total no. surgical procedures	−0.519	0.008
Stay in hospital	−0.523	0.010

ABCS: autologous bioengineered composite skin.

the most common bacteria were *Pseudomonas aeruginosa* and *Staphylococcus aureus*; thirteen patients (52%) were diagnosed with post-engraftment wound infections with the same bacteria as the infectious agents; as before, *P. aeruginosa* was the most common bacterium, followed by *S. aureus*; (2) at the time of engraftment, 14 patients (56%) had serious acute systemic complications; sepsis was the most common systemic complication; (3) a total of 14 patients were receiving intravenous antibiotic therapy at the time of engraftment (56%) for documented infection; and (4) five patients required intravenous vasopressor drug support (noradrenaline) to maintain systolic blood pressure above 90 mmHg at the time of engraftment or in the first 7 postoperative days (PODs).

The assessment of ABCS take was performed at POD 7, 15 and 30, and at discharge from hospital or death of the patient. Take at POD 7 was 34%; at POD 15 and 30, it was 47%; and it was 49% (SD 30) at discharge. We consider that ABCS at the time of discharge is the most clinically relevant, as it corresponds to the ABCS area that did not require regrafting or any other posterior skin-coverage technique. ABCS achieved definitive coverage of a mean 11% (SD 8) of TBSA, and among the prognostic factors of take analysed (Tables 4 and 5), the following were found to be significantly associated with greater take: lack of pre-engraftment skin infection and post-engraftment skin infection and the absence of serious acute systemic complication at the time of the engraftment.

In our series, one patient died on day 97 after admission due to severe sepsis (patient 11). No adverse effects attributable to the use of ABCS were reported and there was no association between its use and any of the in-hospital complications developed by the patients.

Investigators evaluated the cosmetic outcomes after medical examination of the patients. Hypertrophic scarring, texture and cosmetic appearance were evaluated. Medical examination took place at a mean of 46 months after engraftment for the 18 patients included in this assessment. We did not appreciate loss of the epithelialisation in the areas treated with ABCS and none of the patients required subsequent surgery on these areas. Only in one patient was hypertrophic scarring on the grafted area observed. On assessment of the cosmetic appearance of the grafted area, investigators were satisfied or very satisfied with results achieved in 72% of the patients, were neutral in 11%, and not very satisfied in 17% of the cases. On assessment of the texture, investigators were satisfied or very satisfied with the outcome in 61% of the patients, neutral in 22% and not very satisfied in 17%.

Table 5 – Univariate relationship between potential qualitative prognostic factors and ABCS take (tests: Mann–Whitney U, Kruskal–Wallis H).

Prognostic factor Subgroup	No. patients (%)	ACBS take (%)	P
Inhalation injury			
Present	15 (60%)	49 (SD 33)	0.849
Absent	10 (40%)	48 (SD 25)	
Wound bed			
Granulation	11 (44%)	38 (SD 32)	0.336
Autologous dermis	4 (16%)	62 (SD 24)	
Heterologous dermis	10 (40%)	54 (SD 28)	
Residual dermal tissue			
Present	14 (42%)	57 (SD 26)	0.153
Absent	11 (58%)	38 (SD 32)	
PreABCS wound infection			
Present	6 (24%)	24 (SD 29)	0.015
Absent	19 (76%)	56 (SD 26)	
PostABCS wound infection			
Present	13 (52%)	35 (SD 26)	0.016
Absent	12 (48%)	63 (SD 27)	
Systemic complications			
Present	14 (56%)	38 (SD 27)	0.018
Absent	11 (44%)	64 (SD 28)	
I.v. antibiotic therapy			
Present	14 (56%)	39 (SD 25)	0.075
Absent	11 (44%)	61 (SD 32)	
Vasoactive drugs			
Present	5 (20%)	27 (SD 32)	0.060
Absent	20 (80%)	54 (SD 27)	

ABCS: autologous bioengineered composite skin; SD: standard deviation.

Table 6 – Summary of the patients satisfaction (n = 18).

