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Title: A systematic review: current trends and take rates of Cultured Epithelial Autografts (CEA) in the treatment of patients with burn injuries

Short Running Title: Cultured Epithelial Autografts

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Key Words: Cultured Epithelial Autografts, CEA, keratinocyte culture, cultured skin, burn injury

ABSTRACT

Several issues persist in clinical translation and application of cultured epithelial autografts during treatment of patients with massive burn injuries. The aim of this systematic review is to determine (1) current practice and trends in clinical application and (2) clinical efficacy of cultured epithelial autografts. A structured literature search was performed in Ovid MEDLINE from 1946 and Ovid EMBASE from 1974 till present. All published peer-reviewed randomized or non-randomised clinical studies, cohort studies, prospective or retrospective series involving human application of cultured epithelial autografts in the setting of burn injury were included. From 7267 studies initially identified, 77 studies were included in the analysis. Ninety-six percent (74/77) of these series had a sample size of less than 100 patients. In 76.6% (59/77) publications, average burn treated exceeded 40% total body surface area. Overall, cultured epithelial autograft take rates reported in the literature were inconsistent and varied significantly from 0-100%. There was a recent trend for co-application of cultured grafts with autologous skin grafts, achieving relatively high and consistent take rates of 73-96%. Results from cultured epithelial autograft application remained unpredictable. This technology remains an adjunct or biological dressing, and not an alternative to conventional split skin graft.

However, it has contributed to wound closure and it has been life saving in selected circumstances. Skin tissue engineering should continue as the clinical need for skin replacement is foreseeable into the future.

INTRODUCTION

Split skin grafts (SSG) are considered gold standard in wound closure following excision of burn wounds (1). However in patients with deep burns exceeding 40% total body surface area (TBSA), donor sites are limited resulting in conflicting demands for available sites and delayed wound closure. Harvesting of split skin grafts also add further morbidity and healing requirements in these unwell patients.

In the 1970s-80s, Rheinwald & Green were pioneers of cultured epithelial autografts (CEA), laboratory based cell culturing and expansion methods which address the issue of donor site and split skin graft shortage in patients with massive burn injuries (2). The first clinical application of epithelium prepared from autologous epidermal cells were attributed to the same group in 1981 and since then, numerous centres have expanded on their work and published their own experiences (3).

In the clinical translation and application of CEA, several issues persist including the clinical efficacy of CEA, indications for CEA, wound and patient

selection, delivery systems, wound healing and outcomes. Whilst numerous burn units around the world continue to treat patients with major burn injuries with CEA, there are differences in opinion and variations in clinical application.

There is a need to systematically review the literature regarding the clinical application of CEA. Existing reviews are few, either dated or largely not systematic in nature (4-7). The aim of this systematic review is to determine (1) current practice and trends in clinical application of CEA and (2) clinical efficacy of CEA.

METHODS

Literature search strategy

A structured literature search was performed in Ovid MEDLINE from 1946 and Ovid EMBASE from 1974 till present using the keywords "cultured epithelial autograft" OR "CEA" OR "keratinocyte culture" OR "cultured skin". To improve relevance of search results we narrowed the field with NOT "carotid", NOT "cancer", NOT "malignan*", NOT "carcinom*" and NOT "pancreas*". In addition we searched The Cochrane Library and manually searched reference lists of related journal articles and existing reviews for additional studies. All searches were limited to articles in English.

No attempts were made to locate unpublished material or to contact authors of unpublished studies. The review was conducted according to the PRISMA statement for reporting systematic reviews and prospectively registered

in PROSPERO (registration number CRD42018089599), an international register of systematic reviews (8, 9).

Study selection criteria

All published peer-reviewed randomized or non-randomised clinical studies, cohort studies, prospective or retrospective series involving human application of CEA in the setting of burn injury were included. Laboratory based experimental or animal studies, non-English language studies, non peer-reviewed studies, studies not available in full text, unpublished data, clinical practice guidelines and commentaries were excluded. All studies involving culturing of fibroblasts and engineered skin substitutes (ESS), a non-comparable product, were excluded.

Data extraction

Two review authors (CHL, EC) screened records retrieved by the initial search to exclude obviously irrelevant studies. These two authors independently assessed titles and abstracts against the inclusion criteria and selected studies to be included in the review. In all instances, differences of opinion were resolved by discussion among the authors.

The information extracted include characteristics of the study (citation, region), study design, patient demographics (age, gender, % TBSA burn, mechanism of injury), CEA manufacturing process (skin harvest, donor site,

delivery system), CEA recipient sites (timing of burn excision, wound temporization), CEA application (strategy of CEA application), measures to minimise infection risk (intravenous and topical antibiotics, dressings) and outcomes (CEA take rates). Further outcome data extracted include hospital length of stay, follow up and mortality.

Data analysis, management and synthesis

All data were managed and analysed using Microsoft Excel® Mac 2011 and Endnote™ X8.2. Due to the heterogeneity of studies included, absence of randomized controlled trials and inconsistency of outcome reporting measures, pooling of studies and meta-analysis was not possible. A narrative approach was adopted to synthesise findings from included studies.

RESULTS

Search results

The initial search strategy identified 7267 studies for potential inclusion. Independent screening of the titles and abstracts followed by review of articles identified 77 studies included in the study (Figure 1). Most of these publications from 1981-2016 were either retrospective or uncontrolled cohort studies (level III-IV evidence); six had multi-centre involvement. Several publications including the same subset of patients were all included in this comprehensive review to determine the issues discussed over time and the trends involving CEA

application over more than thirty years. Eight case reports were included in this review.

Published clinical series were mostly small in size, with 96% (74/77) of these having had a sample size of less than 100 patients. CEA application has been most widely adopted by burn centres in Europe and United States with 79.2% (61/77) publications originating from these countries. Interest peaked in the early 1990s with commercialization of CEA and availability of Epicel® (Biosurface Technology and Genzyme, United States). Burn centres from Asia have recently started publishing in the English literature, with 80% (8/10) of their clinical series published within the last 10 years. Similarly, this recent interest from burn centres in Asia coincided with development and health insurance funding of JACE® (Japan Tissue Engineering Co, Japan), Keraheal™ (MCTT, South Korea) and Holoderm® (Tego Science, South Korea).

Patient demographics

Summary of clinical series included can be found in Table 1. In 76.6% (59/77) publications average TBSA burn treated was > 40%. The scarcity of these patients with massive burn injuries partly explained the small number of patients included. In 11 publications, CEA was also used to treat other conditions such as giant naevi, non-healing ulcers, scar revisions and tattoo removal (10-20). In 18 publications, CEA was used to treat paediatric burn injuries only (11, 15, 16, 20-34).

Notably, the largest clinical series published by Oshima et al included 430 patients with acute burn injuries, revision of burn scars, giant naevi and tattoos (17). In Matsumura et al's 6 year multi-centre surveillance on the application of JACE® (Japan Tissue Engineering Co, Japan), 515 patients were enrolled to undergo CEA treatment with 216 patients included in the week 4 efficacy analysis (35). Odessey published verified data for 104 patients from different burn centres in United States and Europe (36).

