INTRODUCTION
The emphasis in managing burns and complex non-healing wounds has shifted from merely reducing morbidity and achieving satisfactory survival to ultimately improving the long-term prognosis, function and aethetics of healed wounds. Large skin defects resulting from burns, trauma, congenital giant nevi and disease can lead to skin necrosis and represent a challenging clinical problem that necessitates a novel approach to achieving skin coverage \[1,2\].

The primary problem in this type of wound is usually a shortage of autologous skin. For example, in a patient with a 70% total body surface area (TBSA) burn, the remaining 30% of normal skin is insufficient to provide coverage \[3\]. The secondary problem is scarring, which often results at both the recipient and donor sites from the ‘gold standard’ of using an autologous split thickness skin graft (STSG) (epidermis and part dermis). In certain instances, this can result in hypertrophic or keloid scars that can be further disabling and disfiguring \[See article on p7\] \[4,5\].

Although full-thickness skin grafting (FTSG) (all of dermis) leads to less scarring, it can only be performed if the injured area is approximately 1% of the TBSA. Thus, its widespread use is limited by donor site availability.

Theoretically, both of these issues could be reduced or even eliminated if it were possible to culture a skin substitute that encompassed both epidermis and dermis. In deep wounds, an optimal skin substitute would provide immediate replacement of both these layers with permanent wound coverage \[6\]. The features of an ‘ideal’ skin substitute are shown in \[Box 1\].

Unfortunately, there are currently no engineered skin substitutes that can completely simulate the complexity of human skin, either in form or function. However, with advances in tissue engineering and biotechnology, there are several skin substitutes, both being used and in development \[Fig 1\], that can be used for replacement or reconstruction of one or both layers of the skin.

**References**
THE DEVELOPMENT OF SKIN SUBSTITUTES

Xenografts in the form of frog skin were first used to provide wound coverage as early as 1,500 BC[6] and a product made from the skin of the bullfrog is still used in certain parts of the world such as Vietnam and South America[7]. Water lizard skin was also used in Western culture in the 1600s[8]. More recently, in the 20th century rabbit, dog and pig skin gained acceptance. As the understanding of immunology and principles of critical care management has increased, patients with extensive burns are now surviving well beyond the acute phase[10]. This has paved the way for the development of homografts in the form of cadaveric skin and autografts. Subsequently, evolving technologies led to the production of tissue-engineered skin substitutes (TESS). The first transplantation of cultured epidermal autograft (CEA) took place in the 1980s[11], followed by the transplantation of cultured composite skin substitutes in the 1990s[12]. Research and development in the field have since progressed to include synthetic materials and genetic modifications. The first genetically modified CEA for epidermolysis bullosa treatment was put to use in 2005[13].

FUNCTION

- Provides efficient protection against mechanical disturbances, infection and hazardous substances
- Acts as a sensory organ
- Helps regulate temperature
- Prevents excessive fluid loss and absorption
- Acts an immune organ to detect infections, etc
- Reduces harmful effects of UV radiation
- Helps in the production of vitamin D

Box 2 – The functions of the skin.

TYPES OF SKIN INJURY

The skin is the largest organ of the body and

Figure 1: Examples of skin substitutes currently being used.

Skin Substitutes
 Synthetic
 Biosynthetic
 Suprathel™

Biobrane® (Porcine)
 EZ-Derm™ (Porcine)
 Integra® (Bovine)
 Dermagraft® (Human)

Cultured Autograft
 Epigraft™
 Epiderm™
 Myskin™
 ReCell®
 Bioseed®
 CellSpray*
 StrataGraft™
 Cultured skin substitute

Allograft

Cultured
 Amnion

Cadaveric

Plant

Banana peel
Potato skin

Frog Skin
Lizard Skin

Bovine
Porcine

OASIS®
Wound Matrix
Permacol™

References

performs a number of vital functions (Box 2). The timely restoration of the protective and homeostatic functions of the skin is essential for successful clinical outcomes.

Burn injuries, depending on depth, can be divided into:
- **Superficial (also called epidermal)**
- **Superficial dermal (also called superficial partial thickness)**
- **Deep dermal (also called deep partial thickness)**
- **Full thickness.**

Although this classification is mainly used in burns, the principle nevertheless applies to any tissue defect, eg an abrasion or split skin graft donor site is equivalent to a superficial burn and a grade IV pressure ulcer is equivalent to a full thickness burn. Ulcers of varying aetiologies range from superficial to full thickness.

