Cytotherapy 27 (2025) 475–482

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

International Society ISCT Cell & Gene Therapy®

FULL-LENGTH ARTICLE
Stem Cell Therapy

Human keratinocytes grown at a gas-permeable interface *in vitro* stratify correctly to generate engineered human epidermis

Vaughan Feisst[1,*], Lisa Y.Y. Zhou[1], Chun-Jen Jennifer Chen[1], Eloise Williams[1], Elliott Dunn[1], Inken Kelch[1], Sarah Meidinger[1], John M.T. Hunt[1], Alex du Rand[1], Heidi Robinson[1], Saem M. Park[1], Evert J. Loef[1], Julian Lofts[2], Hilary Sheppard[1], Michelle Locke[3,4], P. Rod Dunbar[1,5,*]

[1] *School of Biological Sciences, University of Auckland, Auckland, New Zealand*
[2] *St Marks Road Surgical Centre, Auckland, New Zealand*
[3] *Department of Surgery, Faculty of Medicine and Health Sciences, The University of Auckland, Auckland, New Zealand*
[4] *Counties Manukau District Health Board, Auckland, New Zealand*
[5] *Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand*

A R T I C L E   I N F O

Article History:
Received 3 October 2024
Accepted 5 December 2024

Key words:
air-liquid interface
epidermal stratification
in vitro skin graft
keratinocyte differentiation
skin engineering
wound treatment

A B S T R A C T

Background: One of the key functions of human skin is to provide a barrier, protecting the body from the surrounding environment and maintaining homeostasis of the internal environment. A mature, stratified epidermis is critical to achieve skin barrier function and is particularly important when producing skin grafts *in vitro* for wound treatment. For decades epidermal stratification has been achieved *in vitro* by culturing keratinocytes at an air-liquid interface, triggering proliferating basal keratinocytes to differentiate and form all epidermal layers.
Results: We show here that culturing keratinocytes at a gas-permeable interface can induce epidermal stratification equivalent to an air-liquid interface.
Conclusions: Culturing skin grafts at a gas-permeable interface has a number of advantages over the traditional air-liquid interface method including: less time input from highly skilled personnel, with consequent cost savings; fewer manipulations, with concomitant reduced risk of cell culture contamination; increased scalability of skin graft size; and improved potential for automation. These advantages confer significant benefits to the use of cell culture devices with gas-permeable interfaces to grow human skin for the treatment of major burns and other skin injuries.

Introduction

Providing a barrier from the external environment is one of the key functions of human skin, protecting the body from biological and chemical hazards in the external environment while also preventing water loss and maintaining homeostasis in the internal environment [1]. In order to form a mature epidermis with barrier function keratinocytes must undergo a programmed terminal differentiation process called epidermal stratification [2]. Keratinocytes residing at the basement membrane (Stratum Basale) proliferate and undergo programmed differentiation resulting in the formation of the upper layers of the epidermis – the Stratum Spinosum, Stratum Granulosum, and Stratum Corneum [2]. The thick skin of the palms and soles also contains a Stratum Lucidum beneath the Stratum Corneum [3]. Morphological changes occur to keratinocytes as they undergo this differentiation process: the "spikey" cells of the Stratum Spinosum begin to flatten in the Stratum Granulosum, accumulating granules containing keratins and glycolipids; then in the Stratum Corneum the nuclei flatten out before being lost entirely as keratinocytes undergo apoptosis [2].

Formation of barrier function through epidermal stratification is critical when producing *in vitro* skin grafts for the treatment of full thickness wounds [4–6]. It has been known for decades that exposing basal keratinocytes to an air-liquid interface (ALI) *in vitro* will induce epidermal stratification [7]. This method mimics the natural ALI of human skin, where the upper layers of the epidermis are

Abbreviations: ALI, air-liquid interface; GPI, gas-permeable interface; DED, acellular de-epithelialized dermis

* Correspondence: Vaughan Feisst, PhD, and P. Rod Dunbar, MBChB, PhD, School of Biological Sciences, University of Auckland, Auckland, New Zealand.
E-mail addresses: v.feisst@auckland.ac.nz (V. Feisst), r.dunbar@auckland.ac.nz (P.R. Dunbar).

https://doi.org/10.1016/j.jcyt.2024.12.005
1465-3249/

exposed to air, and is still widely used to induce epidermal stratification *in vitro* for both laboratory and clinical applications [8−11].

Generating an ALI *in vitro* involves seeding basal proliferating keratinocytes onto a substrate, often a material that simulates the dermal skin layer, and raising this substrate on a platform in a cell culture chamber, so that the surface is exposed to air, but the base of the substrate remains in contact with culture medium (Figure 1). This technique enables nutrient and waste exchange to be maintained but prevents cells from dehydrating and dying. However, keratinocyte cell culture at an ALI suffers from several obvious technical challenges. The first is maintaining a tightly controlled level of cell culture medium in the culture vessels in the face of medium evaporation in 37°C humidified incubators. Clearly if the medium level drops below the level of the substrate, cell viability may be catastrophically reduced. To avoid this situation liquid levels must be closely monitored and intensively maintained—a significant workload for highly-skilled cell culture staff over the two-to-four-week period required to achieve complete epidermal stratification. This intensive manipulation also incurs risk of errors that can result in loss of viability or sterility, for example through microbial contamination. Such costs and risks are particularly important when considering the production of *in vitro* skin for therapeutic purposes. Therefore, a method for growing stratified epidermis that does not involve an ALI could have significant cost and sterility advantages in the clinical context.

