MODULE 1 – Week 2
## Skin Structure & Function/ Burn Pathophysiology – The Structure & Function of Normal Skin [1] – The Epidermis
## Navsaria/ McKenzie

The Structure & Function of Skin

The integument comprises the skin together with its appendages (**Figures 1 & 2**). These include hair and hair follicles, sebaceous and sweat glands, and nails. The skin covers the entire body and is the largest organ of the body. It covers a surface area of more than 1.7 m^2 making up in total about 16% of normal body weight. It has an array of functions. These include acting as a barrier to physical, biological and chemical agents, as well as to ultraviolet (UV) radiation. Skin barrier function also acts to prevent dehydration by controlling loss and gain of fluid. Other functions include sensory and thermoregulatory roles, vitamin D synthesis, immune surveillance, excretion of wastes through sweat glands, socio-sexual communication and reproduction, by virtue of its appearance and smell (e.g. hormones and pheromones).

Skin is divided into glabrous (covering the palms of the hands and soles of the feet) and hairy skin. The skin comprises of 2 layers, the outer most epidermis and the innermost layer, the dermis. Embryologically, these 2 layers of skin are derived from the ectoderm and mesoderm respectively. The epidermis and dermis are firmly attached to each other and together, vary in thickness from around 0.5 to 4 mm or more depending on body site. At the point where the epidermis meets the dermis, evaginations that project into the dermis are formed known as 'rete ridges' or 'pegs'. Complementary projections of the dermis are called dermal papillae.
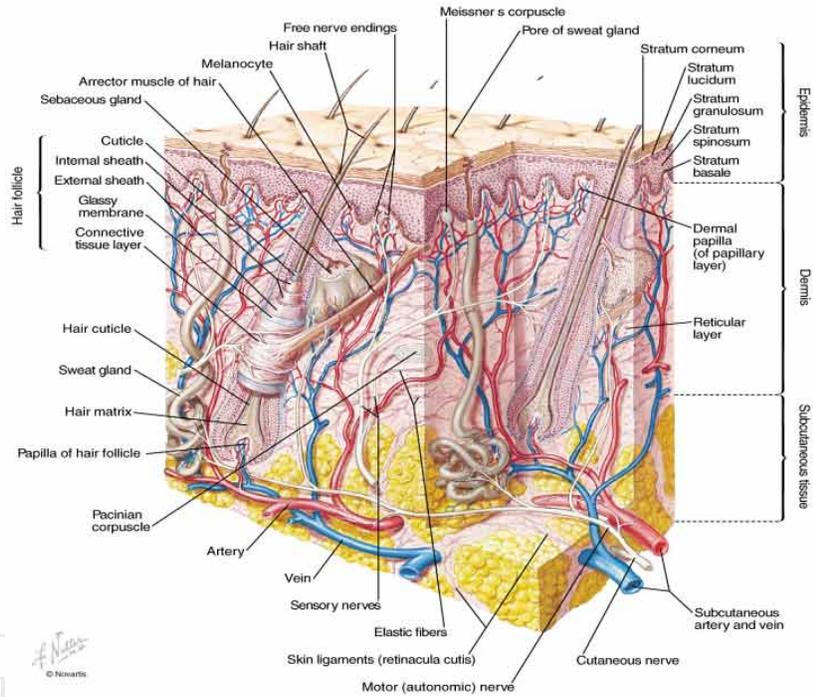
Figure 1 - Diagrammatic representation of hairy skin. This image was taken from http://www.healthandage.com/html/art_popup/skin1.htm.
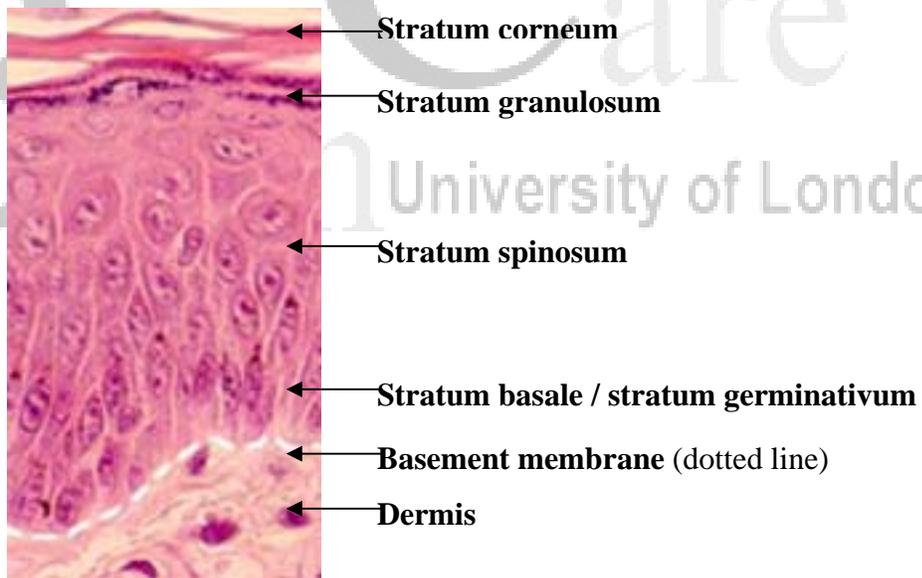
Figure 2 - Haematoxylin and eosin (H&E) histology of the skin.

