World Journal of Surgery and Surgical Research

Research Article
Published: 14 Sep, 2024

Structural Characterization and Safety Validation of Cultured Skin Cells Grown on Routinely Used Dressing in Pediatric Incubators: A Preliminary Report

Kleintjes WG[1,2] and Prinsloo TK[3,4]*

[1]Department of Surgical Sciences, Stellenbosch University Medical School, Francie van Zijl Avenue, South Africa

[2]WC Provincial Adult Tertiary Burns Centre, Tygerberg Hospital, South Africa

[3]Department of Biomedical Sciences, Cape Peninsula University of Technology (CPUT), South Africa

[4]Department of Emergency Medical Sciences, Cape Peninsula University of Technology (CPUT), South Africa

Abstract

Cultured Epithelial Autografts (CEA), have improved wound treatment outcomes and combining it with temporary coverage reportedly increases graft take. In this study, a modified composite technique was established that mitigated several drawbacks experienced in resource-limited settings. The aim was to report on the CEA's preliminary histological findings and demonstrate its safety for routine use. Biopsy-retrieved keratinocytes in autologous plasma were seeded onto routinely-used bacteria- and fungi-binding dressing pads. Incubation in pediatric incubators at 37°C then followed and fresh autologous plasma was applied daily while amorphous hydrogel every third or fourth day. Confluence was reached (2 weeks), and culture samples were histologically evaluated using Light microscopy (4X and 10X magnification) with two stains (Hematoxylin and eosin, and Masson's Trichrome) and transmission electron microscopy (800-4000X magnification) with Reynold's lead nitrate stain. Bacteriological safety was externally assessed by analyzing wound swabs (n=10; 2 each =20 samples) of CEA grown in both pediatric and standard culture incubators before transplant. Light microscopy demonstrated densely packed keratinocytes, collagen fibers, and classical epidermal and dermal layer differentiation. Electron microscopy also displayed the differentiated cellular nature including basal epithelial and dermal cells, keratinocytes and melanocytes. No pathogenic organisms were observed in any of the cell culture specimens tested. The differentiated nature of this CEA emulated the epidermis to a large degree which was not only indicative of functional integrity, but also mechanical integrity outcomes of the technique. No differences were observed between the incubators in terms of the pathogenic bacteria grown or cell culture growth.

Keywords: Burns; Cultured epithelial autografts; Light microscopy; Transmission electron microscopy; Biosafety

OPEN ACCESS

***Correspondence:**
Wayne George Kleintjes, Department of Surgical Sciences, Stellenbosch University Medical School, Francie van Zijl Avenue, Parow, 7505, CPT, WC, South Africa, Tel: +27837090876;

Received Date: *23 Aug 2024*
Accepted Date*: 10 Sep 2024*
Published Date*: 14 Sep 2024*

Citation:
Kleintjes WG, Prinsloo TK. Structural Characterization and Safety Validation of Cultured Skin Cells Grown on Routinely Used Dressing in Pediatric Incubators: A Preliminary Report. World J Surg Surgical Res. 2024; 7: 1569.

***Copyright** © 2024 Kleintjes WG. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Introduction

Early excision of burns followed by wound coverage is essential for improving the survival of burn patients [1]. Wound coverage can be achieved through skin substitutes ranging from biological (*e.g.* allografts and xenografts), to synthetic (*e.g.* polyurethane films) and biosynthetic (combination of the latter) [2,3]. Traditional autografts (skin grafted from the patient) are preferred [4], but coverage is dependent on the burn surface area that requires the graft and the availability of donor sites for harvesting [2]. When donor sites are exhausted from repeated harvesting, allografts and xenografts are temporarily effective in the early wound phase after severe thermal injuries until adequate donor sites are available [5-7]. However, allografts and xenografts provide only a temporary solution and would eventually be rejected by the host's immune system. Permanent wound closure of large burns has been achieved by incorporating Cultured Epithelial Autografts (CEAs) grown *in vitro* using the patient's own skin cells until it can be transplanted as a skin layer onto the excised and prepared burn wound. The best graft take outcome has been observed using a composite technique that incorporates xenografts or allografts during the culture period and is removed before the CEA transplant [8].