Cell culture devices with a gas permeable interface at the bottom of the device have increased gas exchange at the base of the device which has enabled the culture of a number of different cell types at higher densities than possible with standard cell culture plates [12−14]. We therefore sought to determine whether it was feasible to grow stratified human epidermis in cell culture devices that have a gas-permeable interface (GPI) at their base (Figure 1). We hypothesized that the steep gas gradient across the GPI might be sufficient to induce complete epidermal stratification, with keratinocytes differentiating as they grow downwards from their substrate towards the gas supply, rather than upwards towards an ALI. We postulated that major advantages of such a culture system might be that the level of cell culture medium would no longer need to be precisely controlled because the cells were at the base of the device; and that the need for manipulation would be further reduced because the device could be filled with high volumes of culture medium. We show that such GPI-based culture of stratified epidermis is indeed feasible with significant advantages over the ALI method for clinical use.

Methods

Cell isolation

Whole-skin digest single-cell suspensions were isolated from skin tissue as previously described [15]. Briefly, all skin tissue samples were trimmed to remove all adipose tissue and hypodermis before being incubated with 0.5 mg/ml collagenase (Gibco) and 0.1% trypsin/EDTA (Gibco) in DMEM (Gibco) supplemented with 100 U/mL Penicillin/Streptomycin (Gibco) overnight at 37°C with 5% CO_2. Digested tissue was mechanically disrupted with a scalpel and then passed through a 100 μm cell strainer (BD) to produce a single-cell suspension containing a mixture of cells derived from the dermis and epidermis.

Keratinocyte culture

Whole-skin digest single-cell suspensions were cultured in Kelch's medium (DMEM:F12 3:1, 10% FBS (Moregate), 20 ng/mL KGF (Peprotech), 0.625 mg/mL Amphotericin B, 100 U/mL Penicillin/Streptomycin (Gibco), 400 nmol/L SB772077B (Tocris) [15]. Keratinocyte cultures were passaged when they reached 70−90% confluence. To detach keratinocytes the culture medium was removed, and the culture washed once with phosphate-buffered saline (PBS) (Gibco) before incubation with TrypLE (Gibco) for 5−10 minutes at 37°C with 5% CO_2. After the majority of cells had detached, TrypLE was neutralized using Kelch's medium and the cells counted.

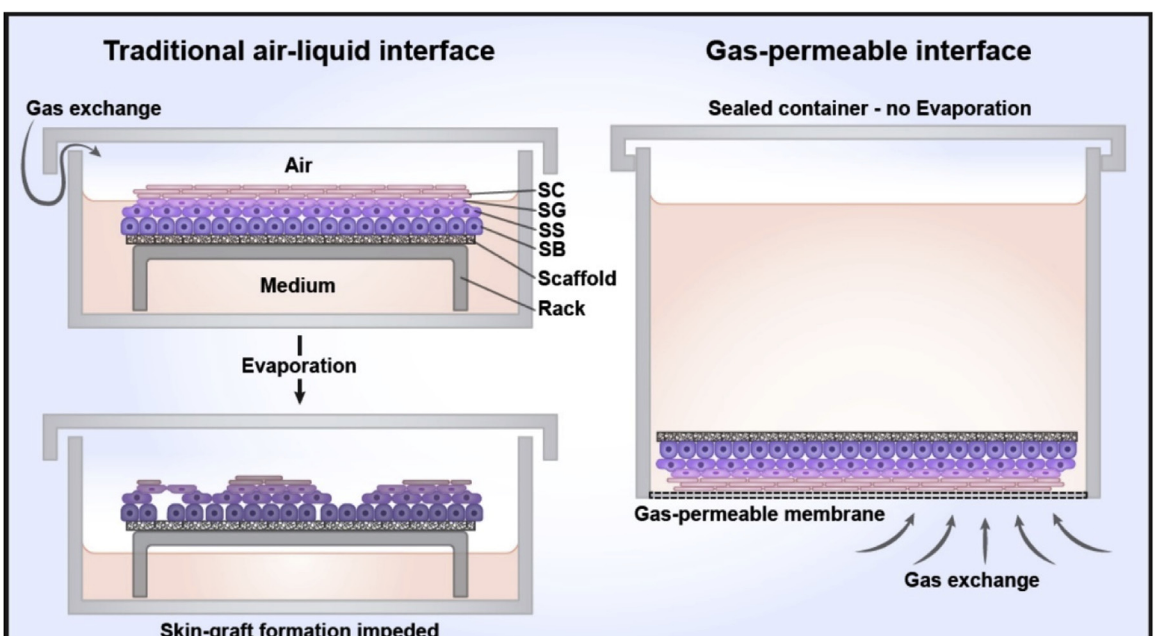

Fig. 1. Diagram of ALI and GPI epidermal stratification methods.
ALI epidermal stratification is induced by exposure of keratinocytes to air with the lower layer of the sample immersed in culture medium to maintain cell viability. In ALI cultures liquid levels can drop due to evaporation of liquid in incubators and result in loss of sample viability. GPI epidermal stratification is induced by keratinocyte exposure to gas exchange occurring through a gas-permeable membrane. Culture medium evaporation does not occur in GPI culture because the vessel can be sealed and filled with enough medium for the entire length of the culture period. (Color version of figure is available online.)

Acellular de-epithelialized dermis (DED) preparation

Acellular de-epithelialized dermis was prepared from human skin tissue based on the protocol described by MacNeil et al. [16]. Briefly, adipose and hypodermis were trimmed away from the dermis of skin tissue. Trimmed skin tissue was immersed in 1M NaCl and incubated at 37°C for 72 hours. Epidermis was removed by pulling it away from the dermis using forceps and incubated in DMEM supplemented with 100 U/mL Penicillin/Streptomycin and 0.625 mg/mL Amphotericin B overnight at 37°C. DED was stored at 4°C in DMEM supplemented with 100 U/mL Penicillin/Streptomycin and 0.625 mg/mL Amphotericin B until required for use.

