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A Novel Technique for Composite Cultured Epithelial Autograft in a Patient with Extensive Burn Wounds: A Case Report

Wayne Kleintjes, Grant Thomas, Brogan Salence Stephan Thaele, Nico Stevenson and Brian Warren*

Department of Surgery, Western Cape Provincial Tertiary Adult Burn Unit, Tygerberg Hospital and Stellenbosch University, South Africa

Abstract

Objective: Our goal was to develop a simple, safe and affordable technique for cultured epithelial autografts (CEA) without delay to save our patients life. The patient was 16 years old and suffered 45% burns, and his condition was steadily deteriorating for three months in the intensive care unit. As a last resort we decided culture his skin with minimal resources.

Method: A skin biopsy of 7x3 cm was taken from the patient's hip area. The next day the patient's wounds were covered with a xenograft, EZ Derm®. The cells cultured were nourished on a daily basis with fresh autogenous platelet-rich plasma (PRP). The cells were grown on Cutimed Sorbact dressing pads® in a paediatric incubator. Some keratinocytes grown in sterile specimen bottles and PRP were explanted on day 7 onto new Cutimed Sorbact Dressing Pads® in a second incubator. At two weeks, EZ Derm® superficial layer was removed and the CEA transplanted with the Sorbact pads directly onto the prepared wound beds. Some cells were squirted onto the wounds and dressed with Sorbact Dressing Pads®.

Results: Within two weeks the whole Cutimed Sorbact pad (green) was almost overgrown with epithelial cells. An initial overall successful graft take was estimated at 75%. The patient's final graft take at three weeks was 78%. He was discharged from the ICU two weeks after CEA application and took 2 years to build up strength to walk alone again.

Conclusion: The technique has proven to be effective for CEA, biologically safe, completely autogenous, and it was life-saving for our patient. The overall graft take rate of 78% is better than the largest series in the literature (72, 7%) and may indicate an improvement in CEA technique. The biggest advantage is the cost-effectiveness, which implicates the possibility for saving serious burn victims on a very large scale.

Keywords: Burn wounds; Epithelial autograft; Cutimed Sorbact

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Correspondence:
Wayne G. Kleintjes, Department of Plastic and Reconstructive Surgery, Tygerberg Hospital, Suite 10, 1st Floor, Bellville Melomed Hospital, c/o Voortrekker & AJ, West Street, Bellville, 7530, South Africa,
E-mail: waynekleintjes@yahoo.com

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Background

A 16 year old male patient was admitted to Tygerberg Hospital on 19 July 2014, via a peripheral hospital, with 46% total body surface area (TBSA) deep partial and full thickness burns to his head, neck, chest, back, right arm and right hand, left hand and left thigh, sustained in a domestic fire. Ten% was added and included for an inhalation burn. He was received intubated and ventilated from the referring facility. After initial dressing and fluid resuscitation, he underwent procedures on days 3, 12 and 25 post-admission, culminating in partial thickness skin grafting of his burn wounds. Amputation of all fingers of his right hand was required at the level of the proximal inter-phalangeal joints due to necrosis of the distal portions of these digits. Although he could initially be extubated on day 7 post-admission, multiple nosocomial infections ensued and his nutritional status, despite optimal support, deteriorated to the extent that his skin grafts did not take and donor sites converted to full thickness skin loss, with the result that by day 51 post-admission, he effectively had 30% TBSA full thickness wounds with no prospect of further donor sites. Re-harvesting from previous donor sites was not an option with the patients Albumin levels of 11, and body weight 19 kg. From this time onwards the possibility of importing and funding the cultured epithelial autograft (CEA) Epicel® (Genzyme Inc, Boston, Massachusets) was explored. It transpired, however, that apart from prohibitive cost, there was an import restriction on the product and plans to use this option had

to be abandoned. It was then decided to attempt in-house epidermal culturing with autogenous platelet-rich plasma (PRP). Expedited approval to embark on this unproven therapy as a potentially lifesaving measure was obtained from the Tygerberg Hospital Clinical Ethics Committee (at a later date approval to publish this case report was granted by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University, reference C15/01/001 letter 30 March 2015). Consent to the proposed procedures was also given by the patient's parents.