A modified composite technique was established and used under emergency ethical approval. The technique was used on premise that the patient has a poor prognosis and that donor sites are

unable to meet the wound coverage requirements. The benefit of the technique was that the cultures could be cultivated on routinely-used wound dressing and incubated in pediatric incubators that was in close proximity to the theatre which mitigated time, cost and infrastructure constraints [9]. With the first CEA treated case, the patient survived despite an initially calculated 10% survival rate, several complications and graft rejections prior to treatment. With this success and that of others experienced at the current center, the technique continues to be incorporated, but only in severe cases with dire outcomes. Verifying the presence of typical skin components and its safety for use would be the first important step that would contribute to validating its routine use as a skin aide in wound therapy. This report therefore aimed to illustrate the preliminary histological findings using two contrasting stains on the CEA cultivated in pediatric incubators, and demonstrate its safety compared with standard incubator-grown CEA.

Materials and Methods

Study design

The research was done as a prospective controlled descriptive *in vitro* study. Ethical approval for publication was granted by the respective ethics committee of the faculty and institution.

Skin tissue and incubator preparation

For the purpose of this scope, the retrieval of skin biopsies and tissue preparation was not included, but a detailed protocol can be observed in a previously published article [9]. Epithelial cells, after trypsinization, were transferred with forceps and seeded centrally on 20 cm × 10 cm routinely-used bacteria- and fungi-binding dressing sheets. Concurrently, autogenous Platelet-Rich Plasma (PRP) was prepared by centrifuging 6 ml of the patient's blood and the resultant supernatant plasma used to supplement the seeded cells. The dressing containing the epithelial cells and plasma was then incubated at 37°C in a pediatric incubator.

State/public hospitals are typically devoid of tissue culture laboratories, therefore, a seminar room within the Burn Centre was converted into a laboratory and two pediatric incubators donated from the pediatric ward were installed. The incubators were sterilized inside and out with chlorhexidine and alcohol solution shortly prior to CEA incubation. Sterile conditions were employed at best throughout the culture period (the immediate areas and related surfaces were wiped down with 70% ethanol). The incubator moisture was maintained daily by adding sterile water to the inlet on the outside of the incubator. The seeded cells were supplemented daily with newly drawn and freshly prepared autogenous PRP for nourishment and to prevent "drying out". The PRP was sprayed onto the cells on the pads and on ribbon gauze, which was placed between the pads to form a border for the cell growth and to prevent the cells from growing over the edges. Every third day, sterile modified sodium carboxymethyl cellulose, hydrogel was applied directly onto the incubated cultures as a further precaution against "drying out". The period taken from biopsy retrieval to the time that cultures were confluent and ready for direct transferal to the prepared wound beds was 2 weeks. Initially the seeded and supplemented cells were grey in color. Culture confluence was macroscopically confirmed by the presence of a light brown gelatinous-like monolayer and shrinkage of the dressing which indicated potential keratinocyte and fibroblast activity, respectively.

Histological assessment

For microscopic evaluation the slides were prepared with this confluent monolayer grown on the dressing pads. The culture sample was scraped from the dressing using a sterile scalpel and placed centrally on the histological slide. Cells were fixed with Cytospray (Isopropyl alcohol, acetone, glycerin and water) onto the slides. The two stains used separately to contrast cellular components were the Hematoxylin and Eosin (H&E) and Masson's Trichrome (MT) stain according to routine histological protocol. The former stain demonstrated nucleic (dark purple/blue) and cytoplasmic components (pink). Masson's trichrome stained connective tissues such as collagen fibers (blue), nuclei (dark red/purple) and cytoplasm (red). Light microscopy (XSZ107BN compound binocular microscope, serial number 001448) was used at 4X magnification for optimal visualization of epidermal and dermal patterns, while 10X magnification was more suitable for the presence of skin cells. Additionally, transmission electron microscopy was performed following standard protocol at the National Health Laboratory Services EM laboratory. For tissue preparation, CEA samples (<1 mm) were fixed in 3% phosphate buffered glutaraldehyde overnight. Post fixed in Osmium Tetroxide. After rinsing, the samples were placed in tissue paper pockets, transferred to processing baskets and loaded into the Leica EM tissue processor (Leica Microsystems GmbH, Wetzlar, Germany). Tissues were then immersed in a series of reagents for dehydration (increasing alcohol concentrations ending in 3 Spurr's resin changes). Samples in Spurr's resin were embedded in gelatine capsules and left in incubator at 60°C to 70°C overnight to polymerize. The resin block was sectioned (190 nm) using EM UC7 Microtome (Leica Microsystems GmbH, Wetzlar, Germany) and placed onto 200 mesh copper grids. The grids were then stained with 1% uranyl acetate and/or Reynold's lead citrate. Grids were scoped using a JEOL JEM 10-11 microscope (Japan Electron Optics Laboratory Company-JEOL, Tokyo, Japan) at 80 KV and micrographs were taken at 800X, 1000X and 4000X magnification.

The primary outcome was to histologically confirm the presence of keratinocytes and demonstrate the normal epidermal and dermal layers in the CEA obtained from this composite technique. In addition, the secondary outcome was to demonstrate comparable safety with CEA grown in standard incubators.

Biosafety assessment

The cultures were compared to those cultured in a control environment; the ISO culture incubator (Scientific Engineering (Pty) Ltd; 40 L Economy incubator; ISO 9001: 2015). These incubators were kept in the same laboratory room for the tests to limit environmental variations. Inclusion criteria consisted of ten consecutive patients with major burns (>20% TBSA) and the exclusion criteria included patients with minor burns, that had infectious complications or patients who died before completion of cultures on day 14. Prior to culturing, functionality for both incubators were checked which included assessing the following parameters: Temperature, humidity, calibration and disinfection. In addition, both incubators were cleaned and disinfected similarly with a Chlorhexidine and alcohol wash, followed by wiping with 75% Isopropyl alcohol (Sterikleen®). On day 14, when confluence was reached, aerobic wound swabs were obtained by applying sterile techniques to extract central gauze portions (1×1 cm) as well as peripheral samples of the CEA cultures from both incubators (n=10 × 2 per incubator) on day 14. The respective specimens were then labelled and an external blinded private laboratory used for pathogenic microscopy and sensitivity culture assessment.