Epidermal culture at an ALI or GPI

Approximately 600,000 keratinocytes/cm^2 were seeded onto the basement membrane side of DED in Kelch's medium without SB772077B. Cell-seeded samples were incubated submerged for 24 hours at 37°C with 5% CO_2. Samples were then either raised to an ALI or transferred to a GPI.

Samples raised to an ALI were transferred to a metal rack in a 6 well plate and the well was filled with Kelch's medium without SB772077B until the liquid level covered the DED of the sample but did not cover the keratinocyte seeded surface of the DED (Figure 1). Culture medium levels were checked daily and additional Kelch's medium without SB772077B was added as required to ensure liquid levels covered the dermis but not the epidermal surface. Samples were cultured at an ALI for 14 days before analysis.

Samples transferred to a GPI were placed into the well of a G-Rex 6 well plate (Wilson Wolf) with the keratinocyte seeded side of the DED in direct contact with the gas permeable membrane in the bottom of the G-Rex 6 well. Each G-Rex 6 well was filled with 20 ml of Kelch's medium without SB772077B and sealed until ready for harvest. Samples were cultured at a GPI for 14 days before analysis.

Sample preparation for Alamar Blue staining and histology

After samples were cultured at ALI or GPI for 14 days samples were cut in half using a scalpel. Half was used for histological and immunohistochemical staining and the remaining half for Alamar Blue live/dead staining. The half of the sample to be used for histology was embedded in OCT (Tissue-Tek) and snap frozen in liquid nitrogen before cryosectioning.

Alamar Blue Live/Dead organotypic epidermis sample staining

Samples were completely immersed in Alamar Blue solution (Invitrogen), diluted 1:10 in DPBS, and incubated at 37°C for 1 hour. Samples were removed from the Alamar Blue solution and imaged.

Histological and immunohistochemical staining

Transverse skin sample cryosections, 5 μm thick, were mounted on positively charged slides, fixed in 95% ethanol followed by staining with Gill's II hematoxylin solution and counter-stained with 2% Eosin Y solution. The sections were dehydrated in ethanol series followed by clearance in xylene before mounted with Eukitt mounting medium (Milton Adams, catalogue number HEUKITT). H&E-stained slides were scanned with a Vectra Polaris.

Transverse skin sample cryosections, 5 μm thick, were fixed with acetone and blocked with 0.25% casein/10% human serum. Samples were probed with primary antibodies (Supplementary Table 1); followed by secondary antibodies (Supplementary Table 2) and 4',6-diamidino-2-phenylindole (DAPI) nuclear staining (Molecular Probes). Samples were mounted using Prolong Gold (Molecular Probes), visualized with an Eclipse Nikon fluorescent microscope (Nikon), a VS200 (Olympus) or PhenoImager (Akoya) and figures were generated using Cytosketch software (CytoCode).

Results

Human keratinocytes were seeded onto human DED and then moved to an ALI or GPI to test induction of epidermal stratification. ALI culture consisted of keratinocyte seeded DED samples sitting on a metal rack in a tissue culture plate with the DED portion immersed in culture medium and the top surface of the DED exposed to air (Figure 1). GPI culture consisted of the keratinocyte seeded side of the DED being placed in direct contact with the gas-permeable membrane in the bottom of a G-Rex 6 well (Figure 1).

Culture medium levels for ALI samples were checked daily and media was topped up if required, otherwise, the culture medium was changed completely every 2–3 days. Once samples were enclosed within the Grex-6 well with 20 mL of culture medium they did not require any monitoring or media changes for the duration of the culture period. After culture at an ALI or GPI for 14 days samples were harvested for histological and immunohistochemical analysis. Alamar blue staining clearly showed the acellular DED as blue and the circular area where keratinocytes were seeded onto the DED and formed an organotypic epidermis as pink to pale yellow (Figure 2B,D). Haematoxylin and eosin staining showed complete epidermal stratification consistently across the whole width of the samples at both ALI and GPI culture conditions (Figure 2A,C and Supplementary Figure 1). In both ALI and GPI culture conditions we could clearly observe keratinocyte differentiation resulting in formation of layered epidermis with a fully differentiated Stratum Corneum (Figure 2A,C).

In human skin Cytokeratin 14 is expressed in the basal layers of the epidermis, while Cytokeratin 10 is expressed in the suprabasal layers (Figure 3). We have also previously observed Cytokeratin 19 expression by a small number of cells scattered throughout the basal layer of the epidermis, as well as in the skin appendages [15]. We therefore used analysis of Cytokeratin 19, Cytokeratin 14 and Cytokeratin 10 to assess the stratification of the epidermis in our samples. In both ALI and GPI samples we observed expression of Cytokeratin 14 in the basal layers of the epidermis, and Cytokeratin 10 in the suprabasal layers, similar to the pattern seen in human skin (Figure 3). Cytokeratin 19 positive cells were present in both ALI and GPI samples, mainly scattered along the basal layer of the epidermis; cells staining for Cytokeratin 19 appeared more frequently in the ALI and GPI engineered epidermis than in human skin (Figure 5).

Claudin-1, Filaggrin and Loricrin are all proteins that are critical for formation of the barrier function of human skin. Claudin-1 is involved in tight junction formation between keratinocytes and is expressed throughout the epidermis [17]. Claudin-1 was expressed by keratinocytes throughout the epidermis of ALI and GPI samples in three out of four donors, indicating the formation of tight junctions which contribute to skin barrier function (Figure 4). In one donor Claudin-1 expression was not expressed in either ALI or GPI samples (Data not shown).