Degree of satisfaction	Appearance		Texture		Flexibility		Sensitivity		Colour	
	ABCS	CA	ABCS	CA	ABCS	CA	ABCS	CA	ABCS	CA
Very satisfied	7	8	15	11	12	10	7	9	8	8
Satisfied										
Neutral	6	2	1	1	1	3	5	2	7	5
Unsatisfied	5	8	2	6	5	5	6	7	3	5
Very unsatisfied										

ABCS: autologous bioengineered composite skin; CA: control area.

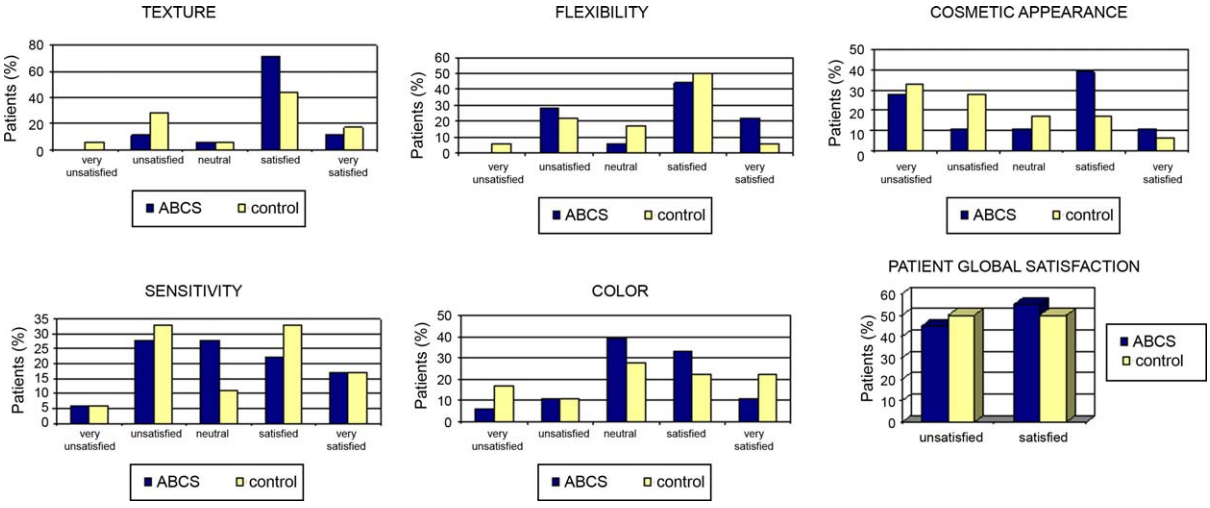

Fig. 1 – Patient satisfaction with cosmetic and functional outcomes. Global satisfaction is evaluated defining a "satisfied patient" when having three or more items with a "very satisfied" or "satisfied" response.

Table 6 and Fig. 1 show data of the 18 patients who reported their satisfaction with the cosmetic and functional outcomes of ABCS and control area. Mean long-term follow-up was 46 months (SD 27, range 2–91 months). Mean ACBS take in these patients was 60% (range, 15–100%), achieving a final coverage of 14% of TBSA (range, 6–36%).

There were no statistically significant differences (McNemar test not significant) between ABCS and the control area outcomes when patients were asked about satisfaction with: colour (p = 1.00); texture (p = 0.12); flexibility (p = 0.69); sensitivity (p = 0.69); and cosmetic appearance (p = 1.00). For both types of coverage, texture was the best-rated aspect, followed by flexibility. The worst ratings were given for general appearance, colour and sensitivity. Patients showed a greater degree of satisfaction with ABCS in general appearance, texture and flexibility. By contrast, a greater percentage of patients were satisfied with the split-thickness autograft compared with ABCS for sensitivity, while satisfaction for colour was the same for both procedures.