Methods and Materials

In preparation for skin harvesting, the seminar room (4 meters x 3.5 meters) within the Tygerberg Hospital Burn Unit was converted into a laboratory and two borrowed paediatric incubators were installed. These were wiped inside and out with chlorhexidine and alcohol solution on sterile gauze. Temperature therein was set to 37°C. Two specimen bottles were sterilised with boiling water and partially filled with a teaspoon of trypsin powder (extracted from beef pancreas and sourced from the Division of Anatomy, Stellenbosch University Faculty of Medicine and Health Sciences) mixed in 20 milliliters (ml) of sterile water. The small sieve from anold Recell® kit (Avita Medical, Perth, Western Australia) was also sterilised with boiling water.

On day 94 post-admission, the patient was taken to theatre and a 7 cm x 3 cm full thickness skin biopsy harvested from the left inguinal area, the defect being closed in two layers with polyglactin 4/0 subcuticular sutures. During the same procedure, a partial thickness skin graft from the right lower leg to the right forearm and anterior neck was performed. The remaining 26% TBSA full thickness wounds were covered in the intensive care unit under sedation the next day with (porcine) xenograft EZDerm® (Molnlycke Health Care, UC LLC, Norcross, Ga). Immediately after the procedure of day 94, the harvested skin was transferred to the "laboratory" in a sterile container and processed on sterile surface (operating theatre instrument pack). With the aid of optical loupes (3.5 x magnification, 45 cm focus) epidermis was separated from dermis using a scalpel with a 15/0 blade and the epithelial fragments were cut into smaller segments. These were then immersed in the trypsin specimen bottles to further separate dermal elements. The trypsin was rinsed off using the Recell sieve and remaining minute specs of epithelial cells were transferred with forceps either to the centre of six 20 cm x 10 cm hydrophobic dressing pads (Cutimed Sorbact®, BSN, Pinetown, and RSA) or returned to the specimen bottles. Meanwhile six acid-citrate-dextrose (ACD) tubes, each containing 6 ml of the patient's blood were centrifuged at 3500 revolutions per minute for 8 min and supernatant plasma withdrawn into a 20 ml syringe via an 18 gauge needle, taking care not to include any red blood cell elements. The withdrawn plasma was sprayed onto the Cutimed Sorbact pads and into the specimen bottles. Sorbact® ribbon gauze (BSN, Pinetown, and RSA) was laid down between the pads in the incubators to form a border for cell growth. Pads, as well as specimen bottles containing epithelial cells in plasma, were thereafter incubated at 37°C. Cells were nourished daily with newly drawn and centrifuged PRP and the incubators were humidified by regular addition of sterile water to the incubators' water inlet. Since the patient was in the ICU, and had a central venous line during the treatment period it was easy to get blood. As a further precaution against cultured cells drying out, modified sodium carboxymethyl cellulose, Intrasite Gel® (Smith & Nephew Pharmaceuticals, Pinetown, KZN) was applied with a sterile glove onto the incubated cultures every third day. Rapid growth of cells in the specimen bottles after 7 days allowed transplantation of the cells in suspension to more specimen jars.

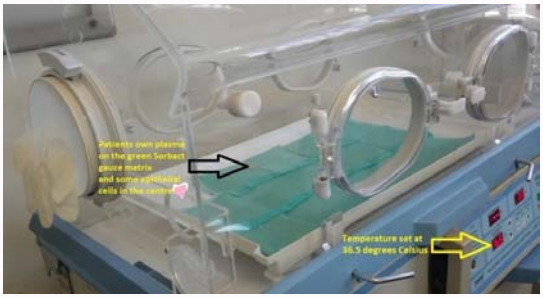

Figure 1: Initiation of the cell culture technique in a paediatric incubator on day 1. A marker points at central bleb of autogenous plasma on the matrix Cutimed Sorbact. The temperature of the incubator is set at 36.5°C, the normal temperature of the body.