Filaggrin expression is required for keratinocytes to differentiate into the flat corneocytes which form the Stratum Corneum [18]. Filaggrin was expressed in the Stratum Corneum of human skin and in the Stratum Corneum of ALI and GPI samples (Figure 4).

Loricrin is a key structural protein that comprises more than 70% of the cornified envelope [19]. Loricrin was expressed in the Stratum Corneum of ALI and GPI samples, with a similar expression pattern to human skin (Figure 5).

Expression of Claudin-1, Filaggrin, and Loricrin in ALI and GPI samples, similar to those of human skin, indicated that both methods had induced keratinocyte terminal differentiation and completed the epidermal stratification process required to produce epidermis with barrier function.

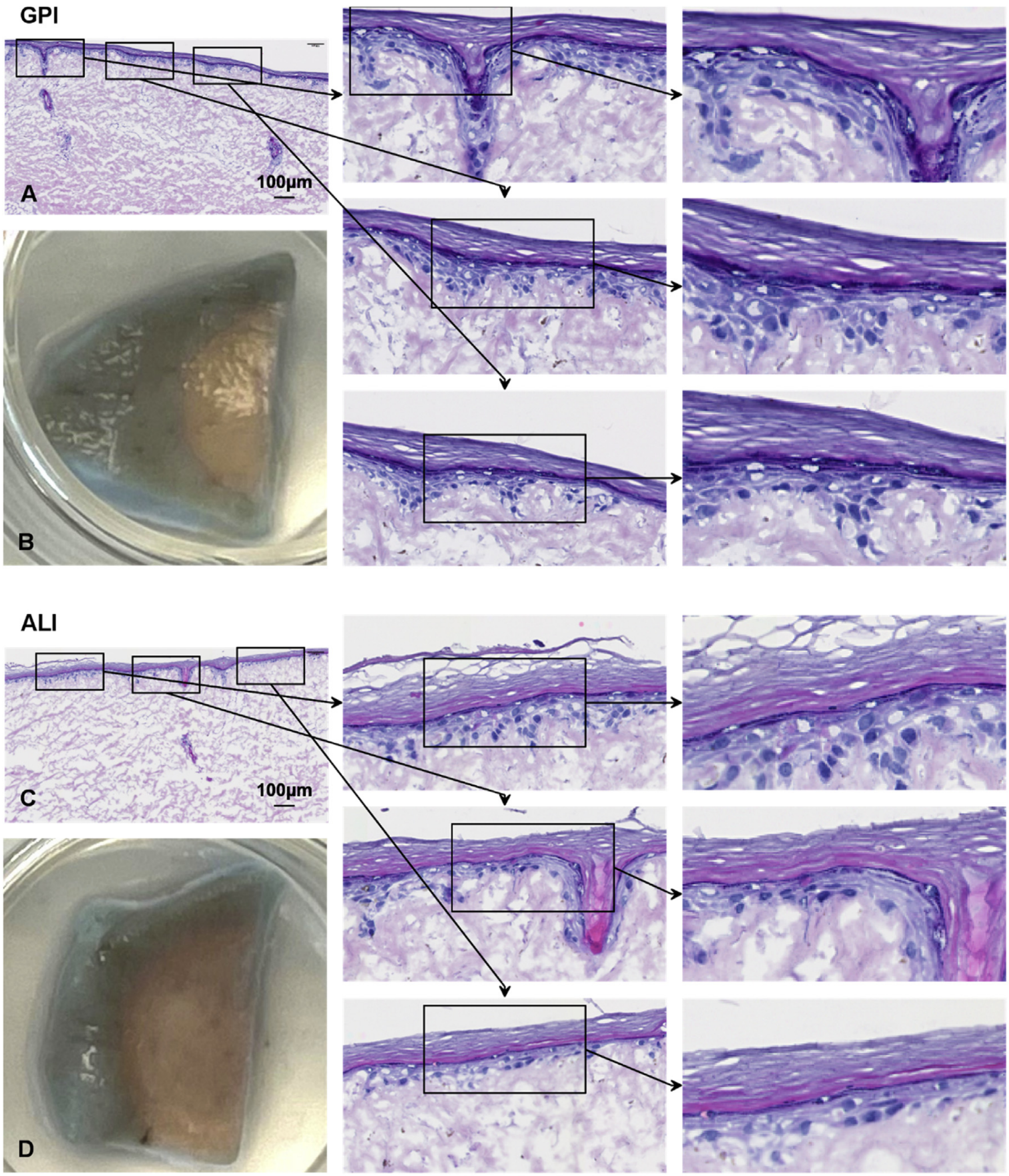

Fig. 2. Alamar Blue and Haematoxylin and Eosin staining of ALI and GPI cultured organotypic epidermis.

Organotypic epidermis was cut in half after 14 days at ALI or GPI. Half of each sample was stained with Alamar Blue (B&D). The remaining half of each sample was used to cut transverse cryosections which were stained for hematoxylin and eosin (A&C). Images are representative of epidermis grown with keratinocytes from four human donors (Supplementary Figure 2). *A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07296*. (Color version of figure is available online.)

