# Dermal Matrices and Bioengineered Skin Substitutes: A Critical Review of Current Options

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**Background:** Over recent decades, scientists and surgeons have collaborated to develop various bioengineered and synthetic products as an alternative to skin grafts. Despite the numerous articles and reviews written about dermal skin substitutes, there is no general consensus.
**Methods:** This article reviews dermal skin scaffolds used in clinical applications and experimental settings. For scaffold evaluation, we focused on clinical and/or histological results, and conclusions are listed. Explanations for general trends were sought based on existing knowledge about tissue engineering principles and wound healing mechanisms.
**Results:** Decellularized dermis seems to remain the best option with no other acellular scaffold being clinically proven to gain better results yet. In general, chemically cross-linked products were seen to be less effective in skin tissue engineering. Biocompatibility could be enhanced by preseeding substitutes with fibroblasts to allow some natural scaffold remodeling before product application.
**Conclusions:** Skin substitutes are a useful tool in plastic and reconstructive surgery practices as an alternative to skin grafts. In the choice of substitute, the general plastic surgery principle of replacing like tissue with like tissue seems to be still standing, and products most resembling the natural dermal extracellular matrix should be preferred. *(Plast Reconstr Surg Glob Open 2015;3:e284; doi: 10.1097/GOX.0000000000000219; Published online 6 January 2015.)*

For many centuries, skin grafts have been used to restore wound defects after trauma, vascular disease, or cancer. However, availability of sufficient healthy skin can be an issue, as well as the additional health risks associated with the procedure. The deforming donor-site morbidity should also be considered when opting for skin grafting.

Disadvantages as such have led to innovations in skin tissue engineering. Over recent decades, scientists and surgeons have collaborated to develop various bioengineered and synthetic alternatives to promote healing in superficial and deep skin wounds. Tissue-engineered skin scaffolds are 3-dimensional structures that are positioned within the defect and provide immediate protection against dehydration, microorganisms, and toxins.[1] The scaffold then gradually becomes incorporated in the wound bed, a process aided by natural wound healing mechanisms such as local inflammation, cell infiltration (neutrophils, macrophages, and fibroblasts), and neovascularization of the scaffold. More recent advances in skin substitutes involve prepopulation of scaffolds with living cells of autologous or allogeneic origin, usually keratinocytes or fibroblasts.

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The choice of an appropriate scaffold is important to guide cell behavior, and cytotoxic products or materials that induce extensive scar formation should be avoided. Scaffolds often have unique physical characteristics due to differences in manufacturing techniques such as decellularization, sterilization, freeze drying, and cross-linking protocols.[2] To resist in vivo forces like wound contraction, scaffold materials are, for example, often freeze dried and/or chemically cross-linked to enhance strength. However, it has been demonstrated that chemical cross-linking can alter clinical results. Non-cross-linked materials exhibit greater cellular infiltration, extracellular matrix deposition, and neovascularization compared with their chemically cross-linked alternatives.[2] They also become less encapsulated[3] and thus more incorporated. Cross-linking a product is a means to enhance strength, but can seriously affect clinical properties.

Despite the numerous articles and reviews written about dermal skin substitutes, there is no general consensus. This article reviews dermal skin scaffolds used in clinical applications and experimental settings. For scaffold evaluation, we focused on clinical and/or histological results, and conclusions are listed. Explanations for general trends were sought based on existing knowledge about tissue engineering principles and wound healing mechanisms.

## REVIEW

Acellular dermal allografts originate from deepithelialized cadaveric skin. The skin is treated to remove cellular, infectious, and antigenic materials.[4] The resultant product is usually freeze dried, allowing it to store easily for many months without refrigeration, and manufacturing generally does not involve chemical cross-linking.[2] AlloDerm (LifeCell, Branchburg, N.J.) has been widely used in several applications for many years. There is an injectable form of AlloDerm marketed as Cymetra (LifeCell),[5] basically a micronized form. AlloDerm is used as a dermal substitute in deep partial- and full-thickness burn wounds, facilitating subsequent autologous split-thickness skin graft take.[6] Successful simultaneous grafting on AlloDerm has, however, also been described.[7] Besides a dermis, AlloDerm has also been successfully used for other sorts of soft-tissue replacement,[8] implantable prosthesis coverage,[9] pelvic and abdominal wall defect repair,[10,11] lip augmentation,[12] laryngoplasty,[13] and vaginal prolapse repair.[14,15] Synthes' alternative is called DermaMatrix (Synthes, West Chester, Pa.). In a comparative study in an in vivo murine model, DermaMatrix was shown to maintain its original shape and consistency, whereas AlloDerm samples become softer and possess a poorly defined character 3 months after subdermal implantation.[16] Furthermore, at 12 months only moderately thickened fibrous implant capsules were seen with DermaMatrix, whereas AlloDerm samples displayed denser connective tissue capsules with apparent chronic inflammation.[17] This experimental study slightly favored DermaMatrix; however, true clinical wound healing studies are lacking.