CEA manufacturing and delivery

Autologous skin was harvested either full thickness or partial thickness for culturing. At least eighteen centres preferred only full thickness hair bearing skin from donor sites such as axilla, groin and scalp (13, 15, 20, 21, 23, 25, 30, 37-47). In general, culturing techniques were based on those described by Rheinwald and Green in 1975, and keratinocytes cultured on a feeder layer of lethally irradiated 3T3 murine fibroblasts (2). Braye et al used irradiated human foreskin fibroblasts instead of murine fibroblasts however the most significant departure from Green's method was perhaps Pittelkow's two-phase technique (32, 48). Proliferation of a basal cell-like population was achieved in serum-free medium followed by induced formation of cohesive, stratified sheets of differentiated keratinocytes for grafting, all in the absence of feeder cells, serum or potentially toxic chemicals (48).

Spray-on CEA was developed by Wood et al and continued to gain popularity in more recent years (4, 33, 34, 45, 46, 49-53). Cultured keratinocytes were applied earlier at the pre-confluent stage and the reduced culturing time in the laboratory led to reduced cost (4). CEA may be sprayed relatively easily and directly onto donor sites and partial thickness wounds, or be applied in association with meshed SSG in deep partial thickness burns (4). Mixed suspension of autologous and allogeneic keratinocytes have also been injected via syringe underneath cadaveric allograft covering full thickness burn wounds (53).

Petrolatum or vaseline gauze were most commonly used as backing for CEA sheets. Other less common yet novel approaches to delivery of CEA sheets included fibrin (43, 54), Biobrane (52), synthetic hydrogels such as poly(2-hydroxyethylmethacrylate) (pHEMA) (18), sterile medical grade polymer coated with plasma polymerized functional surface containing 20% carboxylic acid (PPS) (19), tegapore (55), Myskin™ (55) and chitin membrane (crab shell extract) (17).

Wound bed preparation

Burn wounds were usually excised early. Depending on institutional protocol, it was carried out as early as day of admission and usually completed within 5-7 days. Sites chosen for CEA application included the trunk, upper and lower limbs and depended on pattern of burn injury and surgeon preference. CEA application

over joints, bony prominences and perisphincteric regions were difficult to immobilize and avoided (14). In most instances, weight bearing surfaces such as the back were avoided to minimize shear, pressure and CEA dislodgement. Due to resulting contour deformity and contractures, fascial excision were carried out only if necessary (56). As described by Cuono et al, cadaver allograft was applied in more than two thirds of publications and remained by far the most popular method of wound temporization (32, 57). It is believed that early excision and allograft temporization resulted in low infection rate and higher CEA take (36, 58). However difficulties with application of cadaver allograft included limited availability and inconsistencies in CEA take rate achieved (47, 55, 59, 60). Other wound temporizing measures included Biobrane® (15, 24, 28, 39, 42, 58, 61, 62), Integra® (29, 52, 63), xenografts (11, 24, 39, 40, 42, 64), Kaltostat® (42), saline gauze (42), polyvinyl alcohol hydrogel (23) and cultured allogeneic allograft (47). In addition to burn wound, CEA was being applied to graft donor wounds with increasing frequency (4, 17-19, 33, 47, 52, 65).

Clinical application of CEA

There was a recent trend for co-application of CEA with autologous graft. In the last decade, almost all clinical series involved simultaneous application of CEA with autologous graft (4, 20, 33-35, 45-47, 50-52, 63-66). Autologous grafts were meshed with the meshing ratio ranging from 1:1.5 to 1:12 (33, 47). CEA has also been applied with Meek grafting (33, 50, 63, 65, 67).

The novel approach of applying keratinocyte fibrin glue suspensions underneath allogeneic overgraft led to permanent epithelialization of large fascial wounds and considered better than Cuono method (41).

In terms of dressings, most common contact layers used were Vaseline® petrolatum gauze (42, 43, 45-47, 54, 57, 59, 60, 68) and tulle gras (14, 33, 50, 51, 62, 69). Other non-adherent contact layers used include Adaptic™ (37), N-terface® (13), Comfeel® (70, 71) and UrgoTul® (35). These were supplemented by dry or moist gauze as absorbent secondary dressing.

Infection management

Reporting of microbiological surveillance regimes such as frequency and types of samples tested were inconsistent. There was significant variability in the application of infection prophylaxis measures such as topical antimicrobials and intravenous antibiotics. A wide range of topical antimicrobial agents have been used including silver nitrate (14, 21), silver sulfadiazine (70-72), aminoglycosides (10), bacitracin (37), polymycin (25, 37, 44, 73), vancomycin (44, 74), amphotericin (44), neomycin (73), povidone-iodine (30, 32, 54) and mupirocin (51). Others have noted extensive lists of topical antimicrobial agents tested with CEA (20, 38, 66). Chlorhexidine gluconate was considered toxic to CEA and mafenide acetate inhibited fibroblastic and epithelialization phases of healing and not recommended (58). Topical agents applied also include corticosteroids (73) and antifungal mycostatin (25).

Outcomes: take rate, length of stay and mortality

There was a paucity of quality literature to support the popular but controversial belief that CEA expedites healing, decreases mortality and decreases hospital length of stay.

CEA take rates reported in the literature overall were inconsistent and varied significantly from 0-100% (15, 62, 71, 75). Simultaneous application of CEA and autologous graft achieved relatively high and consistent take rates of 73-96% (32, 33, 35, 45, 65). Chrapusta et al analysed 20 children with 55-65% TBSA full thickness burns (34). Three groups of wounds in this comparative study were treated with meshed split thickness skin graft only, CEA only or a combination of both techniques. The shortest time to wound closure was observed in the group treated with combination of both techniques.

CEA take rate was the most commonly reported outcome measure but poorly defined or standardised. De Luca et al defined CEA take as the presence of viable epithelium following removal of dressings (56). Teepe et al determined graft take as the presence of viable epidermis expressed as a percentage of area treated by two experienced surgeon and one dermatologist (76). Others incorporated areas requiring regrafting into their definition (36, 66). Clinical assessment can be difficult and dressings adherent; epidermis appears thin, transparent or “frosted” and areas of take dull and translucent, but accumulation of fibrous tissue or fibrinous mats can have a similar appearance (36, 59, 73).

Whereas viable CEA usually appear pink and dry, non-epidermised granulation tissue is wet in appearance (44). CEA take was generally determined at approximately 5-10 days after CEA application. Several authors preferred to report CEA take at a different time point (4 weeks after CEA application, or at discharge from hospital) due to delayed formation of anchoring fibrils and anticipated early CEA graft fragility (27, 33, 35, 36, 44, 61, 64, 65, 74). Occasionally, reporting was descriptive only (10, 19, 23, 50, 53, 77); at times it was not reported at all (34, 38, 46, 48, 49, 57, 63, 78). Clinicians were usually not blinded in their asssessments.

Munster reported on their single centre experience and compared 22 patients treated with CEA with 42 controls and found that CEA paradoxically increased hospital length of stay (78). This increase was attributed to the reduction in mortality rate from 48% in the control group to 14% in the CEA group. Barret et al studied all paediatric patients with more than 90% TBSA full thickness burns over a 10 year period and found that patients grafted with CEA had a longer hospital length of stay but no significant difference in mortality rate (31).