Integrin beta-1 and Integrin alpha-6 are critical for basal keratinocyte adhesion to laminins found in the basement membrane of skin [20]. Integrin beta-1 and Integrin alpha-6 are expressed in the Stratum Basale of human skin (Figures 3 and 4). Our high salt concentration DED preparation method, similar to that used to prepare commercial DED product Alloderm^{TM} maintains an intact basement membrane structure [21]. Therefore, residual Integrin beta-1 and Integrin alpha-6 on the DED cannot be distinguished from protein produced by keratinocytes seeded onto the DED. For this reason, positive extracellular staining for Integrin beta-1 and Integrin alpha-6 at the basement membrane was not considered in this analysis. In both the ALI and GPI culture conditions Integrin beta-1 and Integrin alpha-6 were expressed in basal cells in contact with the basement membrane (Figures 3 and 4). For Integrin beta-1 the expression pattern in basal keratinocytes in ALI and GPI samples was similar to human skin (Figure 3). Integrin alpha-6 expression was seen only at the basement membrane side of basal keratinocytes in human skin but was seen in the cytoplasm of basal keratinocytes for GPI and ALI samples as well as the basement membrane.

Discussion

Inducing keratinocytes to undergo programmed terminal differentiation and complete the epidermal stratification process is critical to replicate epidermal layering *in vitro* and a produce a mature

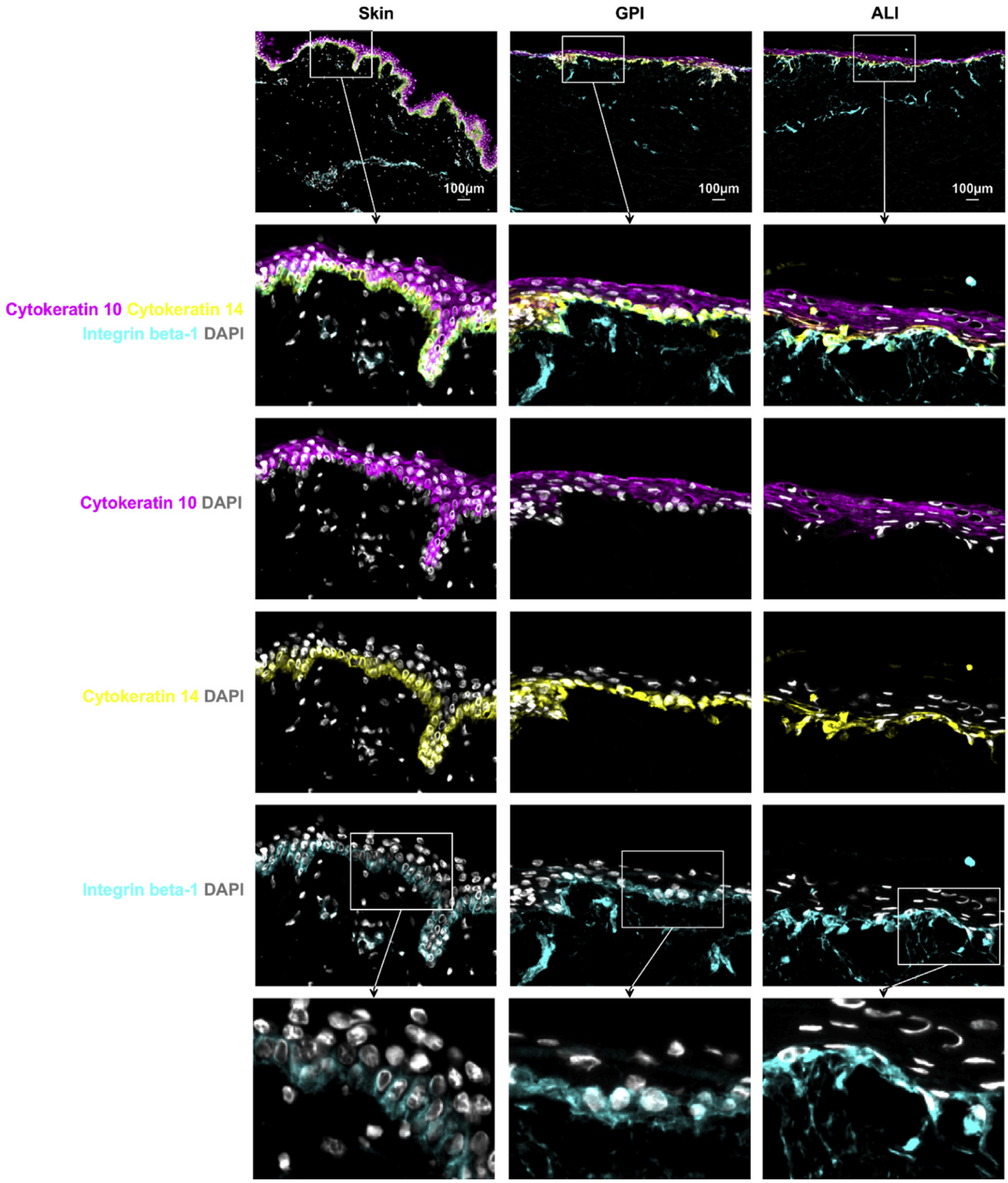

Fig. 3. Cytokeratin 10, Cytokeratin 14, and Integrin beta-1 immunocytochemistry of ALI and GPI cultured organotypic epidermis.
Organotypic epidermis transverse cryosections were stained for Cytokeratin 10 (Magenta), Cytokeratin 14 (Yellow) Integrin beta-1 (Cyan) and DAPI (Grey). Images are representative of organotypic epidermis grown with keratinocytes from four human donors. Controls to demonstrate autofluorescence and non-specific staining are shown in Supplementary Figure 3. *A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07297*. (Color version of figure is available online.)

organotypic epidermis with barrier function. Traditionally epidermal stratification is achieved by exposing keratinocytes to an ALI, the trigger for keratinocyte differentiation and the epidermal stratification process. We have demonstrated that complete epidermal stratification can be achieved at a GPI.