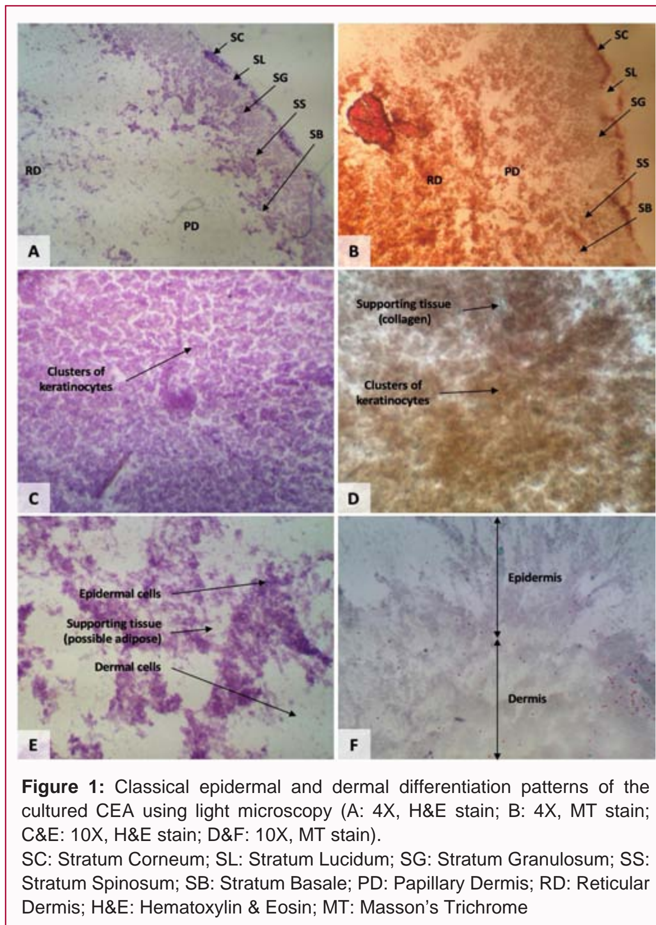

Figure 1: Classical epidermal and dermal differentiation patterns of the cultured CEA using light microscopy (A: 4X, H&E stain; B: 4X, MT stain; C&E: 10X, H&E stain; D&F: 10X, MT stain).
SC: Stratum Corneum; SL: Stratum Lucidum; SG: Stratum Granulosum; SS: Stratum Spinosum; SB: Stratum Basale; PD: Papillary Dermis; RD: Reticular Dermis; H&E: Hematoxylin & Eosin; MT: Masson's Trichrome

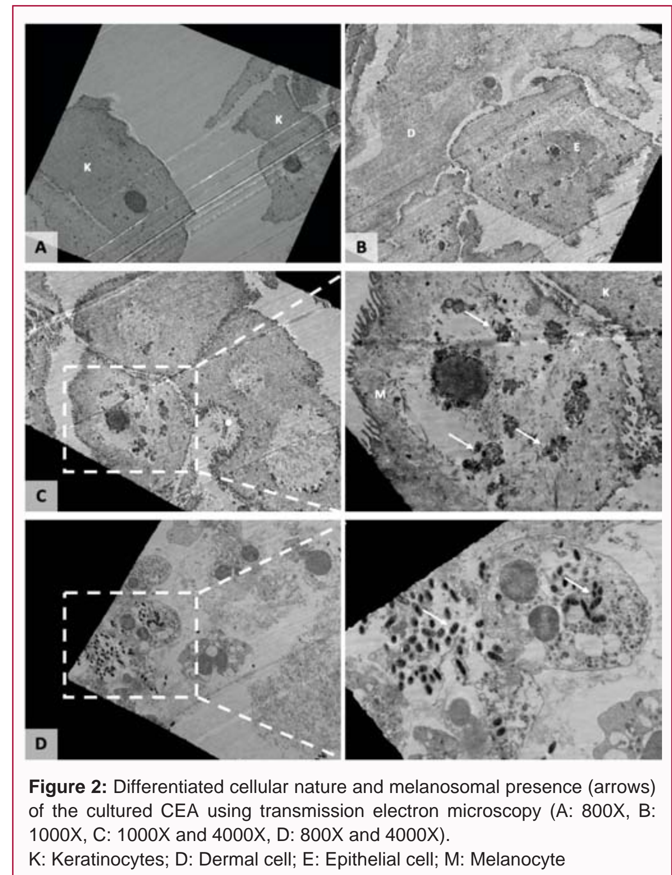

Figure 2: Differentiated cellular nature and melanosomal presence (arrows) of the cultured CEA using transmission electron microscopy (A: 800X, B: 1000X, C: 1000X and 4000X, D: 800X and 4000X).
K: Keratinocytes; D: Dermal cell; E: Epithelial cell; M: Melanocyte