In their retrospective study, Wood et al audited 84 major burn patients between 1992-2002 and identified three non-matched groups totaling 62 patients (4). A decrease in total length of stay was identified in the group of 36 patients who received CEA suspension. However in this group, the mortality rate

(22%) was higher compared to the CEA sheet group (14%) and CEA sheet and suspension group (0%).

Kym et al conducted a prospective observational study of 96 patients treated with and 81 patients without CEA (46). The CEA group showed significantly higher survival than the non-CEA group. However the two groups were not randomized and instead divided according to insurance status, hence potential selection bias. In addition, the percentage TBSA burn of full-thickness burns was significantly higher in the CEA group. Williamson et al published their five year experience with 28 patients comparing the CEA group with a matched control group from the preceding 5 years and found no significant difference in the duration of hospital stay nor survival (59).

DISCUSSION

There has been great enthusiasm for CEA in the published literature (10, 12, 72). Undoubtedly, CEA contributed to wound closure (25, 26, 61). In patients with massive burns, CEA was indeed life saving especially if there were no other treatment options (21, 30). However after more than four decades of research, results from CEA application remained unpredictable (2, 79). Good CEA take was sometimes the exception rather than the norm (59). Others felt CEA exerted no demonstrable effect on the outcome of extensively burned patients (74). Poor outcomes have been attributed to unfamiliarity with technique, poor wound bed

preparation and wound colonization (66). However, a good technique should be reproducible.

Wound colonization was difficult to prevent and infection remained the most common cause of CEA failure (56, 59, 60, 76, 77, 79). The ideal antibiotic or antibiotic combination was uncertain however extensive suggestions have been made (20, 38, 58, 66). Laboratory based investigations (not the focus of this review) have also been done. In one study, combined cytotoxicity-antimicrobial activity assays were examined to determine the most suitable antimicrobials for use with cultured skin (80). Chlorhexidine gluconate was found to be uniformly toxic to cultured cells and microorganisms, and norfloxacin had a dose-dependent toxicity to human cells and broad effectiveness against microorganisms (80). Addition of nystatin to antibiotic formulations did not increase cytotoxicity to cultured cells or reduce antimicrobial activities (80).

The current trend in the literature was for simultaneous application of CEA with autologous graft. There was emerging evidence that take rates were improved by co-application of CEA and expanded autologous graft. It was well accepted that in recipient sites where dermal components were preserved, higher CEA take rates were achieved (10); a similar benefit was derived from the introduction of dermal elements in the autologous graft applied with CEA. However, it was almost impossible to determine the contribution to wound healing from cultured epithelium, from co-applied autograft or even secondary wound healing (69). A randomized, paired site comparison study of full

thickness wounds treated with expanded autologous graft with and without CEA is necessary. Power calculations at the outset of the study would allow robust statistical analyses; clearly defined outcome measures and blinded clinicians would improve reliability of assessments. This is important to direct future directions of CEA application. Despite proposed benefits of reduced keratinocyte culturing time and cost, and ease of delivery, there were no quality publications reporting efficacy of spray technique over CEA sheets (46, 47).

Pandya et al treated one child whose anterior trunk was partially resurfaced with Integra followed by CEA application via a two stage approach (see Table 1) (29). Frew et al treated three cases where at least in one patient, Integra® was applied prior to CEA delivery via a Biobrane® carrier (52). Dorai et al treated nine patients with CEA; however in only two cases was Integra® used and the outcomes not specifically reported (63). Integra® is a trialled and tested product designed for dermal regeneration (81). However, the evidence for successful clinical application of CEA with scaffolds such as Integra® is scant and the practice remains unproven.

It has been estimated that up to 2% of burn scars may undergo underwent malignant transformation, of which squamous cell carcinoma was the most common type (82). Increased trauma or tension of the scar tissue were considered responsible for higher incidence of squamous cell carcinoma in flexor and extensor surfaces, and in areas overlying bony prominences (83). In one study, these accounted for 70% of malignancies after burn injuries (83). At least

six cases (0.26%) of graft site malignancy have been reported in patients treated with CEA (82, 84). It was concluded in one case that the stresses placed upon cultured skin applied in the popliteal fossa led to repeated ulceration and eventually contributed to malignant transformation (82). Another potential contributor to malignant change of CEA treated sites was the use of mitogenic stimulators such as isoproterenol and cholera toxin during in vitro expansion (82). All burn patients should be warned about the possible risk of malignant transformation (82). Even though the frequency of known cases to date does not conclusively indicate further increase in risk for SCC following treatment with CEA, similarly, patients treated with CEA should also be warned of the risk of malignant transformation (84).

The current state is that CEA remains an adjunct or biological dressing, and not an alternative to conventional SSG (59, 79). With cost estimates ranging from $US600 to more than $US13 000 per 1% TBSA cultured CEA, it remained expensive, unviable and arguably unnecessary or inappropriate for most burn centres and most burns (37, 38, 54, 74, 78). We agree with Williamson et al in their recommendation against setting up a culture laboratory for the sole purpose of treating burn patients (59). However, CEA affords experience with culture techniques, wound preparation, graft care and serves as a foundation for development of more sophisticated skin engineering products (59).

Scarcity of funding and regulatory hurdles will continue to present significant challenges, however skin tissue engineering development should

certainly continue (85). Several promising areas deserve further attention to further develop this field. In the current era of antibiotic resistance, phage therapy may prove to be a useful adjunct in wound bed preparation to address the high rates of wound colonization, potential infection and CEA loss (86). Pluripotent, high proliferative capacity and self-renewing nature of stem cells may have much to offer especially if these cells can be isolated consistently from the adipose layer of burned skin obtained during debridement (87).

Since this systematic review was conducted, more clinical data involving treatment of burn patients with CEA have been published. In a prospective observational cohort study of adult burn patients, CEA applied in combination with widely meshed SSG led to the highest take rate of 90.1% at 7-10 days (88). Colonisation of excised burn wounds occurred in nearly a third of treated sites, and was not prevented by temporization of excised wounds with Biobrane® or cadaveric allograft (88). Sites successfully treated did not have any microbiological growth, or grew skin flora only (88). Their experience was consistent with the findings of this systematic review. In another study, a summary of 954 burned patients treated with CEA, the largest series to date, was presented (84). The inclusion of 34% (325/954) paediatric patients likely contributed to the median graft take at discharge of 75% (84). CEA was also commonly used in combination with autologous SSG; contribution of CEA to wound healing was not isolated and most likely augmented by co-application of autologous SSG. The overall survival at discharge reported was 84%, and

comparison to National Burn Repository 2016 Report indicated CEA treatment was associated with reduced mortality ($P<0.001$) (84). However it was acknowledged that reporting to National Burn Repository was voluntary and may have resulted in unintentional bias. Data was summarized from sponsor-sourced datasets and the publication funded by Vericel Corporation (Cambridge, Massachussetts), the manufacturer of commercially available CEA called Epicel® (84). The possibility of conflict of interest and reporting bias should be considered in industry-author affiliations or industry sponsored publications (4, 19, 36, 43, 55, 84).