We observed complete stratification of epidermis grown with both ALI and GPI, including formation of Stratum Corneum. Cytokeratin 14, Cytokeratin 10 and Cytokeratin 19, expressed in different levels of the stratified epidermis, showed similar expression patterns throughout the epidermis in both ALI and GPI samples. Cytokeratin 14 and 10 expression patterns in ALI and GPI samples were similar to those seen in human skin. However, compared to human skin we observed an increase in Cytokeratin 19 positive cells in both ALI and GPI cultures. This may partly relate to the high percentage of Cytokeratin 19 positive cells in the starting populations that were seeded into these cultures, as we had previously noted under these culture conditions [15]. Expression of Cytokeratin 19 is associated with improved potential for self-renewal of skin graft epidermis *in vitro* [22], and this property may be particularly useful for permanent solutions to wounds, such as on burn wounds, where ongoing epidermal renewal will be critical for long term survival of grafts.

Integrin beta-1 expression was observed in the Stratum Basale of both ALI and GPI samples, as found in normal skin. Integrin alpha-6 was expressed in the cytoplasm of basal keratinocytes of both ALI

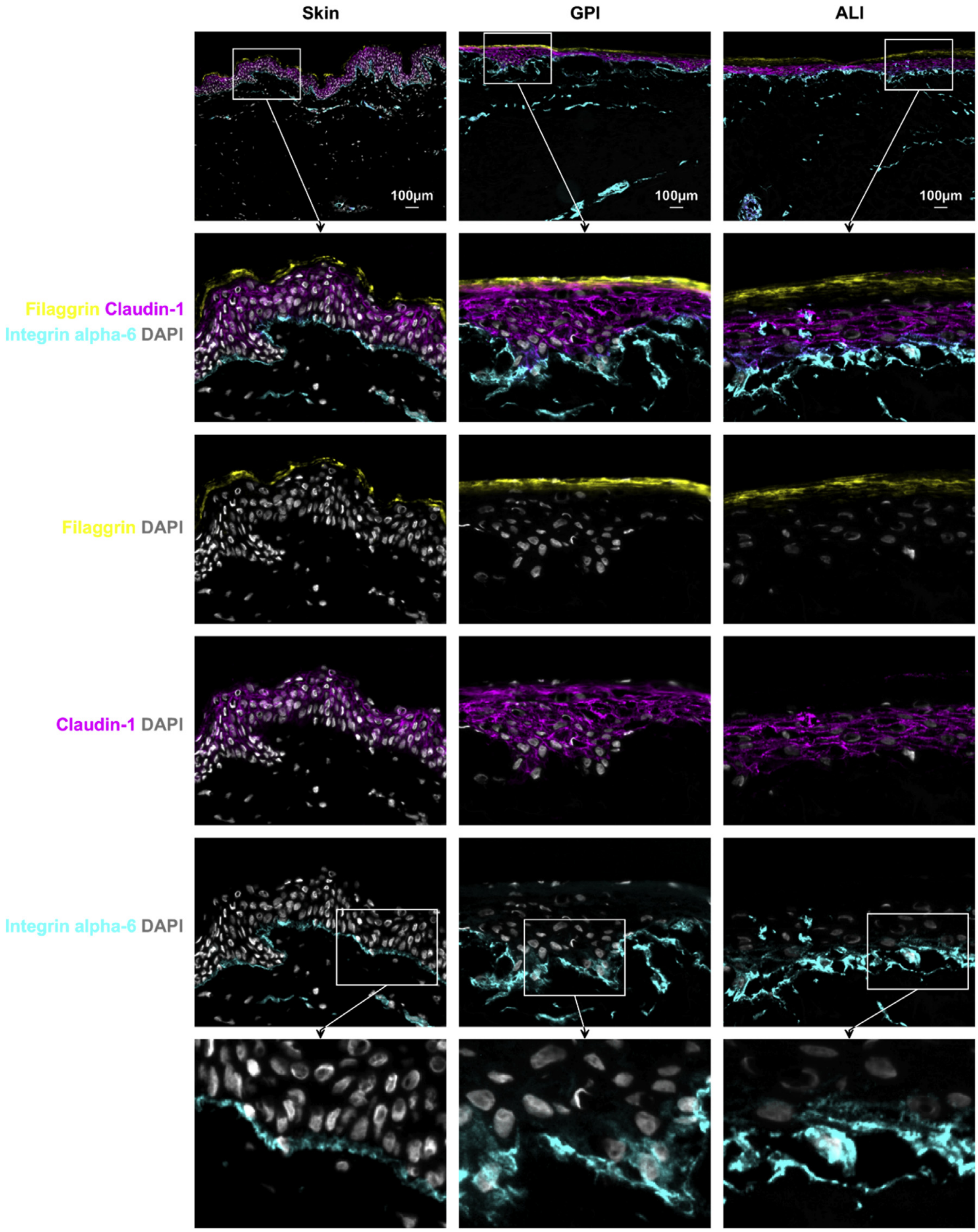

Fig. 4. Claudin-1, Filaggrin and Integrin alpha-6 immunocytochemistry of ALI and GPI cultured organotypic epidermis. Organotypic epidermis transverse cryosections were stained for Claudin-1 (Magenta), Filaggrin (Yellow), Integrin alpha-6 (Cyan) and DAPI (grey). Images are representative of organotypic epidermis grown with keratinocytes from four human donors. Controls to demonstrate autofluorescence and non-specific staining are shown in Supplementary Figure 3. *A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07298*. (Color version of figure is available online.)

and GPI samples. This expression pattern varied slightly from human skin, where Integrin alpha-6 expression was restricted to the interface with the basement membrane. In contrast to the mature, stable basal keratinocytes in human skin, the basal keratinocytes in the ALI and GPI samples have only recently attached to the basement membrane of the DED, so we speculate their relative lack of maturity accounts for their cytoplasmic Integrin alpha-6. Expression of Integrin beta-1 and Integrin alpha-6 in the cytoplasm of basal keratinocytes demonstrated that the keratinocytes expressed both proteins in culture, and positive signal was not restricted to the basement membrane of the DED on which the cells were seeded.