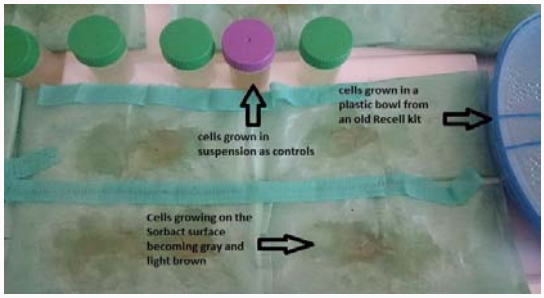

Figure 2: The cells on day 10 covers a larger area of the Sorbact matrix (green), while the control cells in the sterile specimen container and old Recell plastic bowl also grow.

Figure 3: Above can be seen that the cells have grown to the edges (indicated by an arrow) of the Sorbact matrix gauze on day 14 and are ready for transplantation. Compare this image of the cell growth with the previous images to see the progress of the cell growth on the Sorbact.

By day 104 post-admission, after 14 days of culture, sufficient epithelial cell growth had occurred and the patient was returned to theatre. The incubator with cells was also taken to theatre and the cells kept at 37 degrees Celsius. After anaesthesia commencement, 36 ml of blood for PRP was taken from the arterial line. PRP was prepared as usual. The patient was washed with Chlorhexidine soap and draped with sterile clothes. Wound debridement was done by gentle scraping of raw areas with a metal ruler's blunt side/tip. This was done over previously grafted xenograft areas with as well as a donor site on the left medial lower leg. Haemostasis was obtained with swabs soaked in an adrenalin solution (Ringer's lactate 1 litre and 1 ampule of adrenalin and 800 mg Lignocaine). Cutimed Sorbact pads with CEA's were then transplanted directly onto the wounds of the back, left arm, left thigh and knee with the simultaneous application of PRP. For the

patient's right arm, thigh and leg, newly-opened Cutimed Sorbact pads with squirted CEA's (from the cells grown in suspension in the specimen jars) and fresh PRP were applied. A sacral bedsore was also treated with squirted CEA's on Cutimed Sorbact. The back dressings were fixed with iodine containing film dressing, Ioban® (Smith & Nephew Pharmaceuticals, Pinetown, KZN). The rest of the dressings were bandaged with crepe.

Results

The progression of cell growth is shown in Figure 1, 2 and 3 with histological confirmation of cells by simple light microscopy in Figure 4.

The dressings were opened on day 6 post-grafting, when the patient complained of itchiness of the wounds for the first time, and excellent results were observed where the Sorbact Pads with the pre-grown epithelial sheets had been used. The areas where fresh Sorbact Pads squirted with cells and plasma did not show visible confluent epithelial layers at that time. One area on the left thigh had become yellow and sloughy. An initial overall successful graft take was estimated at 75%. It was possible to mobilize him out of his ICU bed, for the first time since admission, one week after CEA. Two weeks after CEA he was transferred to the ward. His final graft take at three weeks post-operatively was 78%. The CEA graft areas are shown in Figure 5.

The major problem after the patient's wounds settled was that of mobility and contractures of virtually all joints, but the CEA remained stable after healing. Three months after the CEA he weighed 34 kg and his Albumin level was 32 g/L (compared to lowest levels prior to embarking on treatment of 19 kg and 11 g/L respectively). He was then also mobilising better. The appearance of CEA donor site area grafted is shown in Figure 6 and other CEA take areas in Figure 7.

The patient at discharge from hospital was in a reasonable state of mental and physical rehabilitation. Total costs directly related to the production of the composite CEA to 40 % of the patient's TBSA were calculated to be 75.26 USD (US dollars) (Table 1). Note the cost of Sorbact Pads in the Table 1was 5.67 USD (US dollars) at that time in 2014, but later in the text 5.97 USD for 2015. The patient only managed to walk alone 2 years after discharge.

Discussion

Composite technique

Use of a composite CEA technique in this case was based on the success of the largest series in the literature by the Indiana burns unit [1]. Different techniques for CEA have been described with variable success since the first description by Rheinwald and Green in 1975 [2]. Different substrates for growing cells have been described in the past. Some of them are extracellular matrix from human umbilical vein endothelial cells on gelatin coated plates, Biobrane® (Smith & Nephew, UDL laboratories, Rockford, IL), which can be used as a skin substitute and polyester filter substrate [3-5]. We were hopeful that the clinical evidence that the Sorbact® dressing was effective in stimulating wound healing via increased fibroblast activity *in vitro* would support the hypothesis that it could be used as a growth scaffold for epithelial cells with the added benefit of the dressing having antimicrobial properties [6-8]. The transfer of cells from the laboratory to the patient also seemed an area of progress in that if the cells grew on the Cutimed Sorbact® it would be able to act as a transfer dressing directly onto the patient.