**HEALING**

Superficial (epidermal) injuries heal by re-epithelialisation from existing keratinocytes or keratinocyte stem cells and scarring in such injuries is minimal. If the injury extends to the superficial layer of the dermis it is possible that regeneration of the epidermis will occur without surgical intervention, provided there are a sufficient number of keratinocyte stem cells. If epidermal keratinocytes are lost, redevelopment may be performed by epithelial stem cells derived from hair follicles and/or sweat glands that are present in the deep layers of the skin (dermis).

However, if the injury extends to the deeper dermis (including the hypodermis, fat, muscle or bone), the injured surface is depleted of its keratinocytes, fibroblasts and any stem cells). Thus surgical excision of the involved tissue is frequently required along with reconstruction using STSGs, which contains all the epidermis and superficial parts of the dermis, thereby transferring self-renewing keratinocyte stem cells to the recipient area.

Deep dermal and full-thickness skin injuries usually require surgical excision and will inevitably result in scarring due to the action of myofibroblasts, even after skin grafting.

**SKIN SUBSTITUTES**

Skin substitutes are a heterogeneous group of products aimed at replacing, either temporarily or permanently, the form and function of lost skin. These products are alternatives to standard wound coverage in circumstances when established wound dressings are not appropriate.

From a practical point of view, skin substitutes are best classified as:
- **Synthetic, biosynthetic or biological**
- **Temporary or permanent**
- **Epidermal, dermal or composite.**

Synthetic skin substitutes are products of tissue engineering and consist of a microengineered biocompatible polymer matrix. If used in combination with cellular and/or extracellular elements such as collagen, they result in a biosynthetic product. Synthetic and biosynthetic constructs are made to be stable, biodegradable and aimed at providing an adequate environment for the regeneration of tissue. They are intended to maintain their three-dimensional structure for a minimum of three weeks to allow for a growth of blood vessels and fibroblasts and coverage by epithelial cells. Biodegradation takes place after this period. This process should preferably occur without extensive foreign body reaction as this will lead to increased scarring.

Biological skin substitutes (eg allografts or xenografts) are similar to the skin, with an intact and native extracellular matrix allowing for restoration of a more natural new dermis. They also support re-epithelialisation due to the presence of a basement membrane. However, natural constructs can exhibit problems with slow vascularisation. The most

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**Box 3 – Definition of different types of grafts.**

<table>
<thead>
<tr>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autograft: tissue grafted to a new position on the same individual</td>
<td></td>
</tr>
<tr>
<td>Allograft: graft of tissue transplanted between genetically non-identical individuals of the same species</td>
<td></td>
</tr>
<tr>
<td>Xenograft: graft of tissue harvested from one species to an unlike species (or genus or family)</td>
<td></td>
</tr>
</tbody>
</table>

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**References**


widely used biological substitutes worldwide are porcine skin, cadaveric skin and amnion.

Depending on their composition, both synthetic and biological skin substitutes can further be divided into dermal, epidermal or dermo-epidermal replacements. Sustainability is an additional factor that decides the temporary or permanent nature of skin substitutes. Temporary skin substitutes provide transient physiologic wound closure by protecting the wound from trauma, providing a barrier to bacteria and pathogens, and maintaining a moist wound environment until repair of the damaged tissue is complete[20]. Conversely, permanent skin substitutes are designed to provide permanent wound closure, replace the lost skin components (epidermis, dermis or both), and integrate with the recipient tissue [Box 3; Table 1].

TISSUE ENGINEERING OF THE SKIN

To culture skin in the laboratory, a skin biopsy from the patient or a donor is obtained to isolate the different cell types [Figure 2]. After trimming all excess fat, it is surface-sterilised in alcohol and placed in an appropriate culture medium. After approximately 24 hours, the epidermis is separated from the dermis and the two layers are then enzymatically treated to digest the bonds that bind the different cell types together (eg the extracellular matrix in the dermis is digested using collagenase to isolate the fibroblasts). Likewise, the epidermal keratinocytes are isolated from the epidermal layer.