The Epidermis

The epidermis is defined as a stratified squamous epithelium made up of several cell types. These include melanocytes (production of melanin pigment), Langerhans cells (immune function), Merkel cells (sensory function) and keratinocytes, of which the latter makes up at least 80% of its cellular population.

The epidermis is arranged into distinct layers, each showing a pattern of keratinocyte proliferation, differentiation and maturation. Four main layers exist. These are strata basale (germinativum), spinosum, granulosum and corneum. In thick skin, a fifth layer called the stratum lucidum is present and is found between the granular and the cornified layers. These layers reflect the sequential differentiation of keratinocytes as they migrate from the basal layer on the onset of terminal differentiation, having lost the ability to proliferate, to the outermost cornified layers where they are sloughed off. The process of terminal differentiation involves a series of biochemical and morphological changes, which result in the production of an anucleated cornified keratinocyte that forms the stratum corneum. Epidermal turnover takes on average 14 to 30 days.

The stratum basale is a single layer of cuboidal basal cells attached to the basement membrane by hemidesmosomes that contain integrins. Adjacent cells are attached by desmosomes that contain cadherins. The majority of the cells in this layer are mitotically active and are required for the continued renewal of the epidermis by upward displacement, replacing the cells of the outermost superficial layer that are lost during normal epidermal turnover. These mitotically active cells derive from a population of putative stem cells that are thought to reside in the deep rete ridges of glabrous skin and at the tips of the dermal papillae in interfollicular epidermis or in the bulge region of the outer root sheath (ORS) of adult human hair follicles in hairy skin. Also arising from stem cells in the basal layer are transit amplifying cells and post mitotic cells which are

displaced into the suprabasal layers. Basal cells contain cytokeratins organised in bundles around the nucleus and insert into desmosomes peripherally.

Above this layer is the **stratum spinosum** consisting of several layers of irregular, polyhedral shaped cells that display spiny projections. Cells from the previous layer lose contact with the basement membrane and are subsequently pushed up to form this layer. Some cells in this layer are still mitotically active. Cells become progressively flattened as they move up towards succeeding layers. Cells contain lamellar granules (for the later provision of epidermal lipids responsible for the barrier properties of the stratified corneum) and more desmosomes for cell-to-cell adhesion.

The next layer, the **stratum granulosum** comprises of three to five layers of flattened cells. In this layer, lamellar granules containing lipids and keratohyalin granules are present within the cells cytoplasm. Keratohyalin granules contain pro-filaggrin, a precursor of filaggrin that bundles the keratin filaments together.

The **stratum lucidum** consists of several layers of flattened cells without nuclei and organelles, and a keratin rich cytoplasm.

The **cornified layer** consists of dead, anucleated, highly keratinised cells called squames or corneocytes. Keratin filaments polymerise by forming strong disulphide bonds. Filaggrin, a protein component of the keratohyalin granule is involved in this process. The cornified envelope forms due to the catalytic activity of the enzyme transglutaminase which crosslinks proteins such as involucrin (an insoluble 70 to 80 kDa cysteine rich protein) in the plasma membrane. Other proteins found as components of the cornified cell envelope include keratolinin, loricrin, small proline-rich proteins, the serine proteinase inhibitor elafin, filaggrin linker-segment peptide, and envoplakin. Lipids, discharged by lamellar granules, fill the intercellular spaces which contribute to the barrier properties of the

epidermis. The lack of desmosomes in the cells that are closest to the outermost layer results in the loss or shedding of corneocytes from the skin.

Keratins

The cytoskeleton of all epithelial cells including keratinocytes is formed from the three groups of filaments, actin (microfilaments), tubulin (microtubules) and intermediate filaments. The keratins belong to the latter group, a multigene family of proteins that form filaments of 10 nm in diameter in which keratins form the two largest groups. These groups which comprise more than 30 members of the keratin family have been designated as type I keratins (acidic), numbered 9-20, and type II keratins (basic), numbered 1-8. Usually, type I and type II keratin subunits pair up and the heterodimers formed are expressed accordingly to epithelial type and in a differentiation-state specific manner. For example, in simple epithelia, keratin (K) 8 and K18 are expressed. Cells in the basal layer of stratified epithelia express K5 and K14. The differentiating suprabasal layers are characterised by the keratin pair K1 and K10. K6 and K16 are not found in normal epidermis except the ORS of hair follicle and junctional region. This keratin pair is also constitutively expressed in certain stratified squamous mucosal epithelia and the skin of palm and sole. Expression of K6 and K16 is induced in wound healing epidermis, hyperproliferative epidermis such as psoriasis, squamous cell carcinomas and hypertrophic scarring. Thus, keratin expression has been used as specific epithelial cell markers to assess epithelial proliferation and differentiation with the use of immunohistochemistry (**Figure 3**).
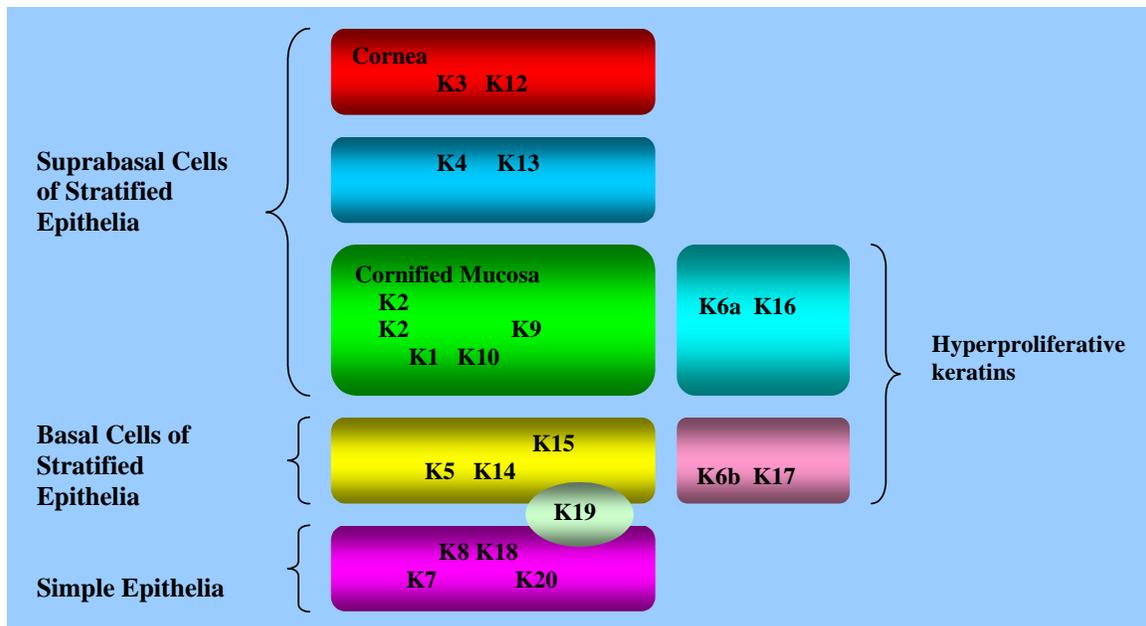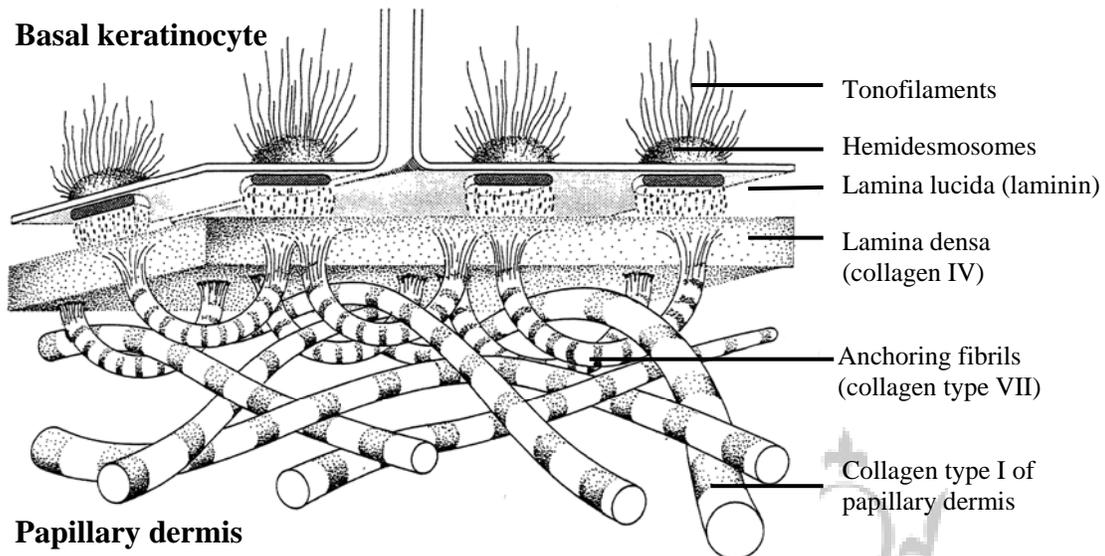
Figure 3 - Schematic representation of keratin expression in normal epithelia. Diagram reproduced by kind permission of Seamus Harrison (Centre for Cutaneous Research, Queen Mary University of London).

The Dermo - Epidermal Junction (The Basement Membrane Zone)

The basement membrane zone lies at the interface between the epidermis and the dermis and functions to strongly attach these layers to each other. It also serves as a support for the avascular epidermis. Four layers have been identified within the basement membrane zone. These layers are:

i. The plasma membrane of the basal keratinocyte, which is penetrated by hemidesmosomes.

ii. The electron-lucent lamina lucida that contains laminin, bullous pemphigoid glycoprotein, anchoring filaments and sub-basal dense plates.

iii. The electron-dense lamina densa that contains collagen type IV and proteoglycans.

iv. The collagen type VII rich-lamina fibroreticularis that contains anchoring fibrils (**Figure 4**).

Fibroblasts or keratinocytes are capable of synthesising many of the components of the basement membrane zone. However, the combination of both cell types leads to the correct assembly of the complex.

Basal keratinocyte

Tonofilaments

Hemidesmosomes

Lamina lucida (laminin)

Lamina densa
(collagen IV)

Anchoring fibrils
(collagen type VII)

Collagen type I of
papillary dermis

Papillary dermis

Figure 4 - The basement membrane zone. Constituent proteins that can be detected by immunohistochemistry are shown in parenthesis. (Reproduced by permission of IBC Ltd., USA from the "Bioengineering of Skin Substitutes" meeting handbook in September 1997).

Epithelial/Mesenchymal Interactions

Evidence is accumulating that the mesenchyme plays a crucial role in epidermal homeostasis. It is known that the epidermis in turn, can influence the dermis with regards to structural remodelling, re-innervation and vascularisation. These epithelial/mesenchymal interactions are orchestrated by a complex network of communications between cell-matrix, cell-cell and cell-diffusible factors.

The hair follicle provides a good system to study epithelial/mesenchymal interactions. For instance, the size and position of the developing hair follicles is

determined by direct mesenchymal signals. During hair follicle morphogenesis epithelial/mesenchymal interactions take place initiated by the first dermal signal which results in the formation of a fully developed hair follicle. Moreover, signalling between dermal papilla and hair follicle epithelium is crucial for hair follicle development and function.

The mesenchyme determines the fate of the epithelium. For example, the formation of epidermis and hair follicles is induced when cultured rabbit corneal epithelium is combined with embryonic hair-forming mouse dermis.

An understanding of the epithelial/mesenchymal interactions that takes place in skin has been brought about by wound healing studies. Initially, Rheinwald and Green (1975) showed the improved growth rates of keratinocytes grown on an irradiated mouse fibroblast feeder layer. Since then, the importance of a dermal component for the improved clinical take of cultured epithelium has been well documented.

In *vitro skin* models have been used to study the importance of epithelial/mesenchymal interactions. These organotypic cultures are composed of keratinocytes and mesenchymal cells grown *in vitro* via an extracellular matrix at an air-liquid interface that induces differentiation. They also share similarities with wound healing, hyperproliferative and psoriatic skin. These models have been useful for elucidating the importance of epithelial/mesenchymal interactions on a number of processes including epidermal homeostasis, production and deposition of basement membrane and ECM components and secretion of diffusible factors.

Burn Wound Management

The roots of the development of Isolagen, and Integra as a dermal filler lie in skin engineering for burn wound treatment.

Burn injuries may result from thermal or chemical injury to the integument which causes local tissue damage to the skin as well as other diverse systemic effects. In the U.K. the depth of the damaged tissue can be classified as (i) superficial partial-thickness, (ii) deep partial-thickness and (iii) full-thickness (**Figure 4**). In superficial burns, the epidermis is damaged and the underlying dermis remains relatively intact or is slightly damaged. Epidermal regeneration occurs spontaneously from the lateral undamaged epidermis and from remnants of skin appendages in the wounded area. In deep partial-thickness burns, the dermis is also injured and in full-thickness burns, all skin components are destroyed to the level of fat or fascia. In most large burn cases both deep partial-thickness and full-thickness wounds have to be treated surgically. Burn tissues consist of purulent exudate produced by immigrating inflammatory cells and organisms, dried blood and denatured skin proteins. These eshars are quickly colonised by endogenous bacteria derived from skin adnexae. Therefore, rapid excision of the dead tissue, leaving behind viable tissue, followed by early wound coverage is a crucial factor for the survival of major burns cases.