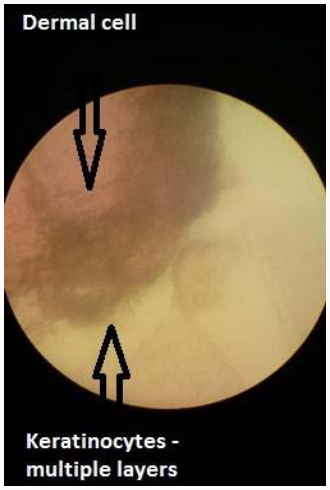

Figure 4: Histological confirmation of cell growth was done by light microscopy and Eosin staining illustrating epidermal and dermal cell differentiation.

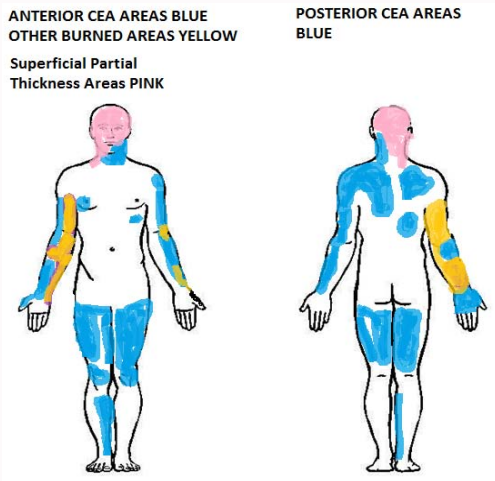

Figure 5: The CEA areas are in blue, while healed superficial partial thickness areas are in pink, and old skin grafts are in yellow.

Figure 6: Upon removal of the Sorbact one week after CEA grafting a completely epithelialized wound is shown with minor punctuate bleeding points where the dressing was stuck to some small epithelial areas.

It has been reported that the composite technique for CEA yields better graft take and a more stable result compared to CEA alone [9]. Either allograft or xenograft can be used as temporary cover over deep burn wounds. At the time of skin biopsy for CEA, the allograft or xenograft can be applied. Two weeks later, during the application of the CEA, the areas of allograft or xenograft can superficially be removed to allow a raw wound bed with some dermal elements. This is then an ideal recipient site for CEA. In the patient's case we used

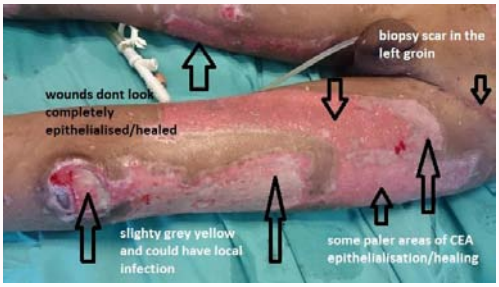

Figure 7: The left thigh initial graft take is shown with some areas that are healing well and others that still need more epithelialisation. The original skin biopsy site for starting the CEA process is visible in the left groin/inguinal area. Possible early signs of wound infection are visible around the knee area and inferior lateral thigh.

a xenograft, EZ Derm®, which was generously donated to us free of charge by the manufacturer.

We recently completed two studies evaluating Cutimed Sorbact in burn wound care [10,11] and the hypothesis was that hydrophobic dressings like Cutimed Sorbact could act as a growth template for epithelial cells in a temperature controlled, relatively sterile environment of an incubator.

The ideal dressing for cell grafting is one that is capable of reducing the effects of shear stress, metabolic stress and tissue infection [12]. Cutimed Sorbact has a hydrophobic coating made from dialkylcarbamoylchloride (DACC). The DACC attracts hydrophobic bacteria and renders them inert [6,10]. It was postulated that the hydrophobic action of Sorbact would allow that PRP could be applied and the cells could grow within the PRP droplets [13].

