CHAPTER 5

SKIN REPLACEMENT IN BURN WOUNDS

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Introduction

Historically, the main goal in burn management was increasing the survival of severely burned patients by rapid debridement and early closure of burn wounds, consequently reducing the infection risk. However, in the last decennia, surgical emphasis has shifted from survival to “quality of survival,” especially by improving the residual scars and preventing contractures. Traditionally, surgeons divide burns into deep burns requiring surgical therapy, and superficial burns which heal spontaneous by re-epithelialization with minimal scarring. Nevertheless, there is a grey zone between those two groups in which therapeutic decision making is difficult. The final decision for surgery generally remains case and surgeon dependent, and will mainly depend on the total burned surface area. Wound closure can be obtained by diverse therapeutic modalities depending on the depth and healing potential of the burn wound. In this article, the main focus is on the surgical treatment of deep dermal and full thickness burns. We endeavour to give a comprehensive overview of the developments in skin substitutes, which is impossible without mentioning some alternative treatments.

The current golden standard for deep burns is surgical debridement and closure with autologous split thickness skin grafts or “STG” (epidermis plus a thin layer of dermis). Nevertheless, donor areas are limited in extended burns, and the residual scars remain unsatisfactory due to the lack of dermis. A more aesthetical reconstruction can be obtained with full thickness skin grafts (epidermis and whole dermis), which are limited in dimension and can only be harvested in a few areas (groin, lower abdomen, etc.). Deep defects with exposed bone or neurovascular structures are currently treated with flap surgery, which gives an optimal aesthetical and functional result. Nevertheless, the severe donor-site morbidity, the technical difficulty, and sometimes severe complications limit its use mostly to secondary reconstructions. Consequently, alternative conservative and surgical treatments were developed to improve the healing and the quality of the residual scars. Several mechanisms are supposed to enhance healing:

(i) providing the ideal wound environment (wound dressings, etc.)
(ii) by assisting the intrinsic healing capacities (growth factors, cytokines, etc.)
(iii) by surgically replacing the damaged skin (“skin substitutes”), which also should reduce scarring in full thickness defects.

A permanent skin substitute is a surgically fixated “long lasting” skin replacement, consisting of naturally occurring skin elements which become incorporated in the normal skin. The main issue of this definition is the longevity of a skin substitute, which seems to be mostly of commercial importance, where terms such as biological dressings, and permanent and temporary skin substitutes are used without a clear distinction. This literature review showed that technically similar products are commercialized as “permanent” by one company and as biological dressing by another. Therefore, we chose to divide all these products in the following categories, depending on the skin layer which is (temporary) replaced: epidermal, dermal and combined skin substitutes (or composite grafts; Figure 1). In the future, a fourth
group might need to be added: the combined skin substitute with a subcutaneous adipose layer. However, the difference between skin replacements and some wound dressings can be small. Wound dressings are intended for coverage instead of replacement, to optimize wound healing. Wound dressings can roughly be divided in dressings containing natural elements (such as honey ointments), synthetic dressings (such as silver-impregnated dressings), and biological dressings containing mammalian cells or cell-derived substances like collagens and growth factors (human donor skin). Synthetically manufactured, naturally occurring elements, such as cellulose membranes, are also synthetic dressings. Wound dressings are not considered as (permanent) skin substitutes because they are not incorporated in the healing wound. Some authors previously named some of these products “skin substitutes” (without mentioning “permanent”) but this only lead to confusing terminology.

Figure 1. Classification of skin replacements.

The most important biological dressing, used since the 1940s, is human donor skin or “cadaver skin”. It contains several beneficial factors (growth factors, cytokines, etc.), and it provides the ideal environment for healing. Because of better preservation techniques (glycerol or cryopreserved), the risk of infection transmission is minimized, and its rejection will be delayed up to 3 weeks to 5 weeks. One of the currently most popular temporary dressings, is Biobrane, a nylon-collagen mesh, often used for partial thickness burns. The skin substitutes can consist of several elements, depending on the skin layers which need to be replaced (Figure 1). The epidermal substitutes consist mainly of keratinocytes, dermal substitutes of major extracellular elements (collagen, elastin,
adhesion glycoproteins, and/or hyaluronic acid\textsuperscript{11}, and sometimes also fibroblasts. Combined skin substitutes or composite grafts contain at least keratinocytes and a dermal matrix. Some authors prefer to use “bilayered skin substitutes” instead, but this terminology is confusing because some silicone-covered dermal substitutes are also labelled as “bilayered.” If skin substitutes contain allogenic cells (from neonatal foreskin, etc.), those will not be incorporated in the healing skin (because of the immunogenic rejection), but they do stimulate healing by secretion of cytokines and growth factors. When allogenic cells are combined with a dermal matrix which becomes incorporated, we consider them nevertheless as permanent skin substitutes. The aim of this study was to give an overview of which types of skin replacements have been developed and which problems still need to be faced. None of these commercialized products can currently claim to be the optimal skin replacement, because clinical evidence is too scarce (several large multicenter trials are currently in process). The number of products becoming commercialized is nevertheless increasing steadily, which pleads for a certain overview, classification, and clear comparison of the available products.

\textbf{Epidermal skin substitutes}

The currently most popular method for epidermal restoration is with STG, which was introduced clinically in 1823\textsuperscript{12}. Because of limited donor area in extended burns, other epidermal replacement techniques were examined. The first attempt to accelerate wound healing with living cells dates from 1870. Healthy skin (containing keratinocytes) was scraped off and applied to the wound bed. The best results were obtained when deeper parts of the skin were used\textsuperscript{12}. However, a good technique for culturing keratinocytes was only obtained in 1975\textsuperscript{13}. To obtain a large amount of autologous keratinocytes, a biopsy (25 cm\textsuperscript{2}) is taken from healthy skin and cultured during 2 weeks to 3 weeks on a nutritional layer\textsuperscript{14,15}. Specialized private or hospital-based laboratories developed several preparation and application techniques - gels, sheets, or sprays - such as Epicel, Laserskin (Vivoderm) (on a hyaluronic acid scaffold), Cellspray, Epidex (keratinocytes derived from hair roots), Bioseed-S, ReCell, and TranCell\textsuperscript{14-21}.

Allogenic keratinocytes (and fibroblasts) can be obtained from fresh human donor skin, neonatal foreskin, and surgical resections (such as abdominoplasties and breast reductions). “Chimerical” keratinocyte cultures, composed of allogenic and autologous cells, can be applied in ratios like 20:1 (less autologous cells needed)\textsuperscript{22,23}, but clinical studies are scarce. Histopathologic follow-up showed only autologous cells in the regenerated epidermis after 1 month to 2 months\textsuperscript{22,24}. There were already attempts to combine the keratinocytes with melanocytes and Langerhans cells to create a more complete epidermal substitute, but the clinical significance remains to be determined, but might be very useful in the treatment of vitiligo\textsuperscript{25}.

The main disadvantages of keratinocyte cultures are the variable take rate, the high susceptibility for infections, the long cultivation time, and the high costs\textsuperscript{26-28}. After
healing, the skin remains fragile with easy blistering for up to 6 months to 12 months, due to the lack of dermis and a dermoepidermic junction. A higher need for reconstructive surgery is also reported for the release of contractures. Therefore, the use is limited to severe burns with limited donor area, for the donor area (usually autologous cells) and chronic wounds (usually allogenic cells). They are often combined with widely meshed STG or human donor skin, and in the future probably with dermal substitutes.

Replacement of the dermal layer

For full thickness skin defects (also severe damage of the dermis), application of epidermal cells is insufficient. Full thickness skin grafts (epidermis + dermis) can be used for small full thickness defects and give better results than epidermal replacement techniques, but donor areas are even more limited (groins, behind the ear, and lower abdomen). The dermis consists mainly of connective tissue (collagen, elastin, hyaluronic acid, etc.) produced by fibroblasts, contributing to strength and elasticity of the skin. The lack of dermis results in severe contraction and hypertrophic scarring especially in regions around joints. Already in the early phases of wound healing, a number of fibroblasts will differentiate into myofibroblasts, but the keratinocytes themselves are also responsible for contraction, even in absence of fibroblasts. After application of a dermal layer, the epidermal layer can be reconstructed by adding a (meshed) STG or cultured keratinocytes.

Nowadays, several dermal substitutes are used clinically (Table 1,2). They can be divided in two large groups: acellular substitutes and cellular substitutes which include living cells (fibroblasts and endothelial cells; Figure 1). The 3D matrices should enable progressive vascularization and invasion of fibroblasts from the surrounding tissues. This should result in a mix of the foreign matrix and “native” material, histologically similar to normal skin. The fibroblasts will synthesize extracellular matrix components, cytokines, etc., which will eventually replace the skin substitute completely after several weeks, months, or even years, depending on the longevity of the material. The cellular skin substitutes are also metabolically active because they contain and synthesize cytokines, which improve healing.

Preventing rejection of human donor skin

The first attempts to reconstruct the dermis were by preventing rejection of human donor skin. Temporary treatment with immunosuppressive drugs like cyclosporine prevented the rejection even permanently, but this method was abandoned because of the side effects. Based on the knowledge that rejection was mainly caused by the more immunogenic potential of the epidermal cells, Cuono et al. proposed to remove the epidermis by abrasion, several days after grafting human donor skin. The dermis of the human
donor skin becomes incorporated into the recipient and provides a dermal bed on which cultured epidermal sheets can be placed\textsuperscript{16,17,45}. However, technical difficulties in consequently removing the epidermal layer and immunologic reactions limit the use.

A third method is the intermingled technique: small pieces of autograft are placed in the interstices of a widely meshed human donor skin. This provides immediate coverage with the human donor skin and allows the autograft epidermis to slowly replace the allograft as rejection proceeds\textsuperscript{46}.

**Development of the first dermal scaffolds**

In the meantime, the first dermal scaffolds were developed, which could be used as such or as scaffold for combined skin substitutes. The initial attempts established to make multi-layered keratinocyte cultures easier to handle, by adding collagen hydrated gels or lattices\textsuperscript{47-51}. Severe shrinkage during culturing restricted the use, which was partially prevented by anchoring methods \textit{in vitro}\textsuperscript{52}. An epidermal layer (STG or keratinocyte cultures) can be placed on top of the applied dermal matrix, immediately or after vascularization of the dermal matrix (1-3 weeks later)\textsuperscript{37}. This “two-step procedure” can be indicated if the donor sites are limited (time to heal before reharvesting) but by postponing full closure, the risk of infection can be increased. The one-step procedure is limited by the slow vascularization of the dermal substitutes, which disables oxygen and nutritional transport to the epidermal layer\textsuperscript{53}. The vascularization is very important to form a dermoepidermic junction, necessary for epidermal survival.

Initially, synthetic scaffold materials such as poly(L)-lactic acid and polyglycolic acid were examined\textsuperscript{54}. These products have predictable and reproducible mechanical and physical properties (tensile strength, pore size, etc.) and can be manufactured with great precision. However, synthetic materials tend to elicit a foreign material type of response, specifically, a fibrous connective tissue deposition leading to formation of dense scars and fibrosis\textsuperscript{54}. Therefore, naturally occurring materials such as hyaluronic acid and purified collagen have been investigated as alternatives to synthetic scaffolds. Collagens provide a unique combination of strength and flexibility, and they are the largest single component in the extracellular matrix and have a low antigenicity. Consequently, collagen is the most popular molecule for dermal scaffolds. The first stable collagen matrix was developed by Bell in 1979\textsuperscript{55} which lead to the development of Integra (cf. infra). Native collagen (from human, bovine, or porcine origin) seemed to be superior to synthetically reconstituted collagen, because native collagen degrades less rapidly\textsuperscript{41,56,57}, but allergic reactions may occur. Reconstituted collagen degrades within 7 days, whereas a native collagen matrix remained detectable almost 6 weeks. Cross-linking improves the survival of collagen and other molecules, leading to an increased tissue half-life and better tensile strength, but it increases rigidity and reduces cellular affinity\textsuperscript{54,58}.

Another important and very stable dermal element is elastin (half-life 70 years), which provides strength and elasticity to the extracellular matrix\textsuperscript{59,60}. Elastin is most
useful in skin replacements when organized in its naturally occurring network. When purified, the matrix can be severely damaged or elastin fibers can be separated, leading to accelerated degradation.

A smaller part of the extracellular matrix consists of hyaluronic acid, which has the significant advantage of structural conservation regardless of the source. Therefore, it does not cause allergic reactions when purified. It has been used therapeutically since 1968\textsuperscript{61,62} and is nowadays available as meshed sheet for wound healing and as viscous hyaluronic gel for instillation into cavities (joint pathologies and eye surgery)\textsuperscript{62}. Hyaluronic acid and collagen are also used widely in cosmetic surgery as dermal soft tissue fillers for softening of wrinkles and or volume restoration\textsuperscript{63}.

Currently available acellular dermal matrices

This group of dermal matrices contains all products mainly based on collagen or hyaluronic acid (\textbf{Table 1,2}). The collagen can be not only obtained from bovine or porcine dermis or tendons but also by processing human donor skin. Collagen can be extracted and remodelled as a 3D scaffold, or the original collagen skeleton can be conserved through a decellularization process. Previously, bovine collagen was more popular then porcine collagen, especially in wound treatment and skin reconstruction (probably due to a higher availability of cattle). Nevertheless, porcine collagen is regaining interest (also in cosmetic surgery), with as big advantage the absence of Prion diseases\textsuperscript{64}. Other porcine viruses also needed to be considered, and porcine collagen might elicit more foreign body reactions than bovine collagen\textsuperscript{65}. In addition, the literature on porcine dermal matrices remains scarce. Finally, religious and cultural differences need to be considered when using porcine and bovine tissues.