Autologous Skin Grafts

Traditional autologous split-thickness skin grafts (SSG) for the resurfacing of large burns are the gold standard and provides permanent wound closure. A SSG contains epidermis and variable amounts of dermis and can be applied either whole or meshed to expand it. The drawback with this technique is the almost inevitable limited availability of donor sites, especially in cases of extensive burns (large Total Burn Surface Area). Moreover, harvesting SSGs create other wounds at donor sites and these donor sites can often be linked to morbidity, give rise to pain and develop some degree of scarring. The availability of other techniques to obtain adequate wound coverage would therefore be of great clinical value. Some of these alternatives include allogeneic skin, synthetic skin and cultured keratinocyte grafts.
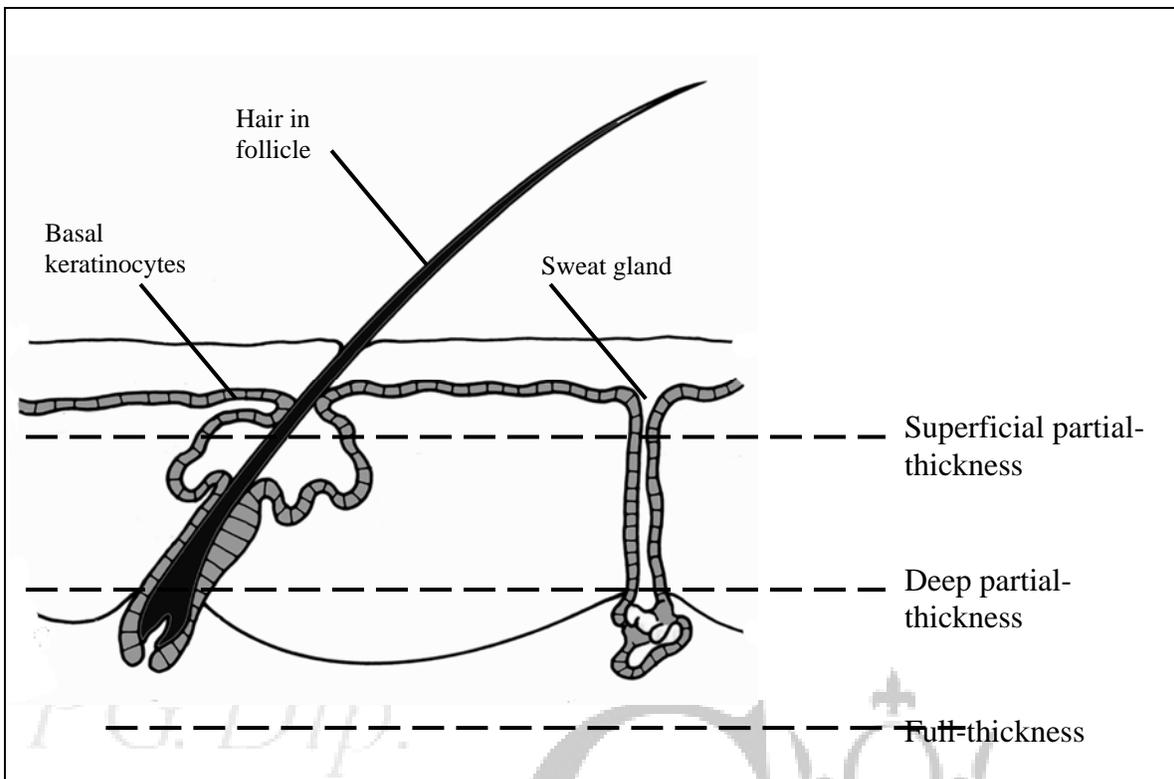
Figure 4 - Diagrammatic representation of the classifications used for burn depth.

Cultured Keratinocyte Grafts

A chance discovery found that primary keratinocytes could be grown rapidly *in vitro* on a feeder layer of lethally irradiated Swiss 3T3 mouse fibroblasts with addition of mitogens to the serum based culture medium. By optimising the growth conditions, the mean keratinocyte cell cycle time was 22-24 hours. This was the major breakthrough that led to the production of keratinocyte sheet grafts (Green sheets). Keratinocytes derived from a small skin biopsy can be expanded to generate large, multilayered (up to 12 layers) epithelial sheet grafts within 3-4 weeks in culture, and enough to cover the whole body. The enzyme Dispase can be used to release the keratinocyte sheets from the plastic although some keratinoctye-derived basement membrane proteins are lost during this process.

The resulting sheets are fragile and supportive backing materials must be used for transfer to the wound bed (**Figure 5**).
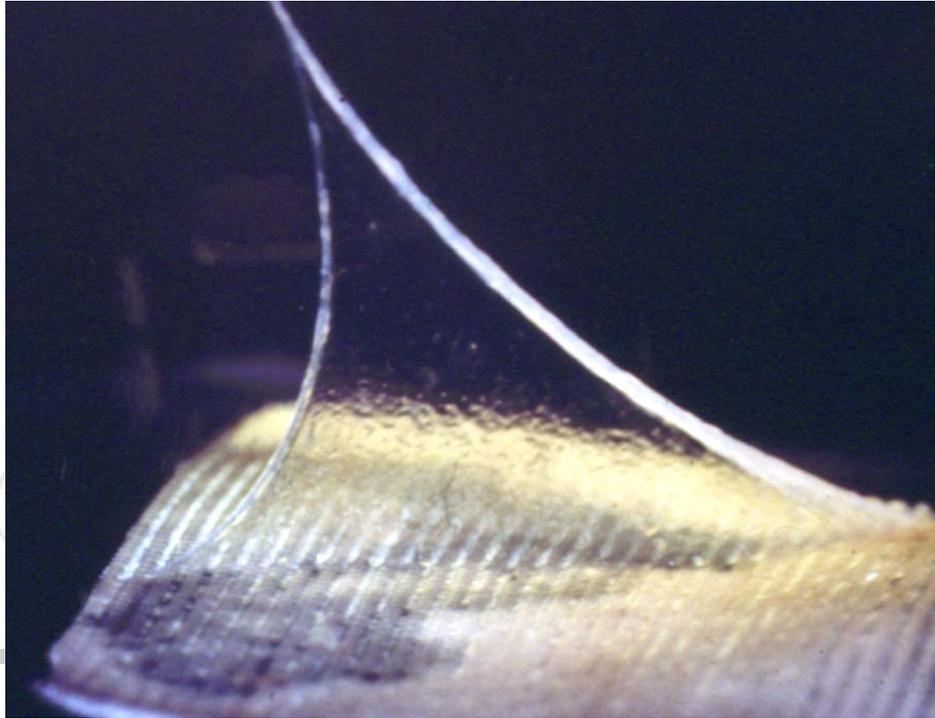
Figure 5 - Cultured keratinocyte sheet being raised from backing material.