Application technique

Two grafting techniques were used. The first and easiest was the direct application of the Cutimed Sorbact pads with the pre-grown CEA. The second and more demanding technique was the application of the cells that were grown in the specimen jars by a squirting technique onto fresh Cutimed Sorbact pads. The former technique produced superior early results in this patient and would appear to be the method of choice.

Biological safety

Animal components should as far as possible be removed from cell culturing [14,15]. In this case, mediums were used that came from the patient himself. An animal feeder layer to culture the cells, as is the case with Epicel® was avoided. Trypsin unfortunately is bovine in origin and could not be avoided in the initial phase of epithelial cell separation and preparation. There remains a possibility that the cells may be infected in the culture period. Strict application of the principles of surgical sterility to the work environment in the laboratory assists to limit unwanted contamination of the cell cultures. The mechanism of action of the Cutimed Sorbact® matrix also assists to limit bacterial colonization by irreversibly binding and deactivating bacteria.

Cost-effectiveness

Munster found that the length of stay in the hospital was longer for patients who had CEA and therefore the cost was greater [16]. However in the group of patients that received CEA, there was a better quality of the burn scars and a reduction in mortality compared with the control, from 48% to 14%. The approximate cost of a 7 cm x 10 cm piece of the CEA product Epicel® was approximately 5 292.49 USD in 2014. It was estimated that approximately 136092.60 USD for Epicel® would have been required to complete the grafting in this patient. A piece of Cutimed Sorbact® dressing pad 10 cm x 20 cm would cost 6 USD. This is also much cheaper than Biobrane used by Frew et al. in 2013 [17]. Biobrane in South Africa would cost approximately 105.85 USD for a 13 cm x 13 cm size. Thus the CEA technique used in this patient was cost effective and biologically safer, with an above average graft take of 75% at one week and 78% at three weeks.

Table 1: The estimated cost breakdown for our CEA technique. This is an estimate created using storeroom prices obtained from the stores at Tygerberg hospital. Many of the items used where donated for the purpose of the case study.

Start up			ZAR single unit	US Dollar Total
	Face masks	1	2.64	0.2
	sterile theater pack	1	0	0
	sterile glove	2	3.15	0.24
	20ml syringe	1	0.61	0.05
	18g jelco	1	0	0
	16g jelco	1	0	0
	ribbon gauze	1	60	4.54
	cutimedsorbact pads	8	75	45.36
	slides	20	0.82	1.24
	blade 15/0	1	0.95	0,07
	Specimen bottles	6	5.8	2.36
	Sterile water 10ml	5	2.5	0.95
Daily	20ml Syringe	14 x 1	0.61	0.64
	Green 18g needle	14 x 1	0.1	0.07
	ACD tubes	14 x 6	0.95	6.03
	sterile gloves	14 x 2	3.15	6.67
	face masks	14 x 2	2.64	5.59
	Non sterile gloves	14 x 1	0.46	0.97
			Total	**75.26 US dollars**

Histology results

The dermal cells that were seen in Figure 4 could be explained by the fact that there was no cell identification techniques used to separate dermal cells from epidermal cells before the cells were cultured. This was due to the fact that the first author did not have the expertise to do so, and hoped that the cells that would be growing would be the keratinocytes since this was the predominant cell type obtained by chopping the skin up into very tiny pieces with a scalpel. Even scraping epidermis off with a scalpel also scratches off some dermal cells with the epidermal cells. Technically then, one could call the technique cultured skin autograft (CSA), and not cultured epidermal autograft.

Conclusion

Cutimed Sorbact® in combination with autogenous PRP has proven to be effective for CEA.

There are mobile skin culture incubators being developed, as well as a fully equipped CEA treatment pack, to assist with the cost-effective use of this technique. It is planned that by the end of 2017 that these products would be available to assist clinicians. The CEA was life-saving for our patient. Mueli and Raghunath (1997) wrote: 'It may

take one or more decades of concerted research, jointly performed by clinicians and tissue culture technology experts in order to fabricate more skin-like grafts which are robust, reliable, and less expensive. Then, "cultured skin" will conquer the world and benefit countless patients [18]. We believe that the technique described in this report is a step closer to this conquest.

Acknowledgement

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