Results

Classical epidermal and dermal layers were visible and mirrored using both stains. The darker outer region to the right denoted the epidermis, while the light inner region to the left denoted the dermal areas (Figure 1A, 1B). The sublayers within the epidermis could also be observed with the outermost and darkest layer forming a border representing the stratum corneum, which was followed by an inferior translucent layer (Stratum Lucidum: SL) and the irregular eosinophilic deeper layer, Stratum Basale (SB). Between the SL and SB layers was the more densely packed Stratum Granulosum (SG) and Spinosum (SS). The green hue in the SG and SS potentially indicates the presence of fibroblast formation and dermal differentiation. Additionally, the papillary and reticular dermis could also be seen deeper to the epidermis (Figure 1A, 1B). The higher magnification (Figure 1C-1F) showed the presence of the eosinophilic cytoplasm of keratinocytes arranged in tightly stacked colonies throughout multiple areas of the CEA. The MT stain also illustrated the presence of collagen as indicated by light blue shaded areas in between the keratinocyte colonies. Interestingly, we could not rule out the possibility that the light "pockets" or "chambers" observed were not fat cell deposits. TEM evaluation of the CEA tissue demonstrated the presence of keratinocytes (Figure 2A), basal epithelial and dermal cells (Figure 2B), melanocytes (Figure 2C) and the presence of monosomes as clusters and rod-like singular spheres (arrows). Moreover, potential endocytosis activity (white dote) of keratinocytes with numerous spinous process (Figure 2C) and monosomal transfer activity (Figure 2D) was also observed. For bacteriological safety, no pathogenic organisms were reported from all the specimens cultivated in both incubators (Table 1). Four out of the 20 specimens had non-pathogenic environmental contaminants (*Bacillus* spp.) from both incubators.

Discussion

In our clinical setting, the cost of commercially available CEAs greatly exceeds the funds available for treatment. In an attempt to overcome these constraints, a more cost effective and convenient method was developed which made use of the patient's own skin cells cultured on a routinely-used local dressing and in pediatric incubators. The culturing ability has previously been demonstrated [9], however, *in vitro* evidence was needed in order to establish a scientific basis that would prove the cultivated CEA had the necessary structural integrity and was safe for clinical application. The basis of this study relied on two important questions that required answering. Firstly, whether the CEA grown in pediatric incubators comprised the anatomical structures comparable to that of typical skin tissue. Secondly, if it would be bacteriologically safe to use pediatric incubators for cell culturing. From the initial single cell cultivation; the proliferation of a confluent, multilayer epithelium was microscopically observed and demonstrated not only by the abundant keratinocyte colonies, but also by the differentiation of the classical skin layers and sublayers that comprise the backbone of normal skin tissue morphology. EM findings corroborated these observations and also demonstrated the presence of differentiated cells such as keratinocytes, epithelial, dermal and melanocyte cells. The highly supportive collagen fibers, potential adipose deposits and melanosome-related activity observed further perpetuated the CEA's potential in skin cell therapy. The presence of the additional supportive components may be present, however, a deep dive beyond the current scope of the study is needed

Table 1: Demographic information and pathogenic outcomes of CEA grown in pediatric and standard incubators (TBSA: Total Body Surface Area; CEA: Cultured Epithelial Autografts).

	Demographic information				Pathogen outcome		
Patient	Age	Gender	TBSA%	Mechanism	CEA day	Pediatric incubator	ISO-incubator
1	22	Male	55	Flame	15	None	None
2	27	Male	23	Flame	14	None	None
3	25	Male	55	Flame	14	None	None
4	22	Male	55	Flame	14	None	None
5	20	Male	50	Flame	14	None	None
6	56	Female	30	Flame	14	None	None
7	27	Male	25	Flame	14	None	None
8	20	Male	55	Flame	14	None	None
9	15	Male	35	Flame	14	None	None
10	30	Male	45	Flame	14	None	None

for confirmation. The findings suggested that the differentiated behavior of the autologous culture structure imitated those normally observed in skin tissue. The novelty lies in this study being the first to report on tissue similarities between normal skin tissue and cells grown in a non-standard and pathogenic-free culture environment.

The use of pediatric incubators was based on the ideal clinically sterile environment and close proximity for CEA cultivation and transfer. It was therefore important to demonstrate the absence of pathogenic organisms in this CEA and demonstrate the safety comparability with CEA grown in standard tissue culture incubators typically used for food and drug administration-approved cultures. No pathogenic organisms were observed, but the technicians determined that few samples had non-pathogenic environmental species (*Bacillus* spp.). Since the private laboratory was not routinely used for cell culture analysis and the samples not easily identifiable as typical cultures, the samples were mistaken for environmental swabs and were first transported to the environmental laboratories in two other cities. This may have compromised the samples and could explain the environmental organisms found on some specimens. It was also possible that the internal standard of cell or incubator care could have dropped during culturing and led to minor environmental contamination. However, these organisms were non-pathogenic and therefore, do not cause disease [10]. Standard culturing techniques remains highly efficient for research purposes, however, in order to utilize and apply these cultures more efficiently and timeously in an under-resourced clinical setting, making use of equipment that is more readily available, in the clinical setting and close to the patients would be even more beneficial to the clinician. Based on these findings, it is plausible that the cultured skin technique is relatively safe to use provided aseptic and sterile techniques are employed throughout the culture period. Moreover, although the primary focus of this study was demonstrating the differentiated morphology of the pediatric incubator-grown CEA and subsequently its safety for application, the satisfactory mechanical quality afforded by the dressing, as a matrix for both the culturing and the direct grafting of the autologous CEA, could not be ignored. The dressing provided a stable and sufficient anchoring matrix for the adherence of the cultivated and actively proliferating keratinocytes.

Conclusion

The initial goal of the novel CEA technique was to cultivate epithelial cells in large quantities to treat severe burn victims. Therefore, it was important to demonstrate evidence of a normal cellular environment in the CEA and that it was clinically safe to transplant. Our findings illustrated the presence of the five epidermal layers, the dermis, along with collagen and potentially adipose cells. The histological evaluation also demonstrated the differentiated cellular nature of skin tissue using both LM and EM. Moreover, no pathogenic organisms were observed in CEAs derived from both standard and non-standard incubator settings. The observed alignment of the CEA's histological characteristics with that of the natural skin barrier and the absence of disease-causing organisms is a step in the right direction for the incorporation of the current CEA technique as a cost-effective and time efficient treatment modality of extensive and deep burns. In-depth research would indubitably validate its routine use in standard clinical practice.

Acknowledgment

The authors would like to thank Tygerberg Hospital management and staff, Prof E. Steyn and the Department of Surgical Services at Stellenbosch University Faculty of Medicine and Health, and Mr. Marcel Golding for support.

References

1. Ong YS, Samuel M, Song C. Meta-analysis of early excision of burns. Burns. 2006; 32(2):145-50.
2. Halim AS, Khoo TL, Mohd Yussof SJ. Biologic and synthetic skin substitutes: An overview. Indian J Plast Surg. 2010; 43(Suppl):S23-8.
3. Oravcová D, Koller J. Currently available skin substitutes. Cas Lek Ces. 2014; 153(1):7-12.
4. Wurzer P, Keil H, Branski LK, Parvizi D, Clayton RP, Finnerty CC, et al. The use of skin substitutes and burn care-a survey. J Surg Res. 2016; 201(2):293-8.
5. Barone AAL, Mastroianni M, Farkash EA, Mallard C, Albritton A, Torabi R, et al. Genetically modified porcine split-thickness skin grafts as an alternative to allograft for provision of temporary wound coverage: preliminary characterization. Burns. 2015; 41(3):565-74.
6. Schlottmann F, Bucan V, Vogt PM, Krezdorn N. A short history of skin grafting in burns: From the gold standard of autologous skin grafting to the possibilities of allogeneic skin grafting with immunomodulatory approaches. Medicina. 2021; 57(3):225.
7. Frame JD, Sanders R, Goodacre TEE, Morgan BDG. The fate of meshed allograft skin in burned patients using cyclosporin immunosuppression. Br J Plast Surg. 1989; 42(1):27-34.

8. 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. J Burn Care Res. 2010; 31(4):559-68.

9. Kleintjes W, Thomas G, Thaele BSS, Stevenson N, Warren B. A novel technique for composite cultured epithelial autograft in a patient with extensive burn wounds: A case report. Clin Surg. 2017; 2:1-5.

10. Pigłowski M. Pathogenic and non-pathogenic microorganisms in the rapid alert system for food and feed. Int J Environ Res Public Health. 2019; 16(3):477.