CEA has been life saving in certain applications, and we have seen glimpses of promise and the clinical need for skin replacement into the future. Moving forward, persistence and collaboration in skin tissue engineering by interested and experienced parties including a burns unit, in the setting of a university teaching hospital will ensure continuing progress towards the manufacture of a true skin substitute.

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LIST OF ABBREVIATIONS

CEA: Cultured Epithelial Autografts

ESS: Engineered Skin Substitutes

pHEMA: poly(2-hydroxyethylmethacrylate)

PPS: Plasma Polymerized Surface

SSG: Split Skin Graft

TBSA: Total Body Surface Area

REFERENCES

1. Lee JO DM, Jimenez CJ, Gallagher JJ, Sayeed S, Sheridan RL et al. Operative wound management. In: Herndon DN, editor. *Total burn care*. Edinburgh: Saunders Elsevier, 2012:157-72.

2. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 1975;6(3):331-43.

3. Gallico GG, O'Connor NE. Engineering a skin replacement. *Tissue Eng* 1995;1(3):231-40.

4. Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: Eleven years of clinical experience. *Burns* 2006;32(5):538-44.

5. Atiyeh BS, Costagliola M. Cultured epithelial autograft (CEA) in burn treatment: three decades later. *Burns* 2007;33(4):405-13.

6. Chester DL, Balderson DS, Papini RP. A review of keratinocyte delivery to the wound bed. *J Burn Care Rehabil* 2004;25(3):266-75.

7. Chua A, Khoo YC, Tan BK, Tan KC, Foo CL, Chong SJ. Skin tissue engineering advances in severe burns: review and therapeutic applications. *Burns & Trauma* 2016;4(3):1-14.

8. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 2009;6(7):e1000100.

9. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6(7):e1000097.

10. Kumagai N, Nishina H, Tanabe H, Hosaka T, Ishida H, Ogino Y. Clinical application of autologous cultured epithelia for the treatment of burn wounds and burn scars. *Plast Reconstr Surg* 1988;82(1):99-110.

11. Coleman JJ, 3rd, Siwy BK. Cultured epidermal autografts: a life-saving and skin-saving technique in children. *J Pediatr Surg* 1992;27(8):1029-32.

12. Krupp S, Benathan M, Meuli M, Deglise B, Holzer E, Wiesner L, et al. Current concepts in pediatric burn care: management of burn wounds with cultured epidermal autografts. *Eur J Pediatr Surg* 1992;2(4):210-5.

13. Siwy BK, Compton CC. Cultured epidermis: Indiana University Medical Center's experience. *J Burn Care Rehabil* 1992;13(1):130-7.

14. Donati L, Magliacani G, Bormioli M, Signorini M, Preis FW. Clinical experiences with keratinocyte grafts. *Burns* 1992;18 Suppl 1:S19-26.

15. Gobet R, Raghunath M, Altermatt S, Meuli-Simmen C, Benathan M, Dietl A, et al. Efficacy of cultured epithelial autografts in pediatric burns and reconstructive surgery. *Surgery* 1997;121(6):654-61.

16. Sheridan RL, Morgan JR, Cusick JL, Petras LM, Lydon MM, Tompkins RG. Initial experience with a composite autologous skin substitute. *Burns* 2001;27(5):421-4.

17. Oshima H, Inoue H, Matsuzaki K, Tanabe M, Kumagai N. Permanent restoration of human skin treated with cultured epithelium grafting--wound healing by stem cell based tissue engineering. *Hum Cell* 2002;15(3):118-28.

18. Dvorankova B, Holikova Z, Vacik J, Konigova R, Kapounkova Z, Michalek J, et al. Reconstruction of epidermis by grafting of keratinocytes cultured on polymer support--clinical study. *Int J Dermatol* 2003;42(3):219-23.

19. Zhu N, Warner RM, Simpson C, Glover M, Hernon CA, Kelly J, et al. Treatment of burns and chronic wounds using a new cell transfer dressing for delivery of autologous keratinocytes. *Eur J Plast Surg* 2005;28:319-30.

20. Sood R, Balledux J, Koumanis DJ, Mir HS, Chaudhari S, Roggy D, et al. Coverage of large pediatric wounds with cultured epithelial autografts in congenital nevi and burns: Results and technique. *Journal of Burn Care and Research* 2009;30(4):576-86.

21. Gallico GG, 3rd, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. *N Engl J Med* 1984;311(7):448-51.

22. Gallico GG, 3rd, O'Connor NE. Cultured epithelium as a skin substitute. *Clin Plast Surg* 1985;12(2):149-57.

23. Bettex-Galland M, Slongo T, Hunziker T, Wiesmann U, Bettex M. Use of cultured keratinocytes in the treatment of severe burns. *Z Kinderchir* 1988;43(3):224-8.

24. Compton CC, Gill JM, Bradford DA, Regauer S, Gallico GG, O'Connor NE. Skin regenerated from cultured epithelial autografts on full-thickness burn

wounds from 6 days to 5 years after grafting. A light, electron microscopic and immunohistochemical study. *Lab Invest* 1989;60(5):600-12.

25. Desai MH, Mlakar JM, McCauley RL, Abdullah KM, Rutan RL, Waymack JP, et al. Lack of long-term durability of cultured keratinocyte burn-wound coverage: a case report. *J Burn Care Rehabil* 1991;12(6):540-5.

26. Herndon DN, Rutan RL. Comparison of cultured epidermal autograft and massive excision with serial autografting plus homograft overlay. *J Burn Care Rehabil* 1992;13(1):154-7.

27. Compton CC. Current concepts in pediatric burn care: the biology of cultured epithelial autografts: an eight-year study in pediatric burn patients. *Eur J Pediatr Surg* 1992;2(4):216-22.

28. Lopez Gutierrez JC, Ros Z, Vallejo D, Perdiguero M, Soto C, Tovar J. Cultured epidermal autograft in the management of critical pediatric burn patients. *Eur J Pediatr Surg* 1995;5(3):174-6.

29. Pandya AN, Woodward B, Parkhouse N. The use of cultured autologous keratinocytes with integra in the resurfacing of acute burns. *Plast Reconstr Surg* 1998;102(3):825-8; discussion 9-30.

30. Chalumeau M, Saulnier JP, Ainaud P, Lebever H, Stephanazzi J, Lecoadou A, et al. Initial general management and surgery of six extensively burned children treated with cultured epidermal autografts. *J Pediatr Surg* 1999;34(4):602-5.

31. Barret JP, Wolf SE, Desai MH, Herndon DN. Cost-efficacy of cultured epidermal autografts in massive pediatric burns. *Ann Surg* 2000;231(6):869-76.

32. Braye F, Oddou L, Bertin-Maghit M, Belgacem S, Damour O, Spitalier P, et al. Widely meshed autograft associated with cultured autologous epithelium for the treatment of major burns in children: report of 12 cases. *Eur J Pediatr Surg* 2000;10(1):35-40.