4. Discussion

ABCS has been used as an adjunct to the treatment of Spanish burn patients since 1999. In this article, we report clinical and functional outcomes obtained after application of ABCS in 25 burn patients from 1999 to 2007. A total of 69 patients treated with ABCS were identified and initially considered for inclusion. Finally, data from 25 patients were included in the analysis, because the information from the other patients was discarded due to lack of informed consent or loss of follow-up. We know there are a large number of patients treated with ABCS but who are lost for this study. We think the main reasons we have lost so many patients are that many of them live far from the Unit where they were treated and could not travel so easily to hospital to sign the informed consent and fill in the satisfaction questionnaire; and also, as we started using ABCS in 1999, some patients have changed their personal data and we were not able to contact them. Intention to treat and follow-up are important in assessing validity to a study, and we acknowledge that loss of follow-up can result in statistic bias.

ABCS used in all patients was produced in similar conditions in a single Tissue Engineering Unit in CCST in Asturias, Spain. The fact that it is produced in a single centre guarantees its homogeneity, and the accumulation of experience can ensure the quality of the product, as is the case with commercially available products [22]. ABCS differs from other bilayered bioengineered products in the fact that the neodermis is produced using fibrin gel prepared from fibrinogen

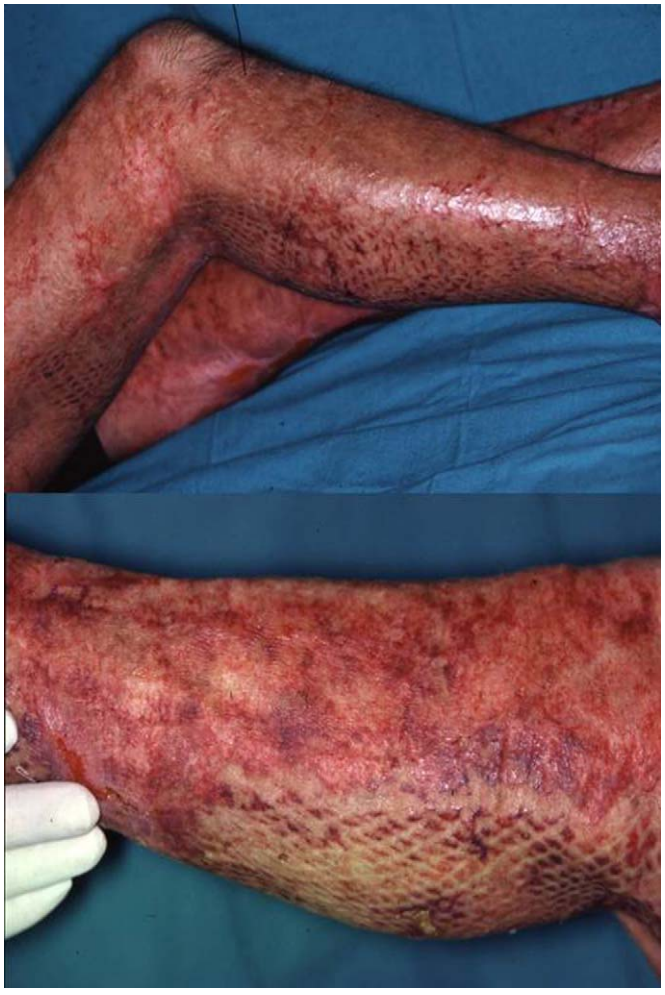

Fig. 2 – Cosmetic appearance of ABCS can be observed in the anterior aspect of the leg vs. cosmetic appearance of meshed autografts in the posterior aspect of the same leg.