Loricrin, Filaggrin and Claudin-1 are all critical for epidermal barrier function. In both ALI and GPI samples Loricrin, Filaggrin and Claudin-1 were expressed in patterns similar to human skin, indicating complete epidermal stratification and likely barrier function.

Several advantages of epidermal stratification using a GPI are apparent. The epidermis remains covered in cell culture medium at all times, with no risk of drying out, or conversely, losing exposure to

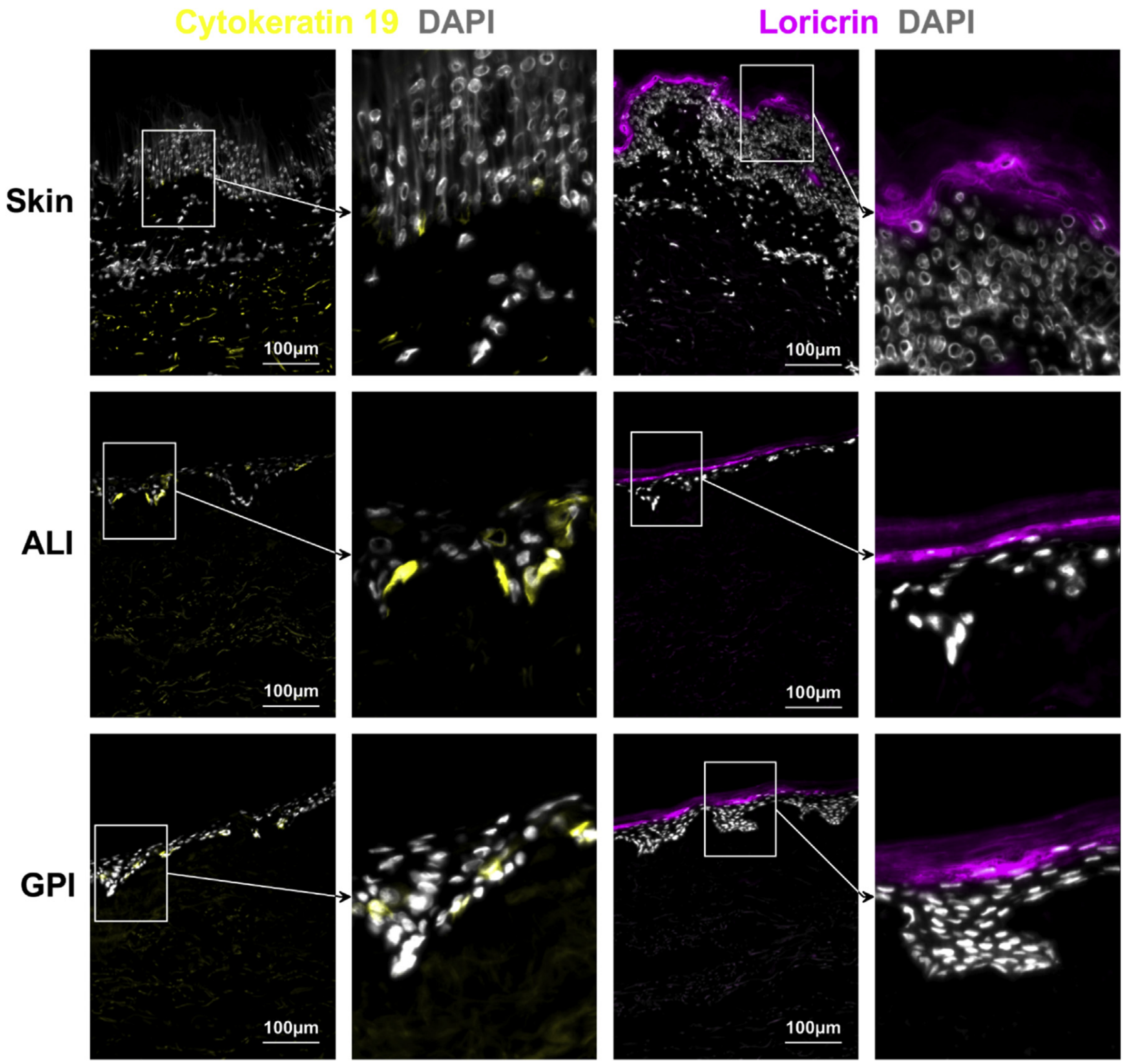

Fig. 5. Cytokeratin 19 and Loricrin immunocytochemistry of ALI and GPI cultured organotypic epidermis.
Organotypic epidermis transverse cryosections were stained for Cytokeratin 19 (Yellow), Loricrin (Magenta) and DAPI (Grey). Images are representative of organotypic epidermis grown with keratinocytes from four human donors. Controls to demonstrate autofluorescence and non-specific staining are shown in Supplementary Figure 3. *A high-resolution version of this slide for use with the Virtual Microscope is available as eSlide: VM07299*. (Color version of figure is available online.)

a strong gas gradient due to excess medium above, as for ALI-based methods. The GPI-based method is therefore less susceptible to loss of viability or stratification signal.