33. Menon S, Li Z, Harvey JG, Holland AJA. The use of the Meek technique in conjunction with cultured epithelial autograft in the management of major paediatric burns. *Burns* 2013;39(4):674-9.

34. Chrapusta A, Nessler MB, Drukala J, Bartoszewicz M, Madry R. A comparative analysis of advanced techniques for skin reconstruction with autologous keratinocyte culture in severely burned children: Own experience. *Postepy Dermatol* 2014;31(3):164-9.

35. Matsumura H, Matsushima A, Ueyama M, Kumagai N. Application of the cultured epidermal autograft "JACE(") for treatment of severe burns: Results of a 6-year multicenter surveillance in Japan. *Burns* 2016;42(4):769-76.

36. Odessey R. Addendum: Multicenter experience with cultured epidermal autograft for treatment of burns. *J Burn Care Rehabil* 1992;13(1):174-80.

37. Munster AM, Weiner SH, Spence RJ. Cultured epidermis for the coverage of massive burn wounds. A single center experience. *Ann Surg* 1990;211(6):676-9; discussion 9-80.

38. Law EJ, Orlet HK, Still Jr JM. Experience with cultured skin in a Georgia regional burn unit. *J Med Assoc Ga* 1992;81(4):185-8.

39. McAree KG, Klein RL, Boeckman CR. The use of cultured epithelial autografts in the wound care of severely burned patients. *J Pediatr Surg* 1993;28(2):166-8.

40. Still JM, Jr., Orlet HK, Law EJ. Use of cultured epidermal autografts in the treatment of large burns. *Burns* 1994;20(6):539-41.

41. Stark GB, Kaiser HW, Horch R, Kopp J, Spilker G. Cultured autologous keratinocytes suspended in fibrin glue (KFGS) with allogenic overgraft for definitive burn wound coverage. *Eur J Plast Surg* 1995;18(6):267-71.

42. Paddle-Ledinek JE, Cruickshank DG, Masterton JP. Skin replacement by cultured keratinocyte grafts: An Australian experience. *Burns* 1997;23(3):204-11.

43. Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y. Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. *Transplantation* 2000;70(11):1588-98.

44. Cirodde A, Leclerc T, Jault P, Duhamel P, Lataillade JJ, Bargues L. Cultured epithelial autografts in massive burns: A single-center retrospective study with 63 patients. *Burns* 2011;37(6):964-72.

45. Yim H, Yang HT, Cho YS, Seo CH, Lee BC, Ko JH, et al. Clinical study of cultured epithelial autografts in liquid suspension in severe burn patients. *Burns* 2011;37(6):1067-71.

46. Kym D, Yim H, Yoon J, Yang HT, Cho YS, Hur J, et al. The application of cultured epithelial autografts improves survival in burns. *Wound Repair Regen* 2015;23(3):340-4.

47. Auxenfans C, Menet V, Catherine Z, Shipkov H, Lacroix P, Bertin-Maghit M, et al. Cultured autologous keratinocytes in the treatment of large and deep burns: a retrospective study over 15 years. *Burns* 2015;41(1):71-9.

48. Pittelkow MR, Scott RE. New techniques for the in vitro culture of human skin keratinocytes and perspectives on their use for grafting of patients with extensive burns. *Mayo Clin Proc* 1986;61(10):771-7.

49. Hartmann B, Ekkernkamp A, Johnen C, Gerlach JC, Belfekroun C, Kuntscher MV. Sprayed cultured epithelial autografts for deep dermal burns of the face and neck. *Ann Plast Surg* 2007;58(1):70-3.

50. James SE, Booth S, Dheansa B, Mann DJ, Reid MJ, Shevchenko RV, et al. Sprayed cultured autologous keratinocytes used alone or in combination with meshed autografts to accelerate wound closure in difficult-to-heal burns patients. *Burns* 2010;36(3):e10-20.

51. Lee H. Outcomes of sprayed cultured epithelial autografts for full-thickness wounds: A single-centre experience. *Burns* 2012;38(6):931-6.

52. Frew Q, Philp B, Shelley O, Myers S, Navsaria H, Dziewulski P. The use of Biobrane as a delivery method for cultured epithelial autograft in burn patients. *Burns* 2013;39(5):876-80.

53. Lu G, Cai L, Zhao P, Gu Z, Zhu Y. Mixed Suspension of Cultured Autologous and Allogenic Keratinocytes in Fibrin Glue for the Treatment of Full-thickness Burns. *Wounds* 2011;23(2):32-7.

54. Pellegrini G, Ranno R, Stracuzzi G, Bondanza S, Guerra L, Zambruno G, et al. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. *Transplantation* 1999;68(6):868-79.

55. Hernon CA, Dawson RA, Freedlander E, Short R, Haddow DB, Brotherston M, et al. Clinical experience using cultured epithelial autografts leads to an alternative methodology for transferring skin cells from the laboratory to the patient. *Regen Med* 2006;1(6):809-21.

56. De Luca M, Albanese E, Bondanza S, Megna M, Ugozzoli L, Molina F, et al. Multicentre experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. *Burns* 1989;15(5):303-9.

57. Cuono C, Langdon R, McGuire J. Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. *Lancet* 1986;1(8490):1123-4.

58. Barillo DJ, Nangle ME, Farrell K. Preliminary experience with cultured epidermal autograft in a community hospital burn unit. *J Burn Care Rehabil* 1992;13(1):158-65.

59. Williamson JS, Snelling CF, Clugston P, Macdonald IB, Germann E. Cultured epithelial autograft: five years of clinical experience with twenty-eight patients. *Journal of Trauma-Injury Infection & Critical Care* 1995;39(2):309-19.

60. Blight A, Mountford EM, Cheshire IM, Clancy JM, Levick PL. Treatment of full skin thickness burn injury using cultured epithelial grafts. *Burns* 1991;17(6):495-8.

61. Herzog SR, Meyer A, Woodley D, Peterson HD. Wound coverage with cultured autologous keratinocytes: use after burn wound excision, including biopsy followup. *Journal of Trauma-Injury Infection & Critical Care* 1988;28(2):195-8.

62. Elliott M, Vandervord J. Initial experience with cultured epithelial autografts in massively burnt patients. *ANZ J Surg* 2002;72(12):893-5.

63. Dorai AA, Lim CK, Fareha AC, Halim AS. Cultured epidermal autografts in combination with MEEK Micrografting technique in the treatment of major burn injuries. *The Medical journal of Malaysia* 2008;63 Suppl A:44.

64. Hayashi M, Muramatsu H, Nakano M, Ito H, Inoie M, Tomizuka Y, et al. Experience of using cultured epithelial autografts for the extensive burn wounds in eight patients. *Ann Plast Surg* 2014;73(1):25-9.

65. Matsumura H, Harunari N, Ikeda H. First experience using cultured epidermal autografts in Taiwan for burn victims of the Formosa Fun Coast Water Park explosion, as part of Japanese medical assistance. *Burns* 2016;42(3):697-703.