Each cell type is allowed to proliferate in its appropriate culture medium. The cultured cell types are then used either in isolation (eg epidermal substitutes such as Epitel™ [Genzyme], Myskin™ [Altrika] and ReCell® [Avita Medical]) or in a collagen construct or scaffold (eg in dermal substitutes such as TransCyte® [Advanced BioHealing] and Dermagraft® [Advanced BioHealing]).

Certain dermo-epidermal substitutes can be constructed with keratinocytes, melanocytes and fibroblasts in a collagen hydrogel. Vascular endothelial cells can also be added into the hydrogel to help form capillaries[18-19]. Acellular dermal scaffold (eg Alloderm™ [LifeCell], Integra® [Integra LifeSciences Corporation], Biobrane® [Smith & Nephew], Matriderm® [EuroSurgical]) can also be engineered to provide coverage of a deep wound or burn. However, an autograft in the form of a STSG is needed for epithelial cover if a dermal (cellular or acellular) product is used in isolation (without epithelial layer or keratinocytes).

MONITORING

Although a reliable indicator or marker to monitor the effectiveness of an engineered skin substitute would be helpful, to date, they remain largely experimental. Cytokeratin 19 (CK19) is a protein that is expressed in basal keratinocytes and is a marker for epidermal homeostasis and an indicator of young keratinocytes or stem cells[20,21]. CK19 expression indicates a thriving and functional epidermis that signals a potentially successful transplantation. Other keratinocyte stem cell markers include integrin α6 chain (high expression) and the protein CD71 (low expression)[22,23]. The accuracy of these markers has to be corroborated with a reliable bioassay, in this case the formation of a normal-looking stratified epidermis approximately 12 weeks after transplantation. No epidermal marker is currently in clinical use — likewise there is no established dermal marker at present.

CONCERNS

Although TESSs have many uses and have established an indispensable role in the management of a variety of wounds — particularly burns — there are a number of concerns that remain to be addressed, which currently preclude their widespread use in routine clinical practice.

Lack of level 1 evidence

Randomised controlled trials are lacking for many TESSs, and the current evidence is based on non-randomised prospective trials, retrospective reviews, small case series (institutional or personal), and isolated case reports. No trials have evaluated the effectiveness of two comparable ‘like-to-like’ products (eg, dermal versus dermal skin substitute). Currently large, multicentre, double-blind randomised trials are underway for some products[24].

Poor integration and multiple procedures

In deep burns or wounds the lack of dermis and epidermis requires a product that can replace these two layers. Although

References

32. Swope VB, Supp AP, Cornelius JR, et al. Regulation of pigmentation in cultured skin substitutes by
## Product Content and description

### Epicel®
Genzyme Biosurgery, Cambridge, MA, USA
- Cultured epidermal autograft
- Severe deep dermal, full-thickness burns
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component

### EpiDex™
Euroderm GmbH, Leipzig, Germany
- Cultured epidermal autograft
- Treatment of chronic leg ulcers
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component Not suitable for deep burns or infected wounds

### Myskin™
Altrika Ltd, Sheffield, UK
- Cultured epidermal autograft
- Treatment of burns, ulcers and other non-healing wounds
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component Not suitable for deep burns or infected wounds

### ReCell®
Avita Medical, Cambridge, UK
- Autologous cell therapy device
- Large surface areas of skin trauma, such as burns, scalds, traumatic wounds, scars, hypopigmentation and vitiligo
- Not suitable for infected or necrotic wounds

### CellSpray®
Avita Medical, Cambridge, UK
- Cultured epithelial autograft suspension
- Can be used alone to treat superficial burns or in conjunction with a dermal product to treat deep dermal burns
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component

### BioSeed®-S
BioTissue Technologies AG, Freiburg, Germany
- Autologous keratinocyte-fibrin glue suspension
- Treatment of chronic leg ulcers
- 2-3 week lag period between biopsy and obtaining cells
- Lacks dermal component Not suitable for deep burns or infected wounds

### Lyphoderm™
Altrika, Sheffield, UK
- Lysate of cultured human keratinocytes
- Treatment of chronic leg ulcers
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component Not suitable for deep burns or infected wounds

### SUPRATHEL™
PolyMedics Innovations GmbH, Heerweg, Germany
- Absorbable, microporous membrane and alloplastic skin
- Treatment of deep dermal burns, abrasions and split-skin donor sites
- 2-3 week lag period between biopsy and obtaining epidermis
- Lacks dermal component Not suitable for deep burns or infected wounds

### AlloDerm®
Regenerative Tissue Matrix LifeCell, Branchburg, NJ, USA
- Processed human cadaver skin with acellular dermal matrix and intact basement membrane
- Hernia repair, abdominal wall reconstruction, breast reconstruction, ENT/head and neck plastic reconstruction, grafts
- May necessitate removal after 2-3 weeks
- Autograft is needed for epithelial cover
- Not suitable for infected wounds

### EZ-DERM™
Brennen Medical Inc, Saint Paul MN, USA
- Porcine derived xenograft in which the collagen has been chemically crosslinked with an aldehyde
- Partial-thickness wounds, donor sites, sandwich autografts and full-thickness wounds before skin grafting
- Not suitable for deep burns
- Not suitable for infected wounds

### References


<table>
<thead>
<tr>
<th><strong>Integra</strong>&lt;sup&gt;®&lt;/sup&gt;</th>
<th>Two-layered skin substitute comprising bovine collagen and an outer silicone layer</th>
<th>Immediate permanent coverage for surgically excised deep or full-thickness burns; as a reconstructive replacement in plastic surgery</th>
<th>Requires healthy and non-infected wound base Autograft is needed for epithelial cover</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biobrane</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Porcine dermal collagen bonded to semipermeable silicone membrane</td>
<td>To cover partial thickness burns and skin graft donor sites</td>
<td>Temporary Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>Matriderm</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Bovine dermal collagen and elastin</td>
<td>Burns and reconstruction, non-healing wounds that require skin grafts, such as diabetic foot ulcers</td>
<td>Autograft is needed for epithelial cover Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>Permacol™</strong></td>
<td>Processed dermal xenograft</td>
<td>Temporary coverage in partial-thickness burn, complex hernias and abdominal wall repair</td>
<td>Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>OASIS</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Processed dermal xenograft</td>
<td>Partial/full-thickness wounds, diabetic, venous, pressure and chronic vascular ulcers, trauma wounds (including burns), surgical and drainage wounds</td>
<td>Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>TransCyte</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Allogenic human fibroblasts cultured on nylon mesh, coated with porcine collagen and neonatal foreskin fibroblasts</td>
<td>To temporarily cover surgically excised full-thickness and deep partial-thickness burns before autograft placement</td>
<td>Autograft is needed for epithelial cover Temporary (may need skin grafting after 2-3 weeks) Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>Dermaplast</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Allogenic human fibroblasts cultured on bioabsorbable scaffold</td>
<td>Full-thickness diabetic foot ulcers</td>
<td>Not suitable for infected wounds or ulcers with sinus tracts</td>
</tr>
<tr>
<td><strong>ICX-SKN</strong></td>
<td>Cultured dermal allograft</td>
<td>To cover surgically excised partial thickness burns</td>
<td>In burns, autograft is needed for epithelial cover Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>Apligraf</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cultured allogenic skin containing neonatal keratinocytes and fibroblasts</td>
<td>Non-healing diabetic foot ulcers and venous leg ulcers</td>
<td>Not suitable for infected wounds or patients allergic to bovine collagen</td>
</tr>
<tr>
<td><strong>OrCel</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cultured allogenic skin containing neonatal keratinocytes, fibroblasts and bovine collagen</td>
<td>Treatment of acute and chronic deep dermal ulcers, partial-thickness burns and donor site wounds</td>
<td>Not suitable for infected wounds or patients allergic to bovine collagen</td>
</tr>
<tr>
<td><strong>Cultured Skin Substitute</strong></td>
<td>Cultured composite autograft</td>
<td>Permanent wound closure in large area burns and other congenital skin disorders</td>
<td>Not suitable for infected wounds</td>
</tr>
<tr>
<td><strong>StrataGraft</strong>&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cultured composite autograft (using NIKS&lt;sup&gt;®&lt;/sup&gt; cells)</td>
<td>Treatment of partial-thickness burns and severe skin wounds</td>
<td>2-3 week lag period between biopsy and obtaining epithemis Temporary coverage before autografting Not suitable for infected wounds</td>
</tr>
</tbody>
</table>

Table 1 — Examples of some commercially available skin substitutes.