Bovine collagen

The main representative of this category, Integra, has been developed by Yannas and Burke\textsuperscript{39,40,66–68}. It is approved for use in burn injuries since 1997 and is currently the most commonly used skin substitute in burn care and reconstructive surgery\textsuperscript{69}. This acellular dermal substitute, also named “artificial dermis,” is made of a bovine collagen matrix (Achilles tendon) and glycosaminoglycans of shark cartilage, with a silicone layer on top to prevent dehydration and infection, although the use of antibiotics is advised\textsuperscript{39,40,66,67,70–72}. Because of the slow vascularization of Integra, the epidermal layer will be applied after 2 weeks to 3 weeks to obtain an optimal take. Recently, Integra Single Layer (without the silicone layer) became commercialized, which enables a one-step procedure\textsuperscript{73}. The vascularization could be accelerated by applying negative pressure therapy\textsuperscript{74}. This one-layer version can also be applied underneath the original Integra to treat full thickness defects. A similar matrix (without the silicone layer) is Duragen, also produced by Integra Life Sciences Inc. and used for neurosurgical interventions\textsuperscript{75}. Five weeks after Integra grafting, the implanted products are biodegraded and replaced by their endogenous analogues\textsuperscript{54}.
Pelna®[^58,72,76 - 84] and Terudermis®[^85- 87] are Japanese artificial dermal matrices based on the same principles, but consist only of “atelocollagen,” covered with a silicone sheet. Atelocollagen is a highly purified trypsin-treated collagen I derived from calf dermis. Suzuki et al.[^39-40,78] found no significant improvement when glycosaminoglycans was added, and therefore they commercialized Pelna® without, leading to lower manufacturing costs. The difference between both products is the cross-linking method. Terudermis® is thermally degraded and cross-linked, which may be favourable for cellular affinity[^87]. Pelna® is chemically cross linked, which would produce a more durable result (higher resistance against the collagenases produced by the fibroblasts)[^58,80].

A French dermal matrix is Renoskin®, which is composed of a reinforced silicone film and a porous matrix made from pure cross-linked bovine collagen[^88]. Another non-cross linked product in this category is Primatrix®, which is based on collagen from fetal bovine dermis. Fetal tissues have been shown to have exceptional regenerative capacity and have a reduced transmissible spongiform encephalopathy infectivity (Creutzfeldt-Jakob disease, etc.), which is also the fact for adult skin[^64]. The first scientific results remain to be published.

In animal studies, the addition of elastin to the collagen matrix resulted in a reduced cellular influx, a decreased number of myofibroblasts and more randomly orientated collagen bundles resembling normal skin[^57,89]. This matrix is commercialized as Matriderm®[^89] and can be used in a one-step procedure[^90-92]. It consists of collagen (bovine dermis) coated with elastin hydrolysate from the ligamentum nuchae. The first clinical results seem to be promising[^91,92]. In burn wounds, MatriDerm® seems to degrade sooner than in reconstructive wounds[^93]; and, after 3 months, results are comparable with the standard STG treatment, with no statistical evidence of long-term clinical effectiveness after 1 year[^90].

**Porcine collagen**

The information about biological materials derived from pigs remain scarce. Some *in vitro* and *in vivo* trials are published already[^94,95], but as far as we know, only Permacol is commercialized as dermal matrix for skin regeneration[^96-98]. Permacol can be applied as dermal substitute but can also be used as combined skin substitute (with cells), but clinical results remain to be published[^96]. In hand surgery, Permacol was studied as interposition graft after trapezoidectomy, but this study was discontinued because of severe tissue reactions[^65]. Some porcine products are currently marketed as biological wound dressings (Oasis, E-Z-Derm®), but they are thought to act as dermal matrices[^99]. E-Z-Derm®[^99] is composed of crosslinked porcine collagen, and Oasis is derived from porcine small intestinal submucosa, which seems to serve as a reservoir for cytokines and cell adhesion molecules, providing a scaffold for tissue growth[^100]. The structure and biochemical composition of small intestinal mucosa supports tissue-specific remodelling, and the first clinical results were promising for partial thickness chronic and acute wounds.
Human donor skin

Human acellular dermal matrices (ADM) are derived from human dermis (Table 1), treated to remove all immunogenic elements: keratinocytes (also present on sweat and sebaceous glands), fibroblasts, vascular endothelium, and smooth muscle. Virus screening is also obliged. However, several different methods for processing those matrices have been developed\textsuperscript{101-104}, all aiming to preserve the integrity of the remaining dermal elements as good as possible. The main elements of all ADM are the collagen and elastin fibers, which serve as a 3D natural matrix for the invasion of the native cellular elements \textit{in vivo}. The amount of remaining growth factors, cytokines, etc., depends on the processing method. The first ADM were processed by trypsin\textsuperscript{101,105,106}, freeze-thawing\textsuperscript{102,104,107-109}, or long incubations with enzymes\textsuperscript{103,110}. Most of those matrices remained highly antigenic, which lead to poor graft survival\textsuperscript{101-106,111,112}. At least five different manufacturing processes are currently registered for wound care. Some other techniques (like freeze- thawing) are still used for the processing of combined skin substitutes, but as far as we know, not commercialized as dermal substitute.

\textbf{Alloderm}\textsuperscript{®} is a freeze-dried cryopreserved acellular dermal matrix on an intact basement membrane complex obtained by processing human donor skin in a saline solution (sodium dodecyl sulfate) and enzymes\textsuperscript{37,53,112-117}. It is decellularized, freeze-dried, and biochemically stabilized, and has been successful alone and in combination with cultured autografts (two-steps procedure) in the treatment of burn wounds and dermal defects\textsuperscript{37}. Additionally, \textbf{Alloderm}\textsuperscript{®} is procured by cryopreservation which may affect the integrity of the elastin matrix, and its manufacturing is expensive.