66. Sood R, Roggy D, Zieger M, Balledux J, Chaudhari S, Koumanis DJ, et al. Cultured epithelial autografts for coverage of large burn wounds in eighty-eight patients: The Indiana university experience. *Journal of Burn Care and Research* 2010;31(4):559-68.

67. Raff T, Hartmann B, Wagner H, Germann G. Experience with the modified Meek technique. *Acta Chir Plast* 1996;38(4):142-6.

68. Haith LR, Jr., Moss BE, Kraut JD, Patton ML, Germain TJ. Trilaminar skin coverage combined with cultured epithelial autografts--a new technique for treating the elderly patient with burn injuries. *J Burn Care Rehabil* 1998;19(1 Pt 1):59-61.

69. Eldad A, Burt A, Clarke JA, Gusterson B. Cultured epithelium as a skin substitute. *Burns Incl Therm Inj* 1987;13(3):173-80.

70. Kaiser HW, Stark GB, Kopp J, Balcerkiewicz A, Spilker G, Kreysel HW. Cultured autologous keratinocytes in fibrin glue suspension, exclusively and combined with STS-allograft (preliminary clinical and histological report of a new technique. *Burns* 1994;20(1):23-9.

71. Stark GB, Kaiser HW. Cologne Burn Centre experience with glycerol-preserved allogeneic skin: Part II: Combination with autologous cultured keratinocytes. *Burns* 1994;20 Suppl 1:S34-8.

72. Haith Jr LR, Patton ML, Goldman WT. Cultured epidermal autograft and the treatment of the massive burn injury. *J Burn Care Rehabil* 1992;13(1):142-6.

73. Carsin H, Ainaud P, Le Bever H, Rives J, Lakhel A, Stephanazzi J, et al. Cultured epithelial autografts in extensive burn coverage of severely traumatized patients: a five year single-center experience with 30 patients. *Burns* 2000;26(4):379-87.

74. Rue LW, 3rd, Cioffi WG, McManus WF, Pruitt BA, Jr. Wound closure and outcome in extensively burned patients treated with cultured autologous keratinocytes. *J Trauma* 1993;34(5):662-7; discussion 7-8.

75. Hickerson WL, Compton C, Fletchall S, Smith LR. Cultured epidermal autografts and allodermis combination for permanent burn wound coverage. *Burns* 1994;20 Suppl 1:S52-5; discussion S5-6.

76. Teepe RG, Kreis RW, Koebrugge EJ, Kempenaar JA, Vloemans AF, Hermans RP, et al. The use of cultured autologous epidermis in the treatment of extensive burn wounds. *Journal of Trauma-Injury Infection & Critical Care* 1990;30(3):269-75.

77. O'Connor NE, Mulliken JB, Banks-Schlegel S, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. *Lancet* 1981;1(8211):75-8.

78. Munster AM. Cultured skin for massive burns. A prospective, controlled trial. *Ann Surg* 1996;224(3):372-5; discussion 5-7.

79. Clugston PA, Snelling CF, Macdonald IB, Maledy HL, Boyle JC, Germann E, et al. Cultured epithelial autografts: three years of clinical experience with eighteen patients. *J Burn Care Rehabil* 1991;12(6):533-9.

80. Boyce ST, Holder IA. Selection of topical antimicrobial agents for cultured skin for burns by combined assessment of cellular cytotoxicity and antimicrobial activity. *Plast Reconstr Surg* 1993;92(3):493-500.

81. Burke JF, Yannas IV, Quinby WC, Jr., Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. *Ann Surg* 1981;194(4):413-28.

82. Theopold C, Hoeller D, Velander P, Demling R, Eriksson E. Graft site malignancy following treatment of full-thickness burn with cultured epidermal autograft. *Plast Reconstr Surg* 2004;114(5):1215-9.

83. Edwards MJ, Hirsch RM, Broadwater JR, Netscher DT, Ames FC. Squamous cell carcinoma arising in previously burned or irradiated skin. *Arch Surg* 1989;124(1):115-7.

84. Hickerson WL, Remmers AE, Recker DP. Twenty-Five Years' Experience and Beyond with Cultured Epidermal Autografts for Coverage of Large Burn Wounds in Adult and Pediatric Patients, 1989-2015. *J Burn Care Res* 2019;40(2):157-65.

85. Green H. The birth of therapy with cultured cells. *Bioessays* 2008;30(9):897-903.

86. Gordillo Altamirano FL, Barr JJ. Phage Therapy in the Postantibiotic Era. *Clin Microbiol Rev* 2019;32(2).

87. Natesan S, Zamora DO, Wrice NL, Baer DG, Christy RJ. Bilayer hydrogel with autologous stem cells derived from debrided human burn skin for improved skin regeneration. *J Burn Care Res* 2013;34(1):18-30.

88. Lo CH, Akbarzadeh S, McLean C, Ives A, Paul E, Brown WA, et al. Wound healing after cultured epithelial autografting in patients with massive burn injury: A cohort study. *J Plast Reconstr Aesthet Surg* 2019;72(3):427-37.

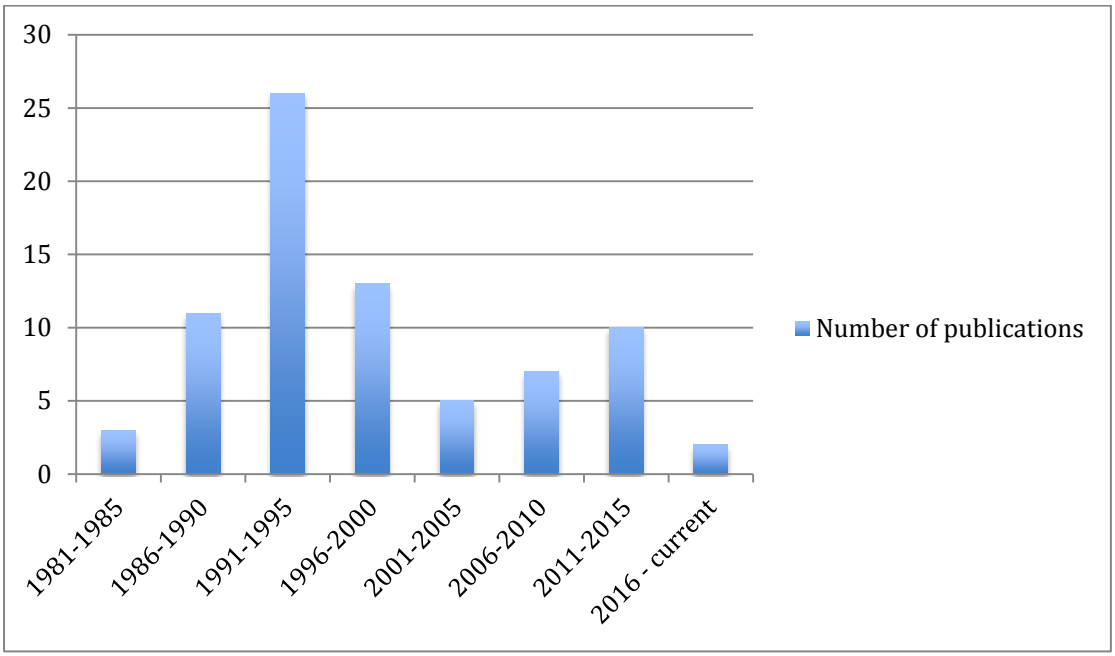
FIGURE DESCRIPTIONS

Figure 1. Summary (five year intervals) of clinical series involving treatment of burn injuries with CEA in the last 35 years.