Animal-derived acellular alternatives populate the market as well. Acellular dermal xenografts are often chemically cross-linked, theoretically making them less suitable for wound healing. Products in this group are Permacol (Tissue Science Laboratories, Hampshire, UK), a porcine-derived acellular dermal matrix, and EZ-Derm (Mölnlycke Health Care AB, Gothenburg, Sweden), a collagen matrix derived from porcine dermis. The use of Permacol as a dermal substitute for wound healing purposes has indeed largely been abandoned, and clinical results of EZ-Derm in wound healing are not convincing (Table 1).[15,18–29]

Maybe more suitable for certain forms of wound healing are the, usually not chemically cross-linked,[30] porcine small-intestine submucosa derivatives. Oasis Wound Matrix (Healthpoint, Fort Worth, Tex.) consists mainly of collagen, glycosaminoglycans (GAGs) (hyaluronic acid and proteoglycans), fibronectin, and growth factors such as fibroblast growth factor-2 and tumor growth factor-β. Advantages are immediate availability, storage at room temperature, and a long shelf life of 2 years.[31] Its main indication is for ulcer treatment. A randomized controlled study in 120 patients with chronic venous leg ulcers showed significantly more wounds (55% vs 34%) healed when OASIS Wound Matrix was combined with compression therapy.[32] In patients with mixed arterial and venous ulcers, complete wound closure was achieved in 82% of OASIS-treated ulcers compared with 46% of ulcers treated with Hyaloskin (Apeldoorn, The Netherlands), a pure hyaluronic acid dermal matrix. Pain reduction and patient comfort were also better.[33] Good results were seen in the treatment of diabetic ulcers as well, where 49% of wounds healed after 12 weeks compared with only 29% treated with Regranex (Johnson & Johnson Wound Management, Somerville, N.J.), a platelet-derived gel.[34]

Human amniotic membrane is derived from human placenta. An example of a commercially available product is Neox (Amniox Medical, Marietta, Ga.), which contains predominantly collagen and fibronectin, resembling dermal skin and is not chemically cross-linked. It is preferred for application on thermal injuries,[35] where it prevents heat and water loss from the wound surface and acts as a bar-

**Table 1. Porcine-derived Acellular Dermis**

| Permacol | EZ-Derm |
|---|---|
| A porcine-derived acellular dermal matrix<br>It is also available as a filler[18] | A collagen matrix derived from porcine dermis<br>It is silver impregnated to improve antibacterial properties[26] and is also cross-linked[27]<br>Unmeshed or premeshed sheets are available[28]<br>EZ-Derm is ready to use and has a long shelf life<br>An increased amount of exudate has been seen when EZ-Derm is applied to the wound[15] |
| The use of Permacol as a dermal substitute for wound healing purposes has largely been abandoned<br>A murine study in full-thickness wounds shows no benefit of using Permacol to support an overlying split-thickness skin graft compared to a graft alone[19]<br>Permacol has, however, been successfully used in hernia repair,[20] endovaginal fistula repair,[21] pelvic floor reconstruction,[22] urodynamic stress incontinence,[18] rotator cuff repair,[23] facial contour augmentation,[24] and rhinoplasty[25] | It is reportedly used in burn treatment[27]; however, to date, no study has shown objective significant benefit of EZ-Derm over standard options in the treatment of split-thickness wounds<br>In a prospective, randomized trial of 32 patients with partial-thickness burns, EZ-Derm was compared to the cheaper Jelonet (Smith & Nephew Healthcare, London, UK). In terms of bacterial colonization rate, need for surgical treatment, time for spontaneous healing, analgesic requirements, and frequency of dressing changes, E-Z Derm was not shown to be better than Jelonet[26]<br>In a prospective trial in split-thickness skin donor-site wounds, EZ-Derm was found inferior to calcium sodium alginate based on healing time, hypertrophic scarring, and patient satisfaction criteria[29] |

rier against bacteria. Furthermore, it reduces pain, and its transparency allows better wound control.[36] Human amniotic membrane dressings are best changed every 2 days.[4]

Manufactured acellular dermal substitutes are produced from natural polymers, synthetic polymers, or a combination of both. Natural polymers are naturally occurring materials, such as collagen, elastin, GAG, fibronectin, chitosan, fibrin, silk, and alginates.[37–39] The extracellular matrix of human dermis consists of many of these polymers, the main constituents being collagen, elastin, and GAGs such as hyaluronic acid (Table 2).[31,40–50]

The advantages of natural polymers are their low toxicity and low inflammatory response.[37] Natural polymers, however, usually have poor biostability and low mechanical strength, facilitating wound contraction.[37,51] To improve biostability and extend durability of the graft, natural polymers are often chemically cross-linked or are cross-linked to other natural polymers, such as GAGs, fibronectin, and chitosan, or to synthetic polymers.[37,51] Cross-linked natural polymers are successfully being used for purposes such as tendon replacement or hernia repair or as fillers, where material durability is much more important than cell infiltration. For wound healing purposes, they are often less suitable because of associated cell cytotoxicity.[2,52]

Examples of absorbable synthetic polymers are polycaprolactone, polylactic acid, polyglycolic acid, polylactic-*co*-glycolic acid (PLGA), poly(ethylene glycol)/poly(butylene terephthalate), and polyethyleneglycol. Examples of nonabsorbable synthetic polymers are polyurethane, nylon, polytetrafluoroethylene (PTFE), and polyethylene terephthalate.[37,38,53] Synthetic polymers have uniformity between samples and are cheaper to produce. They can be fabricated to serve desired physical properties and may be favorable when higher mechanical properties are required. Disadvantages are their limited cellular recognition and tissue compatibility in vivo.[38] Many of the synthetic polymers are used in suture materials such as nylon (Dermalon, Davis and Geck, Gosport, UK; Ethilon, Ethicon, Edinburgh, UK) PLGA (Vycril, Ethicon), polyglycolic acid (Dexon, Davis and Geck), and polycaprolactone (Monocryl, Ethicon).[54,55] Synthetic polymers are often used in wound dressings such as polyurethane in Tegaderm (3M Healthcare, St Paul, Minn.) and Opsite (Smith and Nephew Healthcare, London, UK).[38] As said previously, natural and synthetic polymers are sometimes combined, balancing out advantages and disadvantages of the used polymers.

Some manufactured products have a removable semipermeable silicone layer on top acting as a temporary epidermis preventing moisture loss and infection. They can be grouped as acellular bilayered substitutes.

Biobrane (UDL Laboratories, Rockford, Ill.) consists of a fine nylon mesh cross-linked with porcine dermal collagen (Table 3).[4,56–62] Because of the presence of nylon, Biobrane will never get incorporated and should be considered as a wound dressing rather than as a skin substitute. To date, clinical studies only show Biobrane to be superior to silver sulfadiazine.[59,60] With the use of silver sulfadiazine as a dressing in burn treatment being discouraged according to a recent Cochrane review appointing it an inferior treatment,[63] there is not much clinical evidence to opt for Biobrane at this point. Furthermore, 2

**Table 2. Natural Polymers**

| | |
|---|---|
| Collagen | Collagen is an extracellular matrix protein with excellent biocompatibility and safety due to its biological characteristics |
| | It provides cell adhesive properties and can improve tensile strength |
| | It is widely used in wound dressings and scaffold materials[41] |
| | In normal wound healing, collagen deposition by fibroblasts is one of the key factors in reconstituting a matrix. Final scar quality is largely determined by the nature of this deposition[42] |
| Elastin | Elastin, another extracellular matrix constituent, mainly provides elasticity to tissues[43] |
| | It is less often used than collagen[40] |
| GAGs | GAGs are polysaccharides covalently linked to protein |
| | Examples are hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, proteoglycan, and keratan sulfate[44] |
| | They are important for hydration of the extracellular matrix and bind effector molecules such as growth factors and cytokines.[40] Some GAGs have additional properties that contribute to wound healing |
| | The application of exogenous hyaluronic acid by itself is seen to enhance keratinocyte proliferation, both in vitro and in vivo |
| | Furthermore, it has an important role in reducing scarring |
| Hyaluronic acid | Raised hyaluronic acid levels stimulate fibroblast proliferation but reduce collagen deposition by adult fibroblasts. In regard to scarring, collagen deposition is more ordered and fibroblast contraction is reduced.[42] The lack of scarring and fibrosis seen in regenerating fetal skin wounds may be related to a prolonged presence of hyaluronic acid, possibly due to a lack of hyaluronidase,[45] while application of hyaluronidase in fetal wounds is seen to increase scarring |
| | A non-cross-linked linear scaffold of hyaluronic acid is marketed as Hyaff (Fidia Advanced Biopolymers, Abano Terme, Italy)[47] and has been used in diabetic ulcer treatment |
| Elastin | Elastin, another extracellular matrix constituent, mainly provides elasticity to tissues[43] |
| | It is less often used than collagen[40] |
| Fibronectin | Fibronectin is seen to play an important role in epidermal cell migration and differentiation and in other stages of wound healing such as platelet aggregation and collagen matrix assembly[49] |
| Chitosan | Chitosan is a polymer that stimulates wound healing by enhancing hemostasis and promoting collagen synthesis by fibroblasts[50] |
| | It also enhances growth-factor stability in grafts |

separate studies show no benefit of Biobrane when compared with the 4 times cheaper DuoDerm (ConvaTec, Bristol-Myers Squibb, New York City, N.Y.).[61,62]

Integra dermal regeneration template (Integra LifeSciences, Plainsboro, N.J.) was developed by Burke et al.[64] It consists of a silicone layer on top of a porous matrix comprising a chemically cross-linked coprecipitate of bovine collagen and shark-derived chondroitin-6-sulfate, a GAG (Table 3).[4,15,65–69] When compared with AlloDerm in a mouse wound model, the Integra matrix is seen to induce more foreign body reaction and giant cells, however, again not surprisingly, given the fact that it is a chemically cross-linked material. To allow collagen fibers and others to be deposited within Integra, the scaffold needs to be cleared first by macrophages. This is in contrast with human skin derivatives such as AlloDerm, where there is a much lesser need to clear the area in advance.[70] Given the results of this study, and lacking clinical studies comparing AlloDerm and Integra, Integra is not yet convincing as an option to be preferred above non-cross-linked human skin derivatives such as AlloDerm and DermaMatrix.

More recent advances in skin substitutes involve prepopulation of scaffolds with living fibroblasts. During several weeks, the cells synthesize components of the extracellular matrix within the scaffold. Afterward, the scaffold is usually cryopreserved to allow fridge storage.[4,71] Unfortunately, often a high cost is associated with these types of scaffolds. TransCyte, for example, essentially a Biobrane scaffold that is preseeded with fibroblasts, is seen to be 15 times more expensive than the usual Biobrane. An overview of products is given in Table 4.[4,71–82]

In TransCyte (Advanced BioHealing, La Jolla, Calif.), formerly known as Dermagraft-TC, a scaffold similar to Biobrane (silicon-coated nylon-collagen scaffold) is first populated with neonatal fibroblasts. Over a period of 17 days, the fibroblasts are allowed to proliferate and synthesize growth factors and extracellular matrix components.[4] The addition of a matrix synthesizing fibroblast population seems to be having clinical benefits for local wound bed preparation because partial-thickness burn wounds were seen to gain faster reepithelialization when compared to Biobrane.[59] Furthermore, a multicenter randomized clinical study showed it to be equivalent or even superior to frozen human cadaver allograft for the temporary closure of excised burn wounds, with regard to

**Table 3. Acellular Bilayered Substitutes**

| Biobrane | Integra |
|---|---|
| A fine nylon mesh cross-linked with porcine dermal collagen. Use of the natural polymer collagen enhances the poor bonding of the nonabsorbable nylon to the wound surface[65] | A silicone layer on top of a porous matrix comprising a chemically cross-linked coprecipitate of bovine collagen and shark-derived chondroitin-6-sulfate |
| When the skin has regenerated, Biobrane separates from the wound and can easily be removed[4] | Integra without silicone is also available and marketed as the Integra Matrix Wound Dressing[4] |
| Biobrane is an effective wound covering for clean, superficial partial-thickness burns of limited extent.[65,66] It is also used for donor sites[67] and for temporary coverage of freshly excised deep partial- or full-thickness wounds.[4] Biobrane does not adhere to very deep or contaminated wounds and it shears above joints that are not splinted. Biobrane can also be meshed before application[68] | The Integra pore size of 20–125 μm allows influx of cells |
| A study in children with partial-thickness burns showed that wounds treated with Biobrane reepithelialized slightly faster than when treated with silver sulfadiazine (9.5 days vs 11.2 days). Also the first group required significantly fewer skin grafts later on[69] | The dermal template usually becomes revascularized within 21 days after grafting; at this point, the Silastic sheet can be removed and a split-thickness skin graft can be applied.[75] This time interval often determines the hospital stay. In some circumstances, revascularization is obtained faster, allowing earlier grafting, such as in facial wounds (7–10 days)[76] |
| Another pediatric study confirmed these results, concluding Biobrane was superior to silver sulfadiazine in regard to pain, pain medication requirements, wound healing time, and length of hospital stay.[70] However, treating small intermediate-thickness burns in children with Biobrane showed no difference in pain or time to healing when compared with the cheaper hydrocolloid dressing DuoDerm (ConvaTec, Bristol-Myers Squibb, New York City, N.Y.)[71] | A study in acute and chronic wounds demonstrated that faster revascularization can be reached, by using fibrin glue to anchor Integra to the wound bed and applying negative pressure on the matrix. The take rate in this group was also higher compared with standard Integra application (98% vs 78%)[75] |
| In another clinical trial for the treatment of split-thickness donor sites, Biobrane was even found inferior to DuoDerm in terms of healing time and patient comfort[72] | There is a higher likelihood of seroma formation, but Integra can be meshed allowing wound fluid to drain[76] |
| | A big disadvantage is the high cost[15] |
| | There is a lot of experience with Integra. Integra gained FDA approval for early treatment of patients suffering from severe burns when autografting of the wound is impossible. Integra artificial skin is an effective means of treatment for full-thickness burns.[77] The clinical outcome seems to be superior in terms of final function and cosmesis, with application resulting in softer, more pliable, and hypopigmentated skin.[78] It is also seen to improve graft take of cultured keratinocytes,[79] with the practicality that by the time Integra is vascularized and incorporated, the keratinocytes are ready for application |

split-thickness graft take at postautograft day 14. It was also easier to remove and resulted in less bleeding.[73] Again, due to the presence of nylon, TransCyte cannot be considered as a true skin substitute.

Skin substitutes in this category are Dermagraft (Advanced BioHealing), an absorbable PLGA scaffold seeded with neonatal fibroblasts,[71,74] mostly used to stimulate healing of chronic lesions such as diabetic ulcers,[4] ICX-SKN (Intercytex, Manchester, UK) a fibrin matrix seeded with neonatal human fibroblasts,[79,80] and Hyalograft-3D (Fidia Advanced Biopolymers, Abano Terme, Italy) comprising esterified hyaluronic acid fibers seeded with autologous fibroblasts and covered by a silicone membrane.[81] Proven benefit of Dermagraft use is reported for ulcer treatment,[76–78] whereas ICX-SKN and Hyalograft-3D seem promising as well but lack human randomized controlled trials to date (Table 4).

Attempts to combine a cellular epidermal layer with a cell seeded scaffold have led to the development of the so-called living skin-equivalent grafts. Pioneers in this field were Bell et al,[83] in 1981, reporting successful autografting of a keratinocyte and fibroblast-populated collagen matrix in a rat model.

PermaDerm (Regenicis, New York, N.Y.), previously known as Cincinnati Shriners Skin Substitute, is processed using autologous fibroblasts and keratinocytes in culture with collagen and GAG substrates. Due to the culture period of autologous cells, the product is not readily available. It was successfully used in the treatment of burn wounds with formation of a basement membrane within 9 days after graft placement.[84] To avoid hypopigmentation of the skin after grafting, melanocytes were added to the keratinocyte culture,[85] but pigmentation can still be variable and uneven.[4] In an initial clinical study in burns ($n$ = 17), the outcome of PermaDerm was similar to autologous split-thickness grafts in terms of erythema, blistering, and suppleness of the skin. However, graft take was less, and regrafting was required more frequently in the PermaDerm group.[86] The biggest advantage of PermaDerm over split-thickness skin grafts is that it requires less donor skin, an area of 66 times the original donor site can be closed. PermaDerm can therefore be useful in patients with full-thickness burns greater than 50% of total body surface area.[87] PermaDerm has not gained Food and Drug Administration (FDA) approval but received orphan status in June 2012.

The TissueTech Autograft System (Fidia Advanced Biopolymers) combines 2 products for consecutive application: the dermal substitute Hyalograft 3D, an

**Table 4. Scaffolds Seeded with Fibroblasts**

| | |
|---|---|
| TransCyte | A scaffold similar to Biobrane (silicon coated nylon-collagen scaffold) populated with neonatal fibroblasts. Over a period of 17 days, the fibroblasts are allowed to proliferate and synthesize growth factors and extracellular matrix components such as fibronectin, collagen, and proteoglycans within the matrix,[4] hereby most likely enhancing biocompatibility of the graft<br>Similar to Biobrane, TransCyte is only used as temporary wound coverage[38]<br>It is indicated for wounds that do not require grafting or are planned for grafting at a later time point[4]<br>In March 1997, TransCyte received FDA approval for the treatment of full-thickness burns[81]<br>An absorbable PLGA scaffold seeded with neonatal fibroblasts[80,83] |
| Dermagraft | First experimental results were published in 1991, demonstrating consistent revascularization and epithelialization of these grafts in mice.[83] In full-thickness burn wounds, Dermagraft, however, did not show any significant benefit for graft take of meshed split-thickness grafts[84]<br>Better success is reported in patients with chronic ulcers,[85] including diabetic foot ulcers.[86,87] FDA approval was gained in 2001[81] to stimulate healing of chronic lesions such as diabetic ulcers that are not overlying bone, tendon, muscle, or joint capsule. It may be applied weekly for up to 8 applications[4] |
| ICX-SKN | A fibrin matrix seeded with neonatal human fibroblasts. As fibrin is nature's initial wound matrix after injury before its replacement by extracellular matrix proteins produced by fibroblasts, it is not an odd choice. After freeze drying and gamma sterilization, the scaffold is repopulated with fresh allogeneic human dermal fibroblasts<br>In a murine wound model, an epidermis formed over the graft site, however, wound contraction was also visualized[88]<br>When combined with moist secondary dressings in a human clinical study, less wound contraction was seen, and histological analysis 1 month post implantation showed that the ICX-SKN graft had become vascularized and continuous wound closure was achieved[89]<br>To our knowledge, no other clinical studies have been published so far |
| Hyalograft-3D | Esterified hyaluronic acid fibers seeded with autologous fibroblasts and covered by a silicone membrane[90]<br>It is mainly used in articular cartilage engineering[91]; however, use in diabetic ulcer therapy has been reported[48] |

autologous fibroblast seeded hyaluronic matrix as previously described, and the epidermal autograft Laserskin. It was proven well tolerated and effective in the treatment of 9 diabetic ulcers of the lower limbs.[88] In a larger retrospective study, 70.3% of 401 diabetic ulcers treated with TissueTech Autograft System healed within less than 1 year. One-year healing rates were also high in pressure wounds (71.4%) and posttraumatic ulcers (65.0%) and slightly smaller in arterial (50.4%), venous (56.4%), and arteriovenous ulcers (42.9%).[89]

Apligraf (Organogenesis, Canton, Mass.), also known as Graftskin, is a bilayer product based on the pioneer research of Bell et al.[83,90] Neonatal fibroblasts are seeded onto a bovine type I collagen gel and neonatal keratinocytes are cultured on top of this dermal layer.[91] Apligraf may be applied every 4–6 weeks. It is stored at room temperature. Disadvantages are its short shelf life of 5 days, its fragility, the risk of disease transfer due to its allogeneic constituents, and the high cost.[4,92] In May 1998, Apligraf was approved by the FDA for the treatment of chronic venous and diabetic leg ulcers.[72] Adding Apligraf to compression therapy for chronic venous ulcers doubled the number of healed wounds at 6 months.[93] In chronic diabetic foot ulcers, 56% of patients in the Apligraf group had reached complete healing by 12 weeks compared with only 38% in the control group with moist gauze dressing treatment. Osteomyelitis incidence and progression to amputation were also significantly lower in the Apligraf group (osteomyelitis 2.7% vs 10.4%, amputation 6.3% vs 15.6%).[94] Another diabetic wound study confirmed these results.[95] In a prospective, multicentered open study, in 107 patients with partial- or full-thickness excisional wounds, single Apligraf application was proven to be safe. Graft persistence was good in 73.3% of patients at 1 week, reducing to 56.6% and 53.6% at 2 weeks and 1 month, respectively.[96] Occasional use of Apligraf in other split-thickness skin defects, such as donor-site wounds,[97] epidermolysis bullosa,[98] and cutis aplasia,[99] has been reported. Uneven pigmentation and contracture have also been seen.[100]

Orcel (Ortec International, New York, N.Y.) is a bilayered collagen sponge, comprising a top layer of pepsinized insoluble bovine collagen and a base layer of porous cross-linked type I bovine collagen. The top layer supports neonatal keratinocytes, and the porous layer is seeded with neonatal fibroblasts.[57,92] It has been approved by the FDA to treat split-thickness wounds. There are few clinical data available to support its use. It is reported to be well tolerated, to promote faster healing, and to result in reduced scarring when compared with therapy with Biobrane.[57]

## DISCUSSION

We started this article with a review of the different types of acellular scaffolds used in skin tissue engineering. Human decellularized dermis products such as AlloDerm and DermaMatrix seem to be the best option to date, with no other decellularized or

synthetic scaffold being clinically proven to have better results. This is consistent with the plastic surgery principle of replacing like tissue with like tissue. It indeed seems logical that a dermal skin–derived matrix can easily function as a dermal matrix again and be a hospitable environment for fibroblast and macrophage infiltration and revascularization. Animal-derived acellular dermis would seem a good candidate as well; however, clinical results are more disappointing. Looking further into this phenomenon, we believe that this can partly be contributed to the fact that the listed animal-derived skin products are chemically cross-linked. It is widely known that chemical cross-linking induces a certain degree of cytotoxicity, and when reviewing acellular scaffolds, we noticed indeed a consistency between cross-linked products and their clinical inability to serve as a skin tissue engineering scaffold; results were usually poor or at least not as good and indications focused more toward purposes where strength and scaffold integrity are more desired than scaffold incorporation, such as for hernia repair, tendon replacement, or correction of soft-tissue defects with fillers.

When acellular scaffolds are seeded with fibroblasts, leading to the deposition of extracellular matrix proteins within the scaffold, the remodeled scaffolds may be less cytotoxic and more biocompatible to serve as a tissue engineering matrix. Further research and clinical studies with these products are required, but it is important to realize the associated huge cost of preseeding matrices with cell cultures. Also these quite advanced bioengineered skin substitutes still have their limitations when compared with human skin,[101] and developments in certain fields are essential to reach full therapeutic potential. The lack of built-in vascular or nervous components makes grafts dependent on host neovascularization and reinnervation. Simultaneous growth of vascular networks within skin substitutes by coseeding grafts with endothelial cells or their progenitors looks promising. Various studies have shown facilitation of revascularization of the graft by host vasculature connecting to this prefabricated vascular network.[102] There is a lack of human clinical trials, but improved outcome by enhanced graft take and improved cell survival is expected. Incorporation of other cells, such as dermal papilla cells for hair follicles,[103] and sweat gland cells[104] could be considered as well, especially when grafts are used in areas where these appendages are functionally or aesthetically important. Another disadvantage of current skin substitutes is their sole focus on substituting the epidermal and dermal skin layer while neglecting the subdermal fat layer. Skin mobility is hereby reduced and contour defects are often noticeable. To address this issue, attempts of culturing preadipocytes in skin grafts are being undertaken.[105] Increasing complexity of skin substitutes addressing the deficiencies of skin substitutes over native human skin involves time delays needed for cell culture. It is also important to take the higher costs into account. To give an idea, AlloDerm and Integra both cost about 15–30 USD per square centimeter. Fibroblast-seeded products such as TransCyte and DermaMatrix cost in general about twice as much and Apligraf can even go to 4 times as much.[106] Products where keratinocytes are added are even more expensive. Cheaper options are porcine-derived dermis or small intestine submucosa. It is important to take the costs into account when appointing indications.

## CONCLUSIONS

Various studies show that skin substitutes can reduce morbidity and improve functional outcome. They are considered a useful tool in plastic and reconstructive surgery practices. From this review of the literature, decellularized dermis seems to remain the best acellular skin substitute, with no other scaffold being clinically proven to gain better results yet. The plastic surgery principle of replacing like tissue with like tissue seems to be still standing for now. Chemically cross-linked products are most likely less suitable for skin substitutes; however, biocompatibility can be enhanced by preseeding them with fibroblasts to allow some natural scaffold remodeling before application. More research is, however, needed to fully reach the potential of cell seeded scaffolds while the rising production costs can form a serious issue as well.[101]

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