\textbf{DermaMatrix}\textsuperscript{118,119} is human donor skin processed using a combination of detergent and acid washes and is then freeze dried. It is especially commercialized for reconstructive surgery, but clinical studies in wound care remain to be published.

\textbf{Glyaderm}\textsuperscript{®} is another acellular dermal collagen-elastin matrix, obtained by the treatment of glycerolized human donor skin with a low concentration of NaOH. The elastin matrix is not damaged by this manufacturing and preservation method, which should lead to a more durable effect\textsuperscript{120,121}. Additional advantages of glycerol preservation include inactivation of viruses and ease of storage and handling\textsuperscript{8,9}. \textbf{Glyaderm}\textsuperscript{®} is provided by a non-governmental, non-profit organization, the Euro Skin Bank (the Netherlands) and is intended to be cost-effective, enabling widespread application. \textbf{Glyaderm}\textsuperscript{®} is most effectively applied in a two-step procedure within a 6- to 8-day interval between Glyaderm\textsuperscript{®} application and thin split thickness skin graft engrafting. Initial clinical studies are promising, with randomized and multicenter trials underway.

\textbf{GraftJacket}\textsuperscript{®} is an acellular human dermis commercialized for deep, chronic diabetic foot ulcers\textsuperscript{122-124}. Because these wounds are deep and circulation around the wound is compromised, this product might also be of use for other types of wounds\textsuperscript{124}.

\textbf{SureDerm}\textsuperscript{®} is obtained by sequential treatments with dispase followed by Triton X-100\textsuperscript{125,126}. The enzymatic treatment with dispase removes the epidermal layer. It is freeze-dried and stored at temperatures of 2°C to 8°C. \textbf{SureDerm}\textsuperscript{®} can be applied
together with an STG (one-step), but there is a high risk of infection. Histologic examination showed that this product is completely absorbed within 4 months\textsuperscript{125,126}.

Table 1. Dermal matrices commercialized for acute full thickness wounds and reconstructive surgery.

<table>
<thead>
<tr>
<th>Product</th>
<th>Major Substances</th>
<th>Origin</th>
<th>Contraindications</th>
<th>Price (US $/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloderm (Life Cell Corp.)</td>
<td>Collagen (+elastin) matrix</td>
<td>Human donor skin</td>
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<td>10</td>
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<tr>
<td>DermaMatrix (Synthes)</td>
<td>Collagen (+elastin) matrix</td>
<td>Human donor skin</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>GlyaDerm (Euroskinbank\textsuperscript{1})</td>
<td>Collagen + elastin matrix</td>
<td>Human donor skin</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Integra (Integra LifeSciences Corp.)</td>
<td>Collagen + glycosaminoglycan matrix + silicone layer</td>
<td>Bovine articular cartilage or silicone</td>
<td>Known allergy to bovine collagen or silicone</td>
<td>8</td>
</tr>
<tr>
<td>Matriderm (SkinHealthcare)</td>
<td>Collagen matrix covered with elastin fibers</td>
<td>Bovine dermis and ligamentum nutritae</td>
<td>Known allergy to bovine collagen or elastin</td>
<td>6</td>
</tr>
<tr>
<td>Pelmac (Kowa Company)</td>
<td>Collagen matrix + silicon layer</td>
<td>Bovine dermis</td>
<td>Extremities of children and patients susceptible to keloid formation Known allergy to silicone</td>
<td>4</td>
</tr>
<tr>
<td>Permacol (Tissue Science Laboratories Plc.)</td>
<td>Collagen (+elastin) matrix</td>
<td>Porcine dermis</td>
<td>Known allergy to porcine collagen</td>
<td>14</td>
</tr>
<tr>
<td>Renoskin (Groupe Perouse Plastic)</td>
<td>Collagen matrix + silicone outer layer</td>
<td>Bovine</td>
<td>Known allergy to bovine collagen or silicone</td>
<td>8</td>
</tr>
<tr>
<td>SureDerm (Hans Biomed Corp.)</td>
<td>Collagen (+elastin) matrix</td>
<td>Human donor skin</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Terudermis (Terumo Corp.)</td>
<td>Collagen matrix (+silicone layer)</td>
<td>Bovine dermis</td>
<td>Known allergy to bovine collagen (and silicone)</td>
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</tbody>
</table>

* Price estimates were obtained by e-mail contact with the companies (December 2008) or publically available on the internet. Prices depend on the country and the size of the product. Not all products are available worldwide. NA, price not available.

\textsuperscript{1} Nonprofit organization.