References
certain products have been developed for this purpose, the epidermal component is not always successful due to inadequate in-growth of new blood vessels through the underlying dermis to supply the epidermis. Dermal substitutes thicker than 1mm revascularise poorly[25], resulting in inadequate nutrition to the overlying epidermis and consequent epidermal necrosis. Therefore, most dermal substitutes require a two-step approach to help dermal vascularisation and avoid epidermal necrosis — one operation to initially place the dermal substitute and a second to place an STSG as an autograft over this dermis. Research is currently underway to overcome this limitation[26].

Irregular pigmentation
In intact skin, pigmentation results from the proper distribution and function of melanocytes[27,28]. In cultures of epidermal keratinocytes, melanocytes can sometimes unintentionally persist resulting in hyperpigmentation after grafting[29,30]. By the same phenomenon, a lack of melanocytes can result in hypopigmentation. Although preclinical studies have shown that uniform pigmentation can be achieved[31], irregular pigmentation after usage of a tissue-engineered product with or without skin graft remains a clinical concern.

Graft contraction and lack of elasticity
Most skin substitutes contain only two cell types (fibroblasts and keratinocytes). Thus they lack the connective tissues (extracellular matrix and collagen) that are required for elasticity and pliability of the skin. In addition, the transplanted autograft can ‘contract’, resulting in functional and aesthetic compromise[32].

Transmission of diseases
Although donors’ medical histories are thoroughly evaluated and their blood screened for hepatitis, HIV and syphilis before any tissue is obtained for use in TESSs, products from human sources cannot be terminally sterilised owing to the presence of viable human cells.

References
Existing tests cannot provide absolute assurance that such products will not transmit unknown diseases. Similarly, tissue obtained from animal sources also carries the theoretical risk of transmitting infection, particularly prion-related diseases such as Creutzfeldt-Jakob disease.

**Further concerns**

Many TESSs contain bovine, porcine or human constituents and thus may have religious and ethical implications that need to be addressed (such as obtaining informed consent). Furthermore, the cost-effectiveness of their usage has not been well established[34].

**THE FUTURE**

Tangible concepts are beginning to emerge from the clinical evaluations carried out on TESSs. Biologically active and appropriate matrices and factors, in combination with automated tissue printing techniques[33] designed to produce a new generation of complex skin substitutes, are paving the way to a new concept known as ‘skiningineering’[35]. Other developments include:

- **The use of metabolically active fibroblasts**[34] — these are provided directly to the wound bed to stimulate the healing process.
- **The use of chimeric epithelium** — these comprise allogeneic keratinocytes together with 5% autologous keratinocytes[34,35] or autologous and xenogeneic keratinocytes[36] and can overcome initial problems with immunological rejection.
- **Addition of genetically modified cells** — cultured skin substitutes (CSS) contain keratinocytes that are genetically modified to overexpress vascular endothelial growth factor. This results in enhanced vascularisation of the graft (in animal models)[37,38].
- **Cutaneous gene therapy** — junctional epidermolysis bullosa, a genetic cutaneous disease, can result from the mutation of genes encoding subunits of the protein laminin 5, which helps to anchor filaments in the basement membrane of skin[39]. This disease phenotype can be corrected by gene transfer of LAMB3 using a retroviral gene transfer vector[40,41]. Similarly, genetically modified cultured skin grafts could potentially act as vehicles for cutaneous gene therapy in specific wound healing disorders.
- **Use of genetically modified grafts to treat systemic disorders** — genetically modified keratinocyte grafts have been used for systemic delivery of human growth hormone[42,43], and human factor IX[44-46] (to treat haemophilia B). Proteins secreted by keratinocytes have been identified in the serum after grafting[47,48].

**CONCLUSION**

The healing of burns and chronic wounds is a multi-step process that requires the intricate harmonisation of many different cell types, such as keratinocytes, fibroblasts, melanocytes and endothelial cells within the wound healing environment.

Given the complexity involved, it is unlikely that replacing or supplementing any one cell type or tissue will be successful in achieving satisfactory healing. Identifying specific patient factors and requirements, and developing a mechanism to supplement the lost tissue in its entirety, should be the goal of further research.

Importantly, recent advances and anticipated future developments should be complemented by adhering to the tenets of good basic wound care such as adequate debridement, skin care and infection control. Management of the patient rather than the ‘wound’ should be the primary goal.

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**References**