derived from human plasma [19]. The artificial dermis based on human plasma and fibroblasts avoids the fragility of the sheets and the lack of dermal component, observed with cultured epithelia autografts [21]. Human plasma is an excellent dermal scaffold, as it allows both keratinocytes and fibroblasts to grow rapidly. Plasma is a cheap product obtained from blood banks. In Spain, blood donation is altruistic and fresh-frozen plasma for ABCS production was obtained from voluntary donors from the local blood bank (CCST) according to the standards of the American Association of Blood Banks (AABB). Plasma from blood banks is tested to prevent viral transmission, and it can also be frozen and quarantined to avoid virus transmission during the windows period; human plasma may also be used after methylene-blue viral inactivation without changing its properties as a dermal matrix [21]. For these reasons, along with the great clinical experience using human plasma, we consider it an extremely safe product. Even so, other authors have proposed the use of autologous plasma to further reduce the risk of disease transmission [23]. A mean of 577 ml (SD 310) of plasma was used per patient (range, 200–1700 ml) to produce ABCS in our series. Considering that patients with major burn injury frequently require multiple blood transfusions (in our series, patients received a mean of 29 units of packed red blood cells in 1 month in hospital), we ruled out the possibility of using the patient's own blood to produce ABCS, as this would increase the volume of blood to be extracted.

Our patients had a mean TBSA burned of 74% (SD 23) with a mean full-thickness injury of 61% (SD 19) of TBSA. These figures are similar to those published by other authors, who used bioengineered cultured skin substitutes as a complement to the use of autografts for definitive burn coverage [22,24] (Fig. 2).

Final take percentage in our series (49%, SD 30) is lower than the results published by authors, who use bilayered coverage containing autologous keratinocytes and fibroblasts [24] or commercially available CEA [22,25]. We consider that several factors might influence our lower take. The first possible factor is the age of the patients treated. Carsin et al. [22] found that the only factor significantly associated with better graft take was younger age, with a mean take for patients less than 15 years of 85%, while Boyce et al. [24] reported a mean take of 81% in patients with a mean age of 7 years. In our series, the mean age was 29 years (SD 11). The

second possible factor is the presence of infection in the recipient beds pre- and post-engraftment. Several studies agree that an uncolonised and well-vascularised wound bed is required for high take, given that this type of skin substitute is more vulnerable to colonisation and wound-bed conditions than meshed split-thickness grafts [10,22,24,26]. It is also recommended to prepare the recipient beds before the procedure. Temporary allografting is the most frequent interim coverage after burn excision, along with topical administration of antibiotics after engraftment. In our series, allografts were used in 80% of the cases, but we did not use topical prophylactic antibiotics or antiseptics after engraftment. Our results showed that the presence of pre- and post-engraftment wound infection is one of the factors significantly associated with lower ABCS take, but, when we first started using ABCS, we did not know the possible effects of topical antiseptics/antibiotics on the cultured skin. Some studies have shown that many antiseptics used at therapeutic concentrations are cytotoxic for fibroblasts and keratinocytes, as is the case for the most frequently used antiseptics in our units: povidone iodine, chlorhexidine and silver sulfadiazine with 1% cerium nitrate [27–29]. That is why we were cautious about using them with ABCS. After performing this study, we have checked that ABCS is very vulnerable to local infection; hence, we conclude that we should include the use of topical non-cytotoxic antibiotic solutions at an appropriate concentration in our protocols after ABCS engraftment, to reduce the incidence of wound-bed infection and to improve ABCS final take. On the other hand, we observed a statistically significant association between the presence of serious acute systemic complications at the time of engraftment and the percentage take. These observations highlight two of the drawbacks of ABCS, also applicable to CEA: there is a delay in their availability and the timing of engraftment can only vary by 2–3 days without compromising the viability of the substitute [30,31]. It is thus not possible to select the best time to perform the engraftment according to the general state of the patient and the local conditions of the area to be treated. In our series, the mean time from biopsy until engraftment was 23 days, similar to the time needed to prepare and graft other cultured skin substitutes [22,24]. Often, we were aware that the timing of engraftment was not optimal and that the chances of success with ABCS were limited. We believe that these drawbacks for the use of this type of skin substitute in burn patients are important, and cannot currently be overcome. At present, new approaches are under investigation for optimising the grafts to reduce the production time and take variability by allowing flexibility in timing to coincide with a good general state of the patient and the recipient bed. Some authors envisage extensive automation of production [32]. New dermal matrices to accelerate culture are also under development [33–35]. These dermal matrices use keratinocytes in suspension applied to different beds. They can be cultured to increase the surface to be covered and can be frozen, therefore allowing the moment of application to be chosen according to optimum conditions of the recipient bed. The products can be applied in a single-step approach to decellularised human dermis or Integra grafts [30,36–39].