**Hyaluronic acid**

A third group of dermal matrices consist of hyaluronic acid (Table 2), which is normally produced by fibroblasts\textsuperscript{73,127,128}. After purification, hyaluronic acid is identical in all species and phyla\textsuperscript{129}, and it seems to have a major impact on scar-free fetal wound healing\textsuperscript{11}. It can be obtained from Streptococcus fermentation or extracted from rooster combs. It is used in wound healing, ophthalmology, and joint surgery. Some of the frequently used dermal fillers are also based on hyaluronic acid\textsuperscript{63}. Hyaluronic acid can be esterified to obtain a stable cross-linked matrix which will not liquefy and will postpone degradation\textsuperscript{62}, permitting the application as dermal matrix. Hyaluronic acid is available as a scaffold for keratinocytes (cf. supra: Laserskin), an acellular dermal matrix (Hyalomatrix), and as a cellular dermal matrix (cf. infra Hyalograft-3D). Several variations with different degradation profiles (up to 4-5 weeks) are currently being investigated, even in combination with endothelial cells\textsuperscript{130,131}. Some of the degradation products modulate wound healing\textsuperscript{62} and are proangiogenic\textsuperscript{129,132}.

**Currently available cellular dermal substitutes - dermal equivalents**

The cellular dermal substitutes or “dermal equivalents,” (Table 2) are obtained by culturing fibroblasts on a collagen, hyaluronic acid, or synthetic scaffold. These fibroblasts will synthesize extracellular matrix components and growth factors. The currently available cellular dermal matrices mostly contain allogenic cells, improving healing by production of cytokines, etc. The same principles can also be used for
culturing autologous cells, but the long cultivating time limits the use to chronic wounds and severe burns. Although allogenic fibroblasts themselves do not induce immunogenic reactions in the host, they may accelerate second-set rejection\textsuperscript{133}. Allogenic fibroblasts can be obtained from neonatal foreskin, human donor skin, or surgical “leftovers” (after abdominoplasty, breast reductions, etc.)\textsuperscript{35,134-137}, but infection transmission should always be considered. Autologous cells can be obtained by a skin biopsy, but also of a liposuction aspirate, reducing donor-area morbidity\textsuperscript{35}, or eschar obtained through debridement of burn wounds\textsuperscript{35}. Nevertheless, those “alternative” fibroblasts showed more contraction \textit{in vitro}\textsuperscript{35,138}.

The biological temporary dressing Dermagraft-TC (“Transitional Covering”)\textsuperscript{137}, now named TransCyte, is a porcine collagen-coated nylon mesh with non-viable-cultured foreskin-derived dermal fibroblasts covered with silicone\textsuperscript{137,139,140}. A modification of Dermagraft-TC/Transcyte got the confusing name Dermagraft, which contains viable allogenic neonatal foreskin fibroblasts on a bioabsorbable polyglactin mesh that disappears after 3 to 4 weeks\textsuperscript{135,141,142}. New elastin was not detected after 1 year. Dermagraft showed to be effective in the treatment of chronic wounds like diabetic foot ulcers\textsuperscript{135,143}. The biggest disadvantage is that multiple applications might be necessary, and therefore, the classification as dermal substitute remains questionable.

Hyalograft 3D\textsuperscript{127,130} is based on esterified hyaluronic acid. Fibroblasts are cultured on this non-woven mesh creating a 3D cellular matrix \textit{in vitro}\textsuperscript{131,144}. Two-step interventions, where dermal hyaluronic acid matrices were covered with Laserskin (autologous keratinocyte cultures), already proved useful for chronic and acute full thickness skin defects\textsuperscript{131,145,146}. Fibroblasts were also cocultured with human umbilical vein endothelial cells, leading to the \textit{in vitro} development of capillary-like structures, improving integration\textsuperscript{130}.

<table>
<thead>
<tr>
<th>Product</th>
<th>Major Substances</th>
<th>Origin</th>
<th>Contraindications</th>
<th>Price* (US $/cm\textsuperscript{2})</th>
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<tbody>
<tr>
<td>Apligraf (Organogenesis)</td>
<td>Collagen + glycosaminoglycans + allogeneic fibroblasts + allogeneic keratinocytes</td>
<td>Bovine tendon + neonatal foreskin (cells)</td>
<td>Infected wounds, known allergy to bovine collagen, hypersensitivity to agarose shipping material</td>
<td>32</td>
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<tr>
<td>Dermagraft (Advanced BioHealing)</td>
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<td>Synthetic mesh + neonatal foreskin (cells)</td>
<td>Infected wounds</td>
<td>38</td>
</tr>
<tr>
<td>E-Z Derm (Brennen Medical)</td>
<td>Collagen matrix</td>
<td>Porcine dermis</td>
<td>Known allergy to porcine collagen</td>
<td>3</td>
</tr>
<tr>
<td>GraftJacket (Wright Medical technology Inc.)</td>
<td>Collagen (+ elastin) matrix</td>
<td>Human donor skin</td>
<td>NA</td>
<td></td>
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<td>Hyalograft (Fidia Advanced Biopolymers)</td>
<td>Hyaluronic acid + allogeneic fibroblasts</td>
<td>Streptococcus fermentation + neonatal foreskin (cells)</td>
<td>Hypersensitivity</td>
<td>NA</td>
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<td>Hyalomatrix (Fidia Advanced Biopolymers)</td>
<td>Hyaluronic acid + elastomeric outer layer</td>
<td>Streptococcus fermentation</td>
<td>Hypersensitivity</td>
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<tr>
<td>Oasis (Healthpoint)</td>
<td>Collagen matrix</td>
<td>Porcine small bowel submucosa</td>
<td>Known allergy to porcine collagen</td>
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<tr>
<td>OrCel (FortiCell)</td>
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<td>Primatrix (Tei Biosciences Inc.)</td>
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<td>Foetal bovine dermis</td>
<td>Known allergy to bovine collagen</td>
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</table>

* Price estimates were obtained by email contact with the companies (December 2008) or publically available on the internet. Prices depend on the country and the size of the product. Not all products are available worldwide. NA, price not available.

Table 2. Dermal or combined skin replacements commercialized for the treatment of chronic wounds and/or partial thickness acute wounds (Burns, donor sites, etc.), epidermolysis bullosa.

**Combined skin substitutes**
All combined skin substitutes or composite grafts are manufactured by culturing keratinocytes on a dermal layer, often containing living fibroblasts (Figure 1, Table 2). Several techniques can be used to obtain dermal matrices, but clinical studies remain rare (especially long-term follow-up)\textsuperscript{147}. The first commercialized combined skin substitutes, based on collagen scaffold, already date from the late 80s, and are based on the models of Bell and Boyce.

**The model of Bell**

This combined skin substitute was obtained by incorporating living fibroblasts in a collagen solution with serum, resulting in a resistant and impenetrable layer\textsuperscript{115,136,148-155}. The keratinocytes are cultured on top, forming an epidermal layer, without forming a real dermoepidermic junction. This method is applied in severe burns with a “take” ranging from zero to maximum 70\%\textsuperscript{148,151}. In vivo, an STG or keratinocyte culture needs to be grafted on top during a second operation, resulting in a better take-rate and a better esthetical result\textsuperscript{150}. This technique with living neonatal foreskin-derived keratinocytes and fibroblasts is commercialized as Apligraf (Graftskin)\textsuperscript{115,136,152-154}. It has demonstrated the ability to produce a number of cytokines and growth factors, and it acts very much like human skin\textsuperscript{155}. A more advanced product can be obtained by adding melanocytes, and a hypodermis composed of preadipocytes and adult adipocytes. It might even be combined with hair follicles. The biggest disadvantages are the limited viability, the high cost, and the need for extensive viral screening\textsuperscript{156}.

**The model of Boyce**

Another model is cultured on a matrix of bovine collagen and glutaraldehyde\textsuperscript{157-162}. The fibroblasts and keratinocytes are each cultured on one side of the sponge, forming a complete dermoepidermic junction in vivo\textsuperscript{157-159}. To assure nutrition of the epidermal cells, before vascularization through the dermis, the epidermal cells are exposed to the nutrients in the culture medium. This dermal equivalent with autologous material or “Cultured Skin Substitute” is used clinically since 1989. After the skin biopsy, the preparation takes 20 to 30 days\textsuperscript{161-164}. Clinical results were cosmetically satisfactory and similar to STGs. This model is also available as OrCel with living neonatal foreskin cells\textsuperscript{165,166}. OrCel serves as an absorbable biocompatible matrix that provides a favourable environment for host cell migration, containing several cytokines and growth factors. Resorption appears to take place gradually, with no remnants 2 weeks after treatment. There are limited clinical data available for this product, but large clinical trials are ongoing\textsuperscript{165}. 

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Currently investigated combined skin substitutes

Several dermal matrices, like acellular dermis, collagen matrices, human solidified plasma, and matrices produced by human umbilical vein endothelial cells were already used as template for these composite grafts in vitro and/or in vivo. Several of these composite grafts have been used to study the skin physiology. In particular, the dermoeidermic junction is necessary for the survival of the epidermal layer. The presence of fibroblasts increased the epidermal differentiation and resulted in increased graft take, less contraction, and enhanced vascularization. Skin also contains melanocytes, hair follicles, and sweat glands, which are very difficult to replace. Some research groups are testing composite grafts with melanocytes and even Langerhans cells clinically, but the results remain to be optimized.

Discussion and conclusion

Because of the advancements in tissue engineering, the treatment possibilities for skin defects evolved the last three decades from mainly preventing infection to the use of biologically active products and skin substitutes. These skin replacements can be distinguished from (biological) dressings because they become incorporated in the healing wound and consequently, do not need removal. However, despite of being commercialized as permanent skin replacements, most of these products are completely replaced by autologous tissue within a couple of weeks or months due to normal biological “renewal” processes. Even the most popular skin replacement Integra, is not detectable anymore 5 weeks after application. Yet, those products are often referred to as “permanent,” making it difficult to differentiate them with other “technically” identical products, currently commercialized as “temporary” skin substitutes or biological dressings. Nevertheless, these skin replacements will have a certain influence on the healing process and the quality of the remaining scars. They serve as a matrix for cellular invasion from the surrounding tissue, and some of them will also stimulate healing, similar to biological dressings. To create a more transparent classification of all those “permanent” surgically applied skin replacements, we propose the following categories: epidermal substitutes, dermal substitutes, and combined skin replacements. The dermal substitutes are sub-divided in several categories, depending not only on the main substance (and its origin) but also on the presence of living cells. A fourth group will consist of skin and soft tissue composite grafts (but these products are still in the experimental phase).

The advantage of cultured keratinocytes as epidermal replacement is that closure of the burn wounds is possible with autologous cells even in severe burns where the donor sites for STGs are limited. The main disadvantage is the absence of a dermal layer, which leads to blistering, hypertrophic scarring, and severe contractions when applied to deep wounds. The long cultivation time and the high costs also limit the
clinical use. Keratinocytes can also be used to stimulate healing of the donor sites of STGs, which facilitates early “recropping”.

The dermal substitutes have the main advantage of replacing the dermis, which will lead to a more aesthetical and functional outcome. Nevertheless, the epidermal layer also needs replacement, by thin STGs or keratinocyte cultures. This layer can be restored during the same operation, or after ingrowth of the dermal matrix, which usually takes 1 week to 3 weeks. This two-step procedure is necessary for several matrices, because of the slow vascularization. Highly porous scaffolds with a very diffuse matrix may be more rapidly penetrated by budding neocapillaries than more densely formed scaffolds. However, the turnover of the porous scaffolds may be so high their role as dermal substitutes is questionable. Further research is needed to differentiate between the currently available dermal matrices, and strategies need to be developed to accelerate the invasion of the matrix by fibroblasts and vascular structures from the surrounding tissue. The main goal is to obtain a more optimal healing process (less infections, better “take”) and a further reduction of the scarring and contour deformities.

The combined skin substitutes are a combination of the two previous groups and should be able to restore a full thickness defect in a one-step procedure. Nowadays, those products are mostly used in chronic wounds (with allogenic cells) but often need repeated applications. Because of the living cells and often complicated manufacturing processes, those products remain very expensive (up to 35 US $ per square centimeter).

In full thickness skin and soft tissue defects, restoration of the dermis and epidermis will often be insufficient, because of the remaining depression compared with the surrounding tissue. Currently, two options are available: reconstruction by flap surgery (primary or secondary) or secondary soft tissue augmentation underneath the primary healed skin (dermal fillers, autologous fat transplantation, or prosthesis). Especially, the autologous fat transplantation (“lipofilling”) is gaining importance because subcutaneous fat is present in sufficient amounts in the majority of people, and it is easily accessible182. In the future it might be possible to combine adipocytes with the skin substitutes to close the deep defects in one operation7. But accelerated vascularization becomes even more important163,183,184. Promising allogenic cells are human umbilical vein endothelial cells185,186 and human dermal microvascular endothelial cells183,184. Stem cells (obtained from the bone marrow, subcutaneous fat, etc.) may gain interest to create this vascularized skin-fat-matrix, because of their ability to divide and renew themselves over long periods of time, to differentiate into various cell types, and their relatively easy isolation and expansion187-194. The use of stem cells for acute wounds (burns, etc.) will probably remain limited because of the time needed for cultivation. Ready-to use, off-the-shelve products will probably remain more useful for certain indications.

Other futuristic developments include genetic modifications of transplanted cells to improve wound healing transiently and to deliver gene products systemically160,163,184,195-197. Genetic modification of cells within skin substitutes can hypothetically be used to overcome limitations in anatomy and physiology, resulting
in skin substitutes with greater homology to native human skin and improved performance (improving vascularization, etc.).

In conclusion, 200 years after its discovery, the STG technique remains the preferred method for burn coverage for most surgeons. The currently available skin substitutes and biological dressings are very expensive and their clinical efficacy remains a topic of controversy and continued research. To our knowledge, there are no large controlled, randomized studies attesting the clinical efficacy of any of the currently available dermal substitutes. However, evidence is increasing that wound bed preparation and the use of dermal substitutes contribute to a more optimal wound healing with improved quality of scars, reduced rate of contraction and ultimately, a better quality of life. Researchers continue their quest for the ideal skin substitute, and in the future it should be possible to create such an advanced skin substitute, containing melanocytes, hair follicles, and sebaceous glands. The available products remain rather expensive, because of commercial incentives, high manufacturing, shipment, and storage costs. Nevertheless, accelerated healing and closure of the wound will reduce the labour-intensive dressing changes, hospital stay, and the need for reconstructive surgery. Until the optimal off-the-shelf skin substitute becomes available, the burn surgeon can improve aesthetic and functional outcome by choosing from the gamut of currently available scaffolds for bilayered skin restoration. This classification has intended to facilitate clinical and cost-economic decision making.


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