Show Some Skin

How we make the Human Skin Equivalent Model

Professor Zee Upton

z.upton@qut.edu.au
Phone 07 3138 6185
Skin is an Important Organ

- Largest organ in the body
- Skin fulfils a number of functions:
  - Protects against chemical, mechanical and infectious insults
  - Thermal regulation and fluid balance
  - Immune and sensory systems
  - Blood reserve
Skin is Composed of 3 Key Layers

- Stratified epithelium (epidermis) containing keratinocytes
- Underlying tissue stroma (dermis) containing fibroblasts
- Basement membrane separates the two
- Other key cells types include: melanocytes, microvascular endothelial cells, Langerhan’s cells etc
Zones of the Epidermis

- Stratum corneum
- Stratum lucidum
- Stratum granulosum
- Stratum spinosum
- Stratum basale
- Dermis
Injuries in Skin ➔ Wounds

- Wound healing is complex
- Requires interactions of different cell types and matrix components
- Different phases:
  - Clot formation
  - Inflammation
  - Granulation tissue formation
  - Re-epithelialisation
  - Matrix production
  - Remodelling
- Involves both regeneration of the epidermis and repair of the dermis
Wound Healing is Complicated by:

- Type of wound (acute or chronic)
- Extent and depth of lesion
- Other factors
  - Nutritional status
  - Age
  - Systemic disease
  - Medication
Classification of Wounds

Figure 1. Cross-section of epidermis, dermis, and underlying connective tissue.

Wounds are a Major Challenge for Health Systems Globally

- **Acute wounds**
  - Road accidents, burns, natural disasters, terrorist activities, wars
- **Chronic wounds**
  - Diabetic and venous ulcers, pressure sores
- Increasing incidence with age
- Represent an enormous challenge to our community – physical, personal and emotional
- Currently represents 1–2.5% of total health care spending in developed countries
- …… and we have higher expectations about our quality of life as we age
The Cost in Australia?

- 49% of inpatients in Australian hospitals suffered from a wound, with chronic wounds accounting for 20%;
- Wound care is the second most frequent procedure in general practice;
- Chronic wound care accounts for 22–50% of community nursing time;
- Chronic leg ulcers affect 0.6–3% of those aged over 60 years, increasing to over 5% of those aged over 80 years;
- Patients with chronic leg ulcers report an average ulcer duration of 12 months, 60–70% have recurrent ulcers, 24% are hospitalised with the ulcers and most suffer the condition for 15 or more years;
- More than 3,000 lower limb amputations are performed yearly in Australia as a last resort treatment for non-healing leg ulcers.
So what are we doing about this?

Not much……

• Not a very “sexy” topic
• Most wound care provided by allied health professionals
• Not many deaths directly attributable to wounds
Wounds are Difficult to Study

- Currently few good models are available for skin research
- 2D *in vitro* cell studies do not accurately represent the complex environment
- *In vivo* rodent models heal differently compared to humans
- Pig studies = current gold standard
A Better Approach?

- **Human Skin Equivalents (HSEs)** have recently been developed.
- Created by culturing skin cells and placing them back on to a dermal substitute.
- Unlike organoculture, the HSE system is a “living” model.
- Have been used for a variety of purposes including:
  - clinical skin grafts; modelling physiological processes in skin; phototoxicity; toxicity; absorption; drug transfer; irritancy; and, metabolic studies of topically applied products.
- Reduces animal testing.
Animal Studies

- 115 million animals used in scientific research globally in 2005
- Majority = rodents (83.5%)
- Rodents are not suitable for most wound, or even skin, research
- Even more animals used if take into account testing of “consumer” products
- European Union (EU) regulation (76/768/EEC, Feb. 2003) prohibits the use of animal or animal-derived substances for the development and testing of consumer, cosmetic and pharmaceutical ingredients.
- Will apply to all products imported into EU countries from 2009.
Our 3D Human Skin Equivalent Model

- We use the de-epidermised dermis (DED) HSE model often considered more physiologically relevant
  - Still retains the basement membrane on the de-cellularised dermis
  - Structural integrity of the DED is very similar to native skin
    - studies of the absorption of heat, light or chemicals through the epidermis and into the dermis experience the same physical obstacles/path as native skin
  - Have developed a system where it can be established in a defined media (ie. serum-free and no animal products)
Our 3D Human Skin Equivalent Model

- Can also culture fibroblasts and seed these into the dermal scaffold
- Skin off-cuts collected from consenting patients undergoing breast or abdomen reductions
Our 3D Human Skin Equivalent Model
HSE Retains Essential Features of Human Skin

Enlarged Image
HSE Retains Essential Features of Human Skin

Day 10  
DED Alone  DED + Cells = HSE

Keratin 6
Hyperproliferative

Keratin 14
Basal

Keratins 1/10/11
Differentiated
What we Plan to do Next

- Current drawback of the HSE model = lack of immune system regulation and a blood supply

- increasing the different cell types in this model = it will become even more relevant to the *in vivo* situation
  - Currently we incorporate keratinocytes and fibroblasts
  - Have started studies to include melanocytes, microvascular endothelial cells, Langerhan’s cells etc

- Use cells from edges of ulcers from amputated limbs
  - Have done this successfully with our collaborators at UWA using skin derived from amputated diabetic ulcer
Significance of the Model?

- This is the only HSE model available in Australia

- Due to transport logistics, quarantine issues and cost — Australians cannot purchase HSEs from overseas
  - EU Regulation comes into effect from 2009

- Alternative to animal testing
  - Therapeutics, cosmetics, household products
  - Lower cost
  - Ethical, legal & moral issues
  - High throughput
  - Pre-clinical optimisation

- The following talks will demonstrate its use in
  - studying epithelial cellular events
  - evaluating new dermal therapies