Effect of collagen matrices on dermal wound healