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Tissue engineering for cutaneous wounds: an overview of current standards and possibilities

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Aktueller Stand und Möglichkeiten der Wundbehandlung durch Tissue engineering

Zusammenfassung *Grundlagen:* Die Haut stellt das größte Organ des Menschen dar, wobei sie aus mehreren Schichten aufgebaut ist und den Schichten jeweils spezifische Aufgaben zuzuschreiben sind. Die Haut spielt eine entscheidende Rolle den Körper gegenüber der Umwelt zu schützen. Ein teilweiser Verlust dieser Schutzhülle durch Verletzung oder Krankheit kann zu schwerem physiologischem Ungleichgewicht und schließlich zu wesentlichen körperlichen Einschränkungen oder sogar zum Tod führen.

Methodik: Dieser Artikel dient als Überblick über den derzeitigen Wissenstand zum Thema „Tissue Engineering" bei Wunden.

Ergebnisse: Die häufigsten Ursachen für schwere Hautschädigungen sind thermale Verletzungen. Andere Ursachen für Schädigungen der Haut sind Verletzungen und chronische Ulzeration infolge Diabetes mellitus, Druckeinwirkung und Venenstauung. Während der letzten drei Jahrzehnte wurden bei der Untersuchung der zellulären und molekularen Prozesse bei der akuten Wundheilung und der Pathobiologie von chronischen Wunden enorme Fortschritte erzielt.

Schlussfolgerungen: Dieser verbesserte Wissensstand führte zu Innovationen bei der Wundbehandlung, die eine raschere Abheilung von chronischen und akuten Wunden ermöglichten bzw. zu einem besseren funktionellen und ästhetischen Ergebnis führten. Der Einsatz von Haut bzw. Hautersatzmaterialien hat die Behandlungsmöglichkeiten für komplizierte Wunden deutlich erhöht.

Schlüsselwörter: Epidermaler Ersatz, dermaler Ersatz, Hautersatz, Keratinozyten.

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Summary. *Background:* The skin is the largest organ system in humans, consisting of various distinctive layers, each stratum with a specific purpose. Consequently, our skin incorporates the most essential function, which is to protect our body. Loss of skin integrity because of injury or illness may acutely result in a substantial physiologic imbalance and ultimately in a disability with long-term morbidity or even death.

Methods: This article is an overview of current state-of-the-art concepts and possibilities in the treatment of cutaneous wounds by the use of tissue engineering.

Results: The most common cause of significant skin loss is thermal injury. Other causes of skin loss include trauma and chronic ulcerations secondary to diabetes mellitus, pressure, and venous stasis. Over the past three decades, extraordinary advances have been made in our understanding of the cellular and molecular processes involved in acute wound healing and in the pathobiology of chronic wounds.

Conclusions: This knowledge has led to wound care innovations that have facilitated more rapid closure of chronic and acute wounds, better functional and aesthetic outcome. The use of tissue-engineered skin replacements has upgraded the therapeutic possibilities for recalcitrant wounds and for wounds that are not suitable for primary closure.

Key words: Tissue engineering, epidermal replacements, dermal matrix, dermal replacements, keratinocytes.

Introduction

The skin is the largest organ system in humans, consisting of various distinctive layers, each stratum with a specific purpose. Consequently, our skin incorporates the most essential function, which is to protect our body from environmental influences.

Other critical functions include immune surveillance, sensory detection, the competence of self-regeneration and healing. Loss of skin integrity because of injury or

illness may acutely result in a substantial physiologic imbalance and ultimately in a disability with long-term morbidity or even death.

The most common cause of significant skin loss is thermal injury. Other causes of skin loss include trauma and chronic ulcerations. Over the past three decades, extraordinary advances have been made in our understanding of the cellular and molecular processes involved in acute wound healing and in the pathobiology of chronic wounds [1–3].

This knowledge has led to wound care innovations that have facilitated more rapid closure of chronic and acute wounds, better functional and aesthetic outcome.

Several products for wound treatment that germinated from our increased understanding of fundamental processes underlying wound healing have reached the market for the therapy of recalcitrant wounds [1, 4]. These products include recombinant growth factor (rPDGF-BB) (Regranex, Ortho-McNeil), and several skin substitutes. Most of these interventions demonstrate an increase in closure or healing rates in chronic wounds [4].

Regardless of a specific wound-care product the ideal goal would be to regenerate tissues in order to restore both the structural and functional properties of the damaged layers to the levels before the injury has taken place.

Embryonic wounds, in contrast to wounds in children and adults, undergo real regeneration during the first and second trimester and scarless repair early in the third trimester [5]. Morphogenetic cues from these embryonic phenotypes could be utilized to develop engineered constructs capable of tissue regeneration and not only tissue reparation.

In particular, as cellular response to biological stimuli depends also on the architecture and mechanical strength of the extracellular matrix (ECM) [6, 7], the therapeutic success of tissue-engineered constructs will depend not only on their bioactivity, but also on their mechanical properties.

A possible solution: tissue engineering.

The advent of tissue-engineered skin replacements revolutionized the therapeutic potential for recalcitrant wounds and for wounds that are not amenable to primary closure. This article will introduce the reader to the field of tissue engineering and a review of current state-of-the-art concepts and products from our point of view. Tissue engineering integrates many disciplines of science and engineering in order to design, develop, and test tissue replacement for traumatically lost or disease-damaged tissue. Tissue engineering itself relies essentially on the expertise of scientists and engineers from multiple backgrounds, including cell biology, physiology, chemistry, physics, material science, applied mathematics, biomedical, mechanical and chemical engineering.

Two major approaches, in vitro and in vivo, have been utilized to develop engineered tissue.

- The in vitro method has received considerable attention from the lay-media since it attempts to create organs in tissue culture or bioreactors for implantation and replacement.
- In contrast, the in vivo approach attempts to create an acellular biomaterial that contains matrices capable of stimulating tissue cell recruitment into the biomaterial and inducing cell differentiation to form the tissue, finally needed [8].

Ideally, tissue-engineered skin replacements (Table 1) should facilitate faster healing and promote the development of a new tissue that bears a close structural and functional resemblance to the uninjured host tissue.

Table 1. Commercially created skin substitutes

	Commercial product name	Epidermal component	Dermal component
Cellular epidermal replacement	Epicel	Cultured epidermal autograft	None
	Laserskin	Cultured epidermal autograft in a perforated hyaluronic acid membrane	None
	ReCell, CellSpray XP	Autologous epidermal cell suspension	None
	CellSpray	Cultured epidermal cell suspension	None
Acellular epidermal subsitutes	Biobrane	Silicone film with a nylon fabric partially imbedded into the film	None
	Suprathel	Synthetic copolymer with a porous membrane, mainly based on DL-lactidetrimethytencarbonate, trimethylenecarbonate and ε-caprolactone	None
Engineered dermal substitutes	Alloderm	None	Processed cadaver allograft
	Dermagraft	None	Allogeneic fibroblasts on a bioabsorbable scaffold
	Integra	Synthetic polysiloxane polymer	Bovine collagen and GAGs
	Transcyte	Thin silicone layer	Collagen coated nylon mesh seeded with allogeneic fibroblasts
	Matriderm	None	Bovine collagen and elastin
Engineered skin substitutes	Apligraf	Human allogeneic keratinocytes	Human allogeneic fibroblasts in bovine collagen, ECM proteins and cytokines
	OrCel	Human allogeneic keratinocytes	Human allogeneic fibroblasts in a bovine collagen sponge

Autologous and allogeneic epidermal replacement

Cultured autologous epidermal sheets (CEA) have been used to facilitate repair of both epidermal and partial thickness wounds. Autologous epidermal sheets were first used to cover burn wounds [9], and burns have remained the major clinical target for both autologous and allogeneic epidermal replacement [4, 10–13].

Implantation of epidermal grafts cultured from a small skin biopsy was made possible, when tissue culture techniques were perfected to grow epidermal cells in large quantities [14, 15]. Subsequently, extensive experience has been gained with cultured epidermal grafts for the treatment of burns as well as other acute and chronic wounds [16, 17]. CEA serve as permanent wound coverage, since the host does not reject them, and yield adequate cosmetic results. Furthermore, graft-take can vary widely due to wound preparation and its intrinsic status, patient's underlying disease, and operator's experience.

Cultured epidermal sheet allografts were developed to overcome the necessity to biopsy each patient and its associated 3–4 week cultivation interval between epidermal harvest and final autograft product. With the utilization of techniques for culturing epidermal sheets, epidermal cells from both cadavers and unrelated adult donors [13] have been used for the treatment of burns [10, 11], donor sites of skin grafts [18], and chronic leg ulcers [19]. These allografts promote accelerated healing and pain relief in a variety of acute and chronic skin ulcers without any evidence of immunological rejection; however, the keratinocytes within these allografts are replaced within a few weeks by ingrowth of recipient cells. These allografts serve as biological active dressings in order to promote the epithelisation. To facilitate mass allograft production and wide availability, cryopreserved allografts were developed. These frozen constructs gave comparable results to fresh allografts [20].

Although cultured epidermal autografts and allografts can be used successfully to cover partial thickness burns, they fail to produce a satisfactory response in full-thickness wounds [21].

As an alternative, keratinocyte delivery systems were developed by which cells are delivered to the injury site via a biodegradable scaffold. For example, *Laserskin*®, produced by FIDIA Advanced Biopolymer, Italy, is used to deliver keratinocytes via a chemically modified hyaluronan membrane [22], perforated with micron-sized holes that allow cells to grow to confluence. Alternatively keratinocytes can be delivered to wounds intermixed with fibrin sealant as a spray or cultured on a fibroblast containing fibrin gel [12].

CellSpray®, *CellSpray XP*®, *ReCell*®: Keratinocytes are isolated from a harvested split-thickness skin biopsy and a sprayable cell suspension is generated. In ReCell® and CellSpray XP® exclusively keratinocytes including melanocytes without cell cultivation are isolated. Transplantation is thereafter realised on a debrided wound bed. In order to match skin colour and texture of an appropriate body region, biopsies are harvested either safely from healthy tissue nearby or from the contralateral body hemisphere. The final suspensions are delivered in a culture medium.

CellSpray® contains a keratinocyte suspension of cultivated cells. Transplantation (spray application) is possible after a week. CellSpray XP® carries a single cell laboratory-cultivated suspension of keratinocytes, melanocytes and fibroblasts, ready for use within two days. From a 4 cm^2 biopsy a wound surface of 320 cm^2 can maximally be covered using a drip or spray application of the cell suspension. Clinical indications include acceleration of spontaneous healing after superficial dermal burns, epithelial replacement, scar revision surgery, skin resurfacing after dermabrasion, and vitiligo.

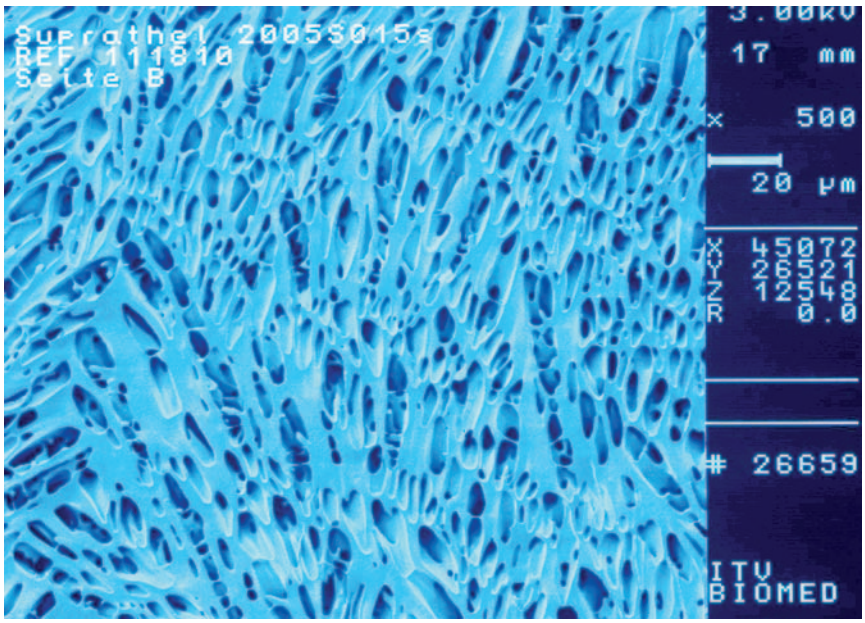

Fig. 1. Suprathel®: Terpolymer (DL-Lactid, ε-Caprolacton and Trimethylencarbonat) (500× magnification)

Acellular epidermal subsitutes

Biobrane® is an acellular biosynthetic skin substitute, which is constructed of an outer silicone film (epidermal analogue) with a nylon fabric (dermal analogue). Both layers incorporate collagen (porcine type 1), which is being chemically cross-linked. The collagen peptides on the nylon bind to the wound surface and promote a healing process. Indications for use include superficial (clean) second degree burns and large area epithelial defects (e.g. Lyell syndrome/toxic epidermal necrolysis).

Suprathel® (Germany) is a promising and fully synthetic copolymer with a porous membrane, mainly based on DL-lactidetrimethytencarbonate (>70%), trimethylene-carbonate and ε-caprolactone, and is available with various pore and surface size (Fig. 1). It can be employed after excision and haemostasis for second degree burned areas (Fig. 2), but also for skin harvest regions. A proposed main advantage of its utilization is the pain-reduced and accelerated epithelialisation with the benefit for employment in joint proximity and functionally stressed regions (eg. for early mobilization) [11, 23–25].

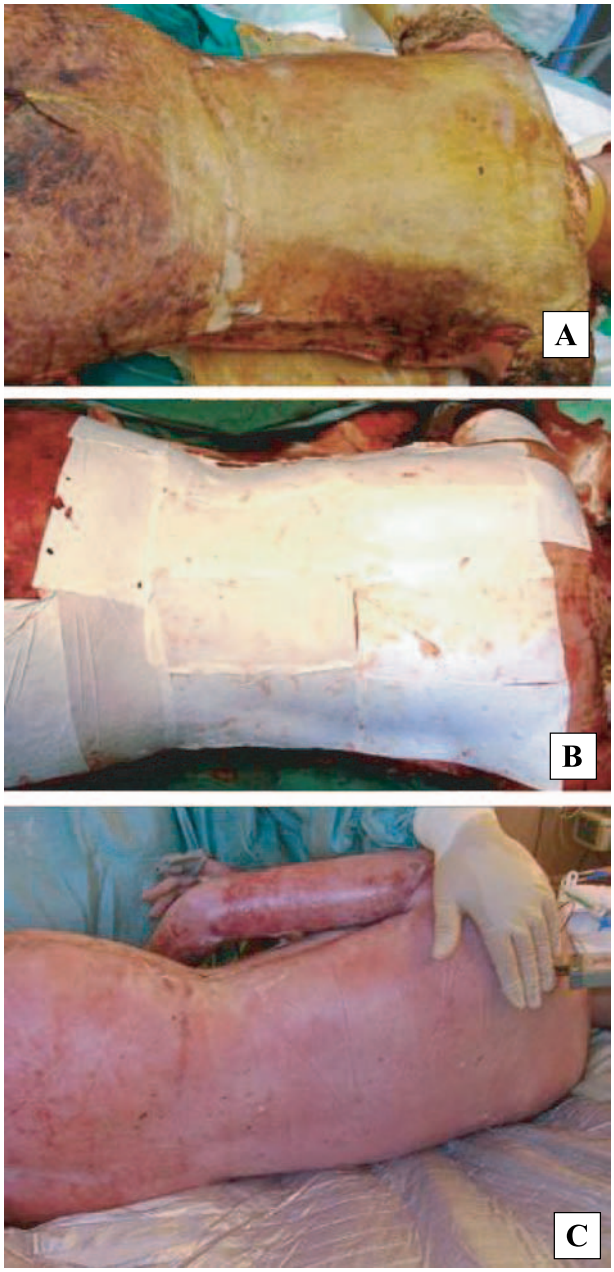

Fig. 2. Deep burn to the back (deep dermal and full thickness burn: 90% TBSA) (A) → Debridement and coverage of the back with Suprathel® (B) → Result 3 months after trauma (C)

Engineered dermal constructs

While cultured epidermal sheets enhance healing, especially of burn wounds, they lack a dermal component that, if present, might prevent wound contraction and provide greater mechanical stability. Cadaver skin allografts containing both epidermis and dermis have been used for many years, but provide only temporary coverage because of host rejection. However, allografts can be chemically treated to remove immunogenic cellular elements, for example, *Alloderm*® (Life Cell Corporation, Woodlands, TX). These ''decellularized'' allografts have been effectively used alone or in combination with cultured autologous keratinocytes for the closure of burns and chronic wounds [26].

In 1981, a composite of bovine collagen and chondroitin-6-sulfate from shark cartilage, with an outer silicone covering, was engineered as an organotypic dermis for skin grafting [27]. After wound coverage, the acellular composite recruited host dermal fibroblasts and was degraded during cell invasion. The silicone sheet was removed 2–3 weeks after placement, and the wound covered with a sheet autograft in a second step. A one-step procedure to cover acute wounds is principally not possible. This organotypic dermis material has been successfully applied in burns [28] and has received FDA approval for this indication (*Integra*®, LifeSciences Corporation, Plainsboro, NJ). This material, however, must be avoided in patients who have developed allergic reactions to bovine products.

Another acellular implant called *Transcyte*® (Dermagraft-TC) was produced by Advanced Tissue Sciences Inc. (ATS, La Jolla, CA). This composite consisted of an inner nylon mesh, in which human foreskin fibroblasts were embedded and an outer silicone layer to limit evaporation. Fibroblasts were allowed to synthesize and secrete ECM material, such as collagen, fibronectin, and glycosaminoglycans; and cytokines, including growth factors. After a few weeks the cells were disrupted by freeze–thawing to create the final product.

Transcyte was successfully used as temporary wound coverage after the excision of eschars from burn wounds [29] and approved by the FDA for this indication. ATS also produced a cellular composite, called *Dermagraft*®. In this construct, human foreskin fibroblasts were cultured in a biodegradable polyglactin mesh and then cryopreserved so that they remained viable. Dermagraft had limited success in the treatment of diabetic foot ulcers [30]. As Dermagraft did not appear to stimulate immune rejection, it was first viewed as a dermal substitute [31]. However, the human foreskin fibroblasts implanted with this material die within a few weeks after implantation;

therefore, the product more likely acts as a delivery vehicle for growth factors and ECM produced by fibroblasts, while they were extant [4]. Transcyte and Dermagraft are currently off the market.

Porcine small intestinal submucosa acellular collagen matrix (*Oasis*®, Cook Biotech Inc., West Lafayette, IN) and an acellular xenogeneic collagen matrix (E-Z-Derm) are also available and have relatively long shelf lives. A recent randomized clinical trial with small intestinal submucosa in 120 patients with venous leg ulcers demonstrated significantly increased healing at 12 weeks (55 versus 34%) in patients receiving small intestinal submucosa dressing weekly plus compression versus patients receiving compression alone [32]. Although swines appear relatively resistant to prion disease, possibly secondary to more inherent structural stability of their prion protein (PrPC), prion disease and porcine retroviruses are a concern that needs to be addressed in these kinds of xenograft products [8].

Recently brought into clinical use is a very promising dermal substitute, which is suitable for single-step repair of dermal tissue defects in combination with thin skin grafts [11, 33, 34]. *Matriderm*® (Dr. Suwelack Skin & Health Care, Germany) is a porous and thin membrane consisting of a native bovine type I, II and V collagen fibre template coated with elastin hydrolysate (derived from bovine ligamentum nuchae) (Fig. 3), which is converted into native host collagen within weeks after application. The matrix can be stored at room temperature and comes as 0.5, 1 and 2 mm thick sheets. Depending on local (intra-operative) conditions after debridement and meticulous haemostasis, it can either be applied after rehydration in 0.9% physiological saline solution or as is onto the wound bed, from which then rehydration can take place. Sheets of 2 mm thickness and above are recommended for two-step repairs, with a time interval of seven days to allow for vascularisation of the matrix before the transplantation of split thickness skin grafts. However, a one-step procedure is feasible in the acute phase after the burn trauma. About a week after dressing removal physiotherapy can be initiated. The take rate does not differ significantly as compared to ''regular'' split-thickness skin grafts and the quality of the resulting scars is reported to be superior if compared to skin grafting alone [33] (Fig. 4).

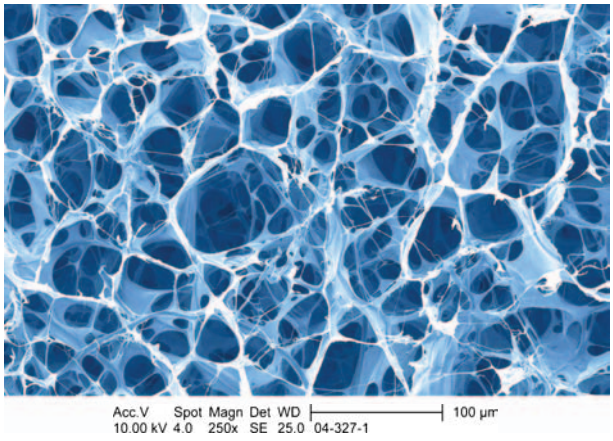

Fig. 3. Matriderm®: Collagen-Elastin-Matrix (250× magnification)

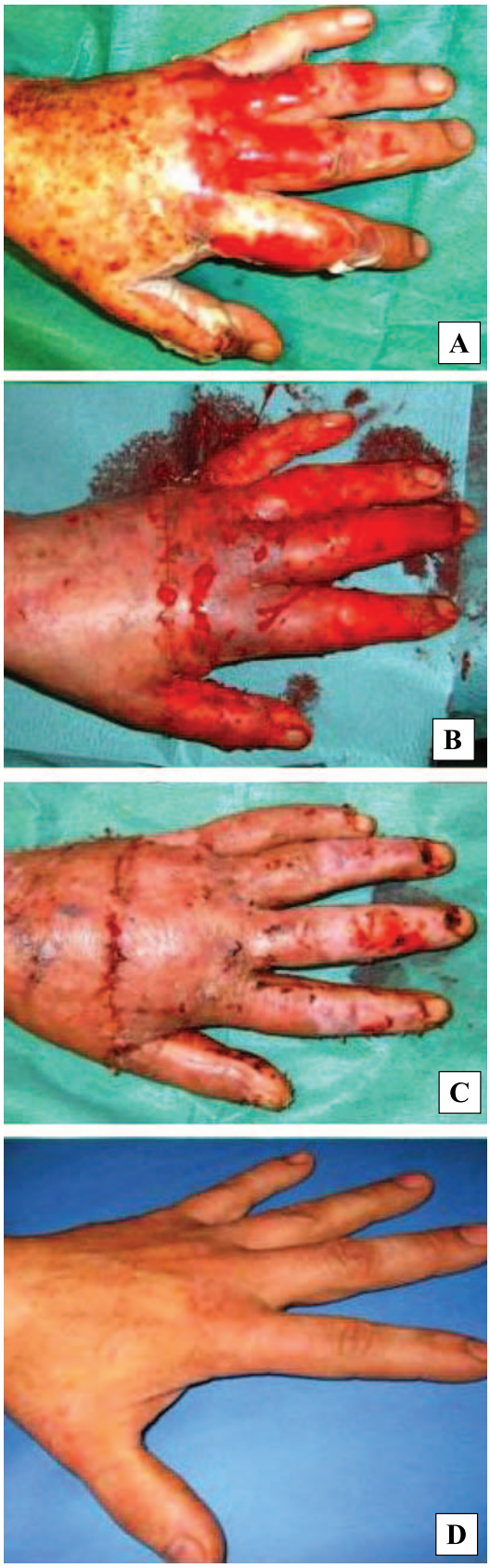

Fig. 4. A: Full thickness burn to the dorsum of the hand → B: Tangential excision and coverage with Matriderm® + unmeshed skin graft (one step-procedure) → C: Early result (10 days after the operation) → D: Long term result (1 year after trauma)

Table 2. Commercially created alloplastic materials

	Commercial product name	Material
Alloplastic materials	Lyomousse	Foils made of polyurethane with a two-layered foam
	Syspurderm	Foils made of polyurethane with a condensated surface
	Epigard	Foils made of polyurethane with a teflon layer

Alloplastic material

Epigard®, *Lyomousse*®, *Syspurderm*® come as foils made of polyurethane with a two-layered foam (Lyomousse ®), with a condensated surface (Syspurderm®) or with a teflon layer upside (Epigard®) and are reserved for temporary cover of debrided wound beds, where biological dressings cannot be employed or are not available (Table 2).

Engineered skin substitutes

Full thickness wounds involve the loss of skin epidermis and dermis. To treat wounds of this depth, a bilayered composite of a contracted collagen lattice containing dermal fibroblasts and an overlying epidermal sheet was designed. Subsequently, a modification of this organotypic skin substitute utilizing type I bovine collagen, live allogeneic human neonatal foreskin fibroblasts and keratinocytes was developed (*Apligraf*, Organogenesis, Canton, MA) and marketed (Norvartis, Zurich, Switzerland). It proved to be beneficial in surgical wounds [35] and venous ulcers and is FDA approved for the latter. In a large multicenter trial this product resulted in approximately 25% accelerated curing of chronic non-healing venous stasis ulcers when compared to standard compressive therapy [36]. Signs of wound infection, however, were observed in 29% of patients receiving Apligraf versus 14% in patients receiving standard care. Apligraf does not result in immunologic rejection [35]; however, donor cells do not remain viable beyond 4–8 weeks. Although Apligraf was first marketed as an organotypic skin substitute, the lack of long-term viable cells contradicts this claim. It is now believed that Apligraf works by delivering growth factors and ECM to the wound bed [4]. The product is provided in a 75-mm tissue culture dish and has a shelf life of 5–10 days. Thus, shipping must be closely coordinated with the patient's admission. Apligraf costs approximately $30 per square cm, mainly because of its associated charges implied in its production, maintenance and delivery, like in Dermagraft [8]. Organogenesis filed for bankruptcy in 2002 after being unable to provide these complex organotypic constructs at the marketed wholesale cost. After undergoing reorganization, Organogenesis is now back in business selling Apligraf, as well as other wound care products.

Several other composite skin substitutes combining dermal and epidermal elements have been developed. A composite cultured skin substitute (*OrCel*®, Ortec International Inc., New York, NY) is composed of both neonatal keratinocytes and fibroblasts embedded on opposite sides of bilayered bovine type I collagen. This product is currently being evaluated in clinical trials for the treatment of burns, in patients with epidermolysis bullosa and on split-thickness donor sites [37].

Tissue-engineered skin replacement: state-of-the-art

For the past several decades, many engineered skin constructs have utilized collagen as a scaffolding material for cell seeding [38]. Collagen's popularity can be attributed to its abundance in skin, its recognition by cell surface receptors, and its ability to crosslink and thereby impart appropriate mechanical strength to the tissue [8]. Collagen, however, appears during the later stages of wound healing after fibroblasts have invaded and filled the wound space [39].

The acellular tissue-engineered constructs discussed so far utilize biopolymers to provide mechanical support for tissue in-growth, and biomimetics to induce key cell functions. The primary goal of this approach is to mimic the attributes of the wound provisional matrix that conducts parenchymal cell migration and induces the appropriate cell differentiation for generation of new tissue. However, a fibrin clot is composed not only of a fibrin/fibronectin scaffold and an array of clotting and fibrinolytic enzymes, but also of multiple growth factors that have been released during platelet aggregation [40]. Growth factors play a crucial role in the healing response, where they function as stimulants to cell migration, proliferation, and differentiation. Growth factor deficiency often leads to impaired healing [8].

As a consequence, several groups have investigated the use of tissue-engineered constructs for local growth factor delivery [41].

It is important to note that despite of growth factor release from platelets and injured cells immediately after wounding, a 3-day delay occurs before granulation tissue begins to form [42]. This fact suggests that growth factors may bind the clot and keep their functional activity. Such 'solid-state' chemical biology is supported by data indicating that basic fibroblast growth factor and vascular endothelial growth factor bind to fibrin and retain their biological activity [43] as well as IGF and vascular endothelial growth factor bind to fibronectin and do the same. Furthermore, studies have shown that PDGF, when preloaded onto hyaluronan hydrogels contain specific domains of fibronectin, preserves its activity at a level typically observed with a higher concentration in solution [8].

Although the finding was counterintuitive from the vantage of glycosaminoglycans, since heparin, rather than hyaluronan, has been demonstrated to bind a variety of growth factors [44], it is consonant with the ability of fibronectin to bind other growth factors. Regardless of the mechanism employed, by incorporating appropriate growth factor-binding materials, a tissue-engineered composite can be used as a growth factor repository, which accentuates cell functions through the bioactivity of bound or released growth factors as well as for mechanical prop-

erties attributable to the biopolymer backbone and for conductive and inductive activity attributable to other tethered biomimetics.

Conclusion

Wound healing is an integral biological response consisting of a dynamic reciprocity among cells, ECM and growth factors that reconstitute tissue after injury. Vigorous cellular activities observed during wound repair are similar to those occurring during embryogenesis and morphogenesis indicating the enormous complexity of this physiological reparative process. That complexity may also explain why, despite over two decades of intense research and development, medical research has still not identified an ''ideal'' therapy and/or the ideal matrix. However, a better appreciation of how natural and synthetic biopolymers affect cell function and multicellular organization, how external biochemical signals interact with the cell membrane in context of the pericellular matrix, and how signals from these physicochemical events are transduced by a solid-state, yet dynamic and integrative, organization of signal transduction proteins in the cytoplasm, is beginning to open new horizons, which should ultimately translate into novel ''break-through'' wound-healing therapeutics.

Today's wound care bears a high potential for interesting new advances and developments in tissue engineering and skin replacement for conservative and surgical therapy. Given the variety of indications and diversity of clinical applications it is essential to cautiously use a specific product for the correct clinical situation and, also as a result of the associated high costs, therefore the required strict handling of these utilities should be reserved to experienced clinical specialists. Only in these hands a sensible and resource-saving application can be realised in order to meet the expectations of our patients. Despite the advances being made until now, the development of a tissue-engineered, universally applicable ''structural and functional normal skin-equivalent'' is nonetheless a challenge for medicine today and in future.

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