The clinical use of cultured keratinocyte autografts to treat extensive burns has been well documented. However, when cultured keratinocyte autografts are applied to full-thickness wounds - granulation tissue, muscle fascia, or fat - they are unstable, fragile, and, due to a flat dermo-epidermal junction, tend to blister, and cause scarring. All these factors influence 'take' which can be highly variable. Optimal time-points for the assessment of completeness of 'take' of cultured keratinocyte autografts have yet to be determined due to a lack of standardisation in the measurement of take rates. Poor take rates led to the discovery that grafting these sheets in combination with a dermal component vastly improved 'take' in a variety of models with better clinical outcome. The realisation that combined engraftment of a dermal and epidermal component

provided efficacious wound closure led to further investigations into dermal equivalents.

Tissue Engineered Skin

Tissue engineered skin replacements are largely grouped into epidermal components, dermal components or a combination of the two (composite skin equivalents). The ideal synthetic skin would be adherent, replace all or most of the functions of skin, minimise contracture and scarring during regeneration, be durable and available in unlimited quantities, have a long shelf life and avoid the risk of transmission of infection. Many products are currently on the market for use as skin substitutes and some of these are listed in **Table 1**. Some of these tissue engineered skin have been used for the treatment of acute wounds (burns, burn scars, congenital anomalies) and chronic wounds (cutaneous ulcers).

Product Name	Layers		Preservation	FDA Approved uses
	Epidermal component	**Dermal component**		
whole skin				
Allogeneic cadaver skin (National skin banks)	Epidermis	Cellular dermis	Cryopreserved for viability, lyophilised, glycerolised	Burns, Scar revision
AlloDerm® (LifeCell, Woodlands, Texas)	None	Acellular dermis	freeze-dried	Burns, Scar revision
De-epidermalised dermis (DED) (National skin banks)	None	Acellular dermis	Cryopreserved, lyophilised, glycerolised,	Burns, Scar revision
HYAFF® technologies				
Hyalograft-3D® (Fidia Advanced Biopolymers srl)	silicone membrane	Esterified hyaluronic acid matrix with autologous fibroblasts	Stored in cool and dry place (<40°C)	Burns, Diabetic foot ulcers
Hyalomatrix® (Fidia Advanced Biopolymers srl)	silicone membrane	Hyaluronic acid matrices with variable esterification	Stored in cool and dry place (<40°C)	Burns, Diabetic foot ulcers
NW HYAFF® 11 (Fidia Advanced Biopolymers srl)	silicone membrane	Total esterified hyaluronic acid matrix	Stored in cool and dry place (<40°C)	Burns, Diabetic foot ulcers
Laserskin® Fidia Advanced Biopolymers srl)	100% esterified hyaluronic acid membrane with laser drilled micropores	None	Stored in cool and dry place (<40°C)	Biodegradable keratinocyte delivery system

Table 1 - Skin substitutes (continued on next page).

Product Name	Layers		Preservation	FDA Approved uses
	Epidermal component	**Dermal component**		
Dermal Substitutes				
Dermagraft® (Advanced Tissue Sciences Inc)	None	Bioabsorbable polyglactin mesh with living cultured neo-natal foreskin-derived fibroblasts	Cryopreservation	Burns, Diabetic foot ulcers
TransCyte® (Advanced Tissue Sciences Inc.)	Silicone membrane	Nylon mesh coated with porcine collagen with non-viable cultured neo-natal foreskin-derived fibroblasts	Cryopreservation	Burns, Diabetic foot ulcers
Integra® (Integra LifeSciences Corporation)	Silicone membrane	Bovine collagen I and shark chondroitin-6-sulphate	Isopropyl alcohol at 2-8$^{\mathrm{o}}$C	Burns, scar revision
Composite skin Equivalents				
Apligraf® (Novartis Pharmaceuticals/ Organogenesis)	Living neo-natal foreskin-derived keratino-cytes	Bovine collagen I with living cultured neo-natal foreskin-derived fibroblasts	Stored at 37$^{\mathrm{o}}$C	Burns, Venous ulcers, Diabetic ulcers, chronic ulcers
Orcel™ (Ortec International Inc.)	Living keratino-cytes cultured on bilayered collagen matrix	Bilayered matrix of bovine collagen I with living cultured fibroblasts	Stored at 37$^{\mathrm{o}}$C	Burns
Epidermal substitutes				
Epicel™ (Genzyme tissue repair corporation)	Viable cultured autologous keratino-cytes	None	Stored at 37$^{\mathrm{o}}$C	Burns, chronic venous ulcers, diabetic leg ulcers, skin graft donor sites, scar revision

In Vitro Skin Organotypic Model

With major advances in the field of keratinocyte culture, many aspects of epidermal cell growth have been elucidated. Conventional keratinocyte cultures grown on plastic substrates under submerged conditions have facilitated progress in the understanding of epidermal cell biology. However, these culture conditions are not optimal as the degree of stratification, differentiation and basement membrane formation is still poor compared to native skin *in vivo*. Thus the need to develop a suitable *in vitro skin model* would be of great benefit. Such a skin model would resemble native skin morphologically, displaying a fully differentiated epidermis, as well as biochemically and to some extent, functionally.

Freeman *et al*. (1976) first described a method of recombining epidermal and dermal tissue in an attempt to mimic native skin. A few years later, Prunieras *et al*. (1983) found that by culturing epidermal keratinocytes on collagen gels maintained on a rigid support or on nitrocellulose filters coated with collagen or with a basement membrane equivalent at an air-liquid interface, epidermal differentiation was stimulated. However, keratinocytes cultured on DED dermal substrate exhibited a far greater degree of differentiation. The workers also found that raising cultures to the air-liquid interface improves differentiation as the cultures are fed from underneath and are exposed to the oxygenated air creating a more physiologic condition, similar to that *in vivo*, for the epidermal cells. The resulting epidermis has been shown to express all the morphologic markers of epithelial differentiation (keratins, involucrin, lipids) except the constitutive expression of the hyperproliferative markers K6 and K16. They have also been shown to exhibit some form of permeability barrier function. These complex cultures termed 'organotypic' cultures have thus been used as *in vitro* skin models.

Various dermal substrates have been used in combination with cultured keratinocytes and all have shown varying degrees of consistency. To date, the most widely used systems that generate a similar epidermal morphology and differentiation to native skin have been achieved when keratinocytes are grown on DED, and this is now considered the organotypic gold standard model, or, on collagen lattices contracted with dermal fibroblasts. The organotypic gold standard model uses a combination of fibroblasts and keratinocytes grown on DED.

Organotypic cultures provide an *in vitro* skin model that can be subjected to histological examination after experimental manipulation and modulation under defined conditions e.g. analysis of specific cell types which can be incorporated into these models such as melanocytes, endothelial cells, Langerhans cells and appendageal structures (hair follicles). Moreover, results obtained from these models may help to assess or predict *in vivo* results. *In vitro* models are useful tools in which to study skin biology and physiology for example: (1) epidermal homeostasis including keratinocyte growth and differentiation, (2) epithelial/mesenchymal interactions including cell-cell and cell-matrix interactions (3) basement membrane production and their cellular origin, (4) epidermal barrier function, and (4) toxicity.

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