We have found that evaluation of ABCS take at POD 15 is more similar to final take (at the time of discharge) than evaluation at POD 7. We agree with Williamson et al. [11] that take cannot be reliably determined at POD 7. This could be explained by the absence of a vascular plexus in bilayered engineered skin substitutes that require regeneration of the blood supply *de novo*, and, therefore, an additional time for take is required compared with split-thickness skin grafts [40,41].

With the use of ABCS, we did not find some of the limitations reported with CEA: mechanical fragility or difficult graft handling, formation of blistering lesions or poor long-term durability with ongoing loss of epithelialisation in treated areas. Like other authors, we attribute these advantages to the presence of a dermal layer in the substitute [26,42].

In addition, the use of human plasma as the base for the dermal matrix of ABCS and the fact that it is produced in a non-profit centre helped to greatly reduce costs. This is important given that treating burns is expensive, particularly in those patients with extensive and severe burns, who require multiple surgical procedures [43,44]. Currently, numerous artificial skin substitutes are available [6,7,45]. In many cases, there is a commercial interest in the product and their effectiveness is not sufficiently proven in clinical practice. Some authors have therefore questioned their cost-effectiveness, pointing out that skin substitutes are not widely used in burn units, where their use depends on the initiative of the director of the unit [46,47]. The ABCS used in Spain has been paid for by public health institutions (public Spanish hospitals) at a price of 1.33 € cm^2 (approximately 1.7 $ cm^2), so that the mean cost per patient was 5758 € (approximately 7385 $) and the cost of 1% of TBSA permanently covered by ABCS was 523 € (approximately 670 $). These costs are much lower than those published by other authors. Meuli and Raghunath [9] reported a mean cost per 1% of TBSA definitively closed with CEA of 6520 $. The cost was 9299 $ in the case of Rue et al. [48] and 5000 $ in the case of Barret et al. [25] per 1% of TBSA covered with Epicel®. Our costs are much lower, and hence, the cost of ABCS did not limit its use and contributed to a better cost-effectiveness ratio.

With regard to cosmetic outcomes, the characteristic scarring of mesh interstices of meshed split-thickness skin grafts is avoided with the use of cultured skin substitutes. In addition, the dermal component aids skin pliability. The result is smoother and there is less raised scar tissue than healed autograft. We therefore might expect clearly better cosmetic results with the use of ABCS than split-thickness autografts, as affirmed by authors who used cultured skin substitutes [9,25,26,41,42]. Nevertheless, the results of our study suggest that for the patients, there is no difference between the results obtained with ACBCS and split-thickness autografts in terms of satisfaction with the general appearance, texture, flexibility, sensitivity and colour. We are aware that the series is small and that we cannot draw firm conclusions. Another finding of note is that in the same group of patients, the physician-reported satisfaction with the cosmetic appearance of ABCS was greater than that reported by the patients. These results cast doubts on whether ABCSs are better than autografts in terms of the cosmetic results for the patients.

Finally, we conclude that: (1) in our experience, ABCS does work and is indicated as an adjunct to the treatment of massive burns >50–60% TBSA, but is vulnerable to secondary

loss by infection; (2) therefore, we think that the final take obtained with ABCS could be improved with the use of non-cytotoxic topical antibiotics following engraftment; (3) the cost-effectiveness of this skin substitute would support more widespread use as an adjunct to the treatment of extensive burns, as cost is not a limitation to its use; and (4) in terms of patient satisfaction, cosmetic and functional outcomes achieved with ABCS are not significantly better than those obtained with split-thickness autografts.

Conflict of interest statement

The authors declare no financial or personal conflicts of interest.

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