Epidermal cells grown at a GPI can be covered with large volumes of culture medium without negatively affecting the amount of nutrient gases cells can obtain from the culture medium [12]. This is because the GPI enables efficient gas exchange with the culture medium at the surface where the cells are located, regardless of the medium volume above it. The ability to load large volumes of culture medium into GPI-based culture vessels therefore reduces the need for manipulation. There is no need to regularly check and maintain culture medium levels as for ALI culture, reducing microbial contamination risk from culture handling, and the workload of highly skilled cell culture staff required for highly controlled liquid level maintenance. Using a GPI based culture vessel for epidermis growth and maturation enables simple scaling of the size of the tissue, all physical support for the tissue being grown is provided by the GPI itself.

All of these advantages could simplify and improve *in vitro* skin graft production for burn wound treatment, skin graft applications, and other tissue engineering applications.

Declaration of competing interest

The authors have been granted a patent for epithelial stratification at a gas permeable interface.

Funding

This work was supported by The William and Lois Manchester Trust (New Zealand), the Maurice Wilkins Centre and Cure Kids (New Zealand).

Author Contributions

Conception, experimental design, experimental work, manuscript preparation: VF. Experimental work: LZ, JC, EW, ED, IK, SM, JH, ADR, HR, SP. Manuscript preparation: EL. Clinical liaison, tissue sourcing: JL. Manuscript preparation: HS. Clinical liaison, tissue sourcing, manuscript preparation: ML. Conception, experimental design, manuscript preparation: RD.

Ethical Approval

Informed consent was obtained from patients who donated human skin tissue. Patients were undergoing elective breast reduction, breast reconstruction or abdominoplasty procedures with planned skin excision, following protocols approved by the New Zealand Northern A Health and Disability Ethics Committee (approval number NTX/08/09/086).

Acknowledgments

The authors wish to thank all the patients, nurses and surgeons from Middlemore Hospital, Auckland, New Zealand who donated their time and tissue to support this research.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2024.12.005.

References

[1] Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol 2008;17:1063–72.
[2] Koster MI, Roop DR. Mechanisms regulating epithelial stratification. Annu Rev Cell Dev Biol 2007;23:93–113.
[3] McLafferty E. The integumentary system: anatomy, physiology and function of skin. Nurs Stand 2012;27:35–42.
[4] Schmidt FF, Nowakowski S, Kluger PJ. Improvement of a three-layered in vitro skin model for topical application of irritating substances. Front Bioeng Biotechnol 2020;8:388.
[5] Regnier M, Caron D, Reichert U, Schaefer H. Barrier function of human skin and human reconstructed epidermis. J Pharm Sci 1993;82:404–7.
[6] du Rand A, Hunt JMT, Feisst V, Sheppard HM. Epidermolysis bullosa: a review of the tissue-engineered skin substitutes used to treat wounds. Mol Diagn Ther 2022;26:627–43.
[7] Prunieras M, Regnier M, Woodley D. Methods for cultivation of keratinocytes with an air-liquid interface. J Invest Dermatol 1983;81(1 Suppl):28s–33s.
[8] Oh JW, Hsi TC, Guerrero-Juarez CF, Ramos R, Plikus MV. Organotypic skin culture. J Invest Dermatol 2013;133:1–4.
[9] Silva S, Bicker J, Falcao A, Fortuna A. Air-liquid interface (ALI) impact on different respiratory cell cultures. Eur J Pharm Biopharm 2023;184:62–82.
[10] John S, Kesting MR, Paulitschke P, Stockelhuber M, von Bomhard A. Development of a tissue-engineered skin substitute on a base of human amniotic membrane. J Tissue Eng 2019;10:2041731418825378.
[11] Gragnani A, Morgan JR, Ferreira LM. Differentiation and barrier formation of a cultured composite skin graft. J Burn Care Rehabil 2002;23(2):126–31.
[12] Bajgain P, Mucharla R, Wilson J, Welch D, Anurathapan U, Liang B, et al. Optimizing the production of suspension cells using the G-Rex "M" series. Mol Ther Methods Clin Dev 2014;1:14015.
[13] Vera JF, Brenner LJ, Gerdemann U, Ngo MC, Sili U, Liu H, et al. Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). J Immunother 2010;33:305–15.
[14] Kim SM, Kim DH, Oh JD. Development and evaluation of cell culture devices with the gas-permeable membrane. Biotechnol Bioproc Eng 2020;25:62–70.
[15] Feisst V, Kelch I, Dunn E, Williams E, Meidinger S, Chen CJ, et al. Rapid culture of human keratinocytes in an autologous, feeder-free system with a novel growth medium. Cytotherapy 2023;25:174–84.
[16] MacNeil S, Shepherd J, Smith L. Production of tissue-engineered skin and oral mucosa for clinical and experimental use. Methods Mol Biol 2011;695:129–53.
[17] Kirschner N, Rosenthal R, Furuse M, Moll I, Fromm M, Brandner JM. Contribution of tight junction proteins to ion, macromolecule, and water barrier in keratinocytes. J Invest Dermatol 2013;133:1161–9.
[18] Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 2009;122(Pt 9):1285–94.
[19] Nithya S, Radhika T, Jeddy N. Loricrin - an overview. J Oral Maxillofac Pathol 2015;19:64–8.
[20] Hegde S, Raghavan S. A skin-depth analysis of integrins: role of the integrin network in health and disease. Cell Commun Adhes 2013;20:155–69.
[21] Dussoyer M, Michopoulou A, Rousselle P. Decellularized scaffolds for skin repair and regeneration. Appl Sci 2020;10:3435.
[22] Pontiggia L, Biedermann T, Meuli M, Widmer D, Bottcher-Haberzeth S, Schiestl C, et al. Markers to evaluate the quality and self-renewing potential of engineered human skin substitutes in vitro and after transplantation. J Invest Dermatol 2009;129:480–90.