Table 1. Summary of clinical series included in this review.

CONFLICT OF INTEREST STATEMENT

All authors declare that there are no financial or personal relationships with other people or organizations that could inappropriately influence (bias) our contribution to this manuscript.



Publication	Author	Year	City (Country)	Number of patients treated with CEA	Paediatric patients only	Average (range) % TBSA burn[c]
1	O'Connor	1981	Boston (United States)	2	No	60 (40-80)
2[a]	Gallico	1984	Boston (United States)	2	Yes	
3[a]	Gallico	1985	Boston (United States)	10	Yes	74 (69-79)
4	Pittelkow	1986	Rochester (United States)	1	No	>99
5	Cuono	1986	Connecticut (United States)	1	No	>55
6	Eldad	1987	London (England)	2	No	15 (2-55)
7	Bettex-Galland	1988	Bern (Switzerland)	1	Yes	40
8	Herzog	1988	North Carolina (United States)	8	No	54 (16-88)
9[a]	Kumagai	1988	Kanagawa (Japan)	7[b]	No	50 (5-75)
10	De Luca	1989	Turin (Italy)	40	No	7-95
11[a]	Compton	1989	Boston (United States)	23	Yes	80 (53-98)
12	Khalfan	1989	Bahrain	2	No	23 (10-35)
13	Teepe	1990	Rotterdam (Netherlands)	17	No	56 (31-85)

14 [a]	Munster	1990	Baltimore (United States)	7	No	70
15	Desai	1991	Texas (United States)	1	Yes	98
16	Blight	1991	Birmingham (United Kingdom)	26	No	55
17	Ronfard	1991	Lille (France)	2	No	70 (50-90)
18[a]	Clugston	1991	Vancouver (Canada)	18	No	49 (15-81)
19[a]	Munster	1992	Baltimore (United States)	10	No	72
20[a]	Odessey	1992	Cambridge (United States)	104	No	70 (25-99)
21[a]	Law	1992	Augusta (United States)	5	No	59-70
22	Coleman	1992	Indianapolis (United States)	6[b]	Yes	15-85
23	Herndon	1992	Galveston (United States)	1	Yes	100
24	Haith	1992	Upland (United States)	6	No	70 (44-93)
25	Krupp	1992	Lausanne (Switzerland)	21[b]	No	***not stated***
26	Nave	1992	Kalamazoo (United States)	3	No	80 (70-90)
27[a]	Siwy	1992	Indianapolis (United States)	8[b]	No	45-85
28	Barillo	1992	Allentown (United States)	3	No	59

29[a]	Compton	1992	Boston (United States)	22	Yes	***not stated***
30	Donati	1992	Milan (Italy)	26[b]	No	***not stated***
31	Rue	1993	San Antonio (United States)	16	No	68 (42-85)
32	McAree	1993	Akron (United States)	7	No	66
33[a]	Still	1994	Augusta (United States)	15	No	54-95
34	Hickerson	1994	Memphis (United States)	5	No	60 (48-70)
35[a]	Kaiser	1994	Cologne (Germany)	3	No	46 (40-54)
36[a]	Stark	1994	Cologne (Germany)	3	No	47 (40-54)
37[a]	Stark	1995	Cologne (Germany)	1	No	88
38	Lopez Gutierrez	1995	Madrid (Spain)	4	Yes	75-85
39[a]	Sheridan	1995	Boston (United States)	5	No	94 (90-96)
40[a]	Williamson	1995	Vancouver (Canada)	28	No	52
41	Raff	1996	Ludwigshafen (Germany)	3	No	68
42[a]	Munster	1996	Baltimore (United States)	22	No	72
43	Gobet	1997	Zurich (Switzerland)	12[b]	Yes	57 (18-70)

44	Paddle-Ledinek	1997	Melbourne (Australia)	37	No	77 (55-95)
45	Haith	1998	Upland (United States)	1	No	15
46	Pandya	1998	East Grinstead (England)	1	Yes	60
47[a]	Dvorankova	1998	Prague (Czech Republic)	2	No	***not stated***
48	Pellegrini	1999	Catania (Italy)	7	No	20-75
49	Chalumeau	1999	Clamart Cedex (France)	6	Yes	82 (70-94)
50	Barret	2000	Galveston (United States)	8	Yes	93
51[a]	Braye	2000	Lyon (France)	12	Yes	65 (42-88)
52	Ronfard	2000	Paris (France)	7	No	>50
53[a]	Carsin	2000	Clamart (France)	30	No	78
54[a]	Sheridan	2001	Boston (United States)	7[b]	Yes	76
55[a]	Oshima	2002	Kawasaki (Japan)	430[b]	No	***not stated***
56	Elliott	2002	Sydney (Australia)	2	No	80
57[a]	Dvorankova	2003	Prague (Czech Republic)	17[b]	No	***not stated***
58	Zhu	2005	Sheffield (England)	7[b]	No	34 (28-40)

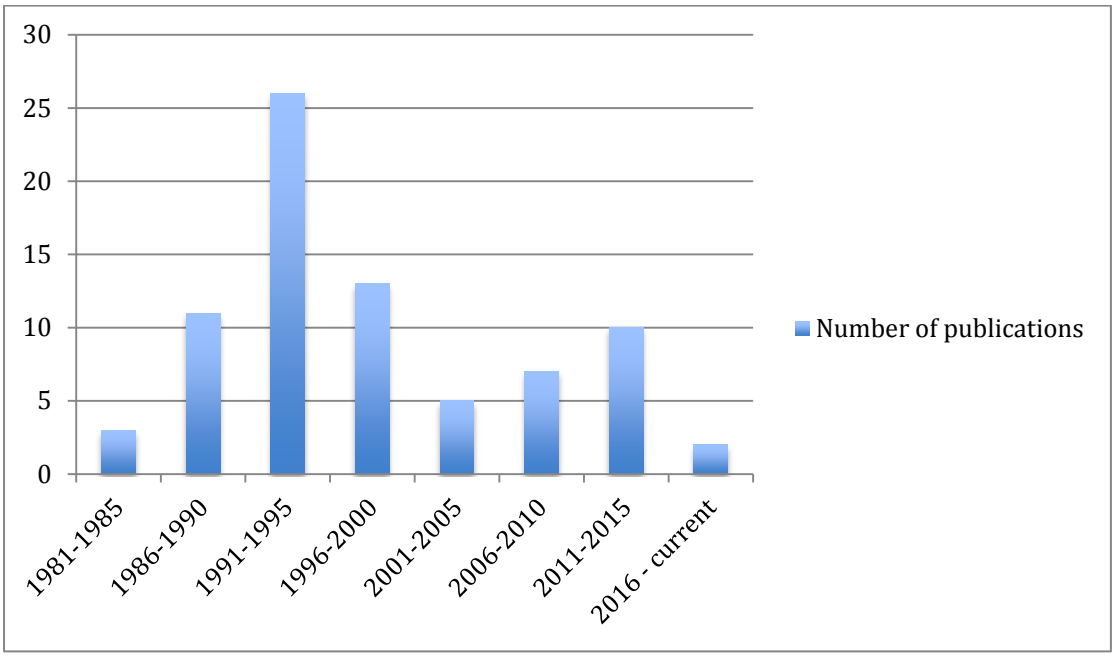
59	Hernon	2006	Sheffield (England)	25	No	***not stated***
60	Wood	2006	Perth (Australia)	62	No	>50
61	Hartmann	2007	Berlin (Germany)	19	No	15 (7-46)
62	Dorai	2008	Kelantan (Malaysia)	9	No	35
63[a]	Sood	2009	Indianapolis (United States)	29[b]	Yes	53
64[a]	Sood	2010	Indianapolis (United States)	88	No	59 (28-98)
65	James	2010	East Grinstead (England)	5	No	32 (8-90)
66[a]	Cirodde	2011	Clamart (France)	68	No	81 (71-91)
67	Lu	2011	Jiangsu (China)	10	No	4
68[a]	Yim	2011	Seoul (South Korea)	29	No	55
69	Lee	2012	Seoul (South Korea)	16	No	51
70	Frew	2013	Chelmsford (England)	3	No	80 (75-85)
71	Menon	2013	Westmead (Australia)	7	Yes	46
72	Chrapusta	2014	Krakow (Poland)	20	Yes	55-65
73	Hayashi	2014	Gunma (Japan)	8	No	52

74[a]	Kym	2015	Seoul (South Korea)	96	No	67
75[a]	Auxenfans	2015	Lyon (France)	63	No	71
76	Matsumura	2016	Tokyo (Japan)	216	No	50
77	Matsumura	2016	Tokyo (Japan)	5	No	81

[a] clinical series appeared to include the same subset of patient data used in multiple publications

[b] clinical series included patients treated for scar revisions, giant naevi, chronic ulcers, hypomelanosis and tattoo removal

[c] in studies where the average % TBSA burn is ***not stated***, the range % TBSA burn is reported wherever possible



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4	Pittelkow	1986	Rochester (United States)	1	No	>99
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7	Bettex-Galland	1988	Bern (Switzerland)	1	Yes	40
8	Herzog	1988	North Carolina (United States)	8	No	54 (16-88)
9[a]	Kumagai	1988	Kanagawa (Japan)	7[b]	No	50 (5-75)
10	De Luca	1989	Turin (Italy)	40	No	7-95
11[a]	Compton	1989	Boston (United States)	23	Yes	80 (53-98)
12	Khalfan	1989	Bahrain	2	No	23 (10-35)
13	Teepe	1990	Rotterdam (Netherlands)	17	No	56 (31-85)
14 [a]	Munster	1990	Baltimore (United States)	7	No	70
15	Desai	1991	Texas (United States)	1	Yes	98
16	Blight	1991	Birmingham (United Kingdom)	26	No	55

17	Ronfard	1991	Lille (France)	2	No	70 (50-90)
18[a]	Clugston	1991	Vancouver (Canada)	18	No	49 (15-81)
19[a]	Munster	1992	Baltimore (United States)	10	No	72
20[a]	Odessey	1992	Cambridge (United States)	104	No	70 (25-99)
21[a]	Law	1992	Augusta (United States)	5	No	59-70
22	Coleman	1992	Indianapolis (United States)	6[b]	Yes	15-85
23	Herndon	1992	Galveston (United States)	1	Yes	100
24	Haith	1992	Upland (United States)	6	No	70 (44-93)
25	Krupp	1992	Lausanne (Switzerland)	21[b]	No	***not stated***
26	Nave	1992	Kalamazoo (United States)	3	No	80 (70-90)
27[a]	Siwy	1992	Indianapolis (United States)	8[b]	No	45-85
28	Barillo	1992	Allentown (United States)	3	No	59
29[a]	Compton	1992	Boston (United States)	22	Yes	***not stated***
30	Donati	1992	Milan (Italy)	26[b]	No	***not stated***
31	Rue	1993	San Antonio (United States)	16	No	68 (42-85)
32	McAree	1993	Akron (United States)	7	No	66
33[a]	Still	1994	Augusta (United States)	15	No	54-95
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43	Gobet	1997	Zurich (Switzerland)	12[b]	Yes	57 (18-70)
44	Paddle-Ledinek	1997	Melbourne (Australia)	37	No	77 (55-95)
45	Haith	1998	Upland (United States)	1	No	15
46	Pandya	1998	East Grinstead (England)	1	Yes	60
47[a]	Dvorankova	1998	Prague (Czech Republic)	2	No	***not stated***
48	Pellegrini	1999	Catania (Italy)	7	No	20-75
49	Chalumeau	1999	Clamart Cedex (France)	6	Yes	82 (70-94)
50	Barret	2000	Galveston (United States)	8	Yes	93
51[a]	Braye	2000	Lyon (France)	12	Yes	65 (42-88)
52	Ronfard	2000	Paris (France)	7	No	>50

53[a]	Carsin	2000	Clamart (France)	30	No	78
54[a]	Sheridan	2001	Boston (United States)	7[b]	Yes	76
55[a]	Oshima	2002	Kawasaki (Japan)	430[b]	No	***not stated***
56	Elliott	2002	Sydney (Australia)	2	No	80
57[a]	Dvorankova	2003	Prague (Czech Republic)	17[b]	No	***not stated***
58	Zhu	2005	Sheffield (England)	7[b]	No	34 (28-40)
59	Hernon	2006	Sheffield (England)	25	No	***not stated***
60	Wood	2006	Perth (Australia)	62	No	>50
61	Hartmann	2007	Berlin (Germany)	19	No	15 (7-46)
62	Dorai	2008	Kelantan (Malaysia)	9	No	35
63[a]	Sood	2009	Indianapolis (United States)	29[b]	Yes	53
64[a]	Sood	2010	Indianapolis (United States)	88	No	59 (28-98)
65	James	2010	East Grinstead (England)	5	No	32 (8-90)
66[a]	Cirodde	2011	Clamart (France)	68	No	81 (71-91)
67	Lu	2011	Jiangsu (China)	10	No	4
68[a]	Yim	2011	Seoul (South Korea)	29	No	55
69	Lee	2012	Seoul (South Korea)	16	No	51
70	Frew	2013	Chelmsford (England)	3	No	80 (75-85)

71	Menon	2013	Westmead (Australia)	7	Yes	46
72	Chrapusta	2014	Krakow (Poland)	20	Yes	55-65
73	Hayashi	2014	Gunma (Japan)	8	No	52
74[a]	Kym	2015	Seoul (South Korea)	96	No	67
75[a]	Auxenfans	2015	Lyon (France)	63	No	71
76	Matsumura	2016	Tokyo (Japan)	216	No	50
77	Matsumura	2016	Tokyo (Japan)	5	No	81

[a] clinical series appeared to include the same subset of patient data used in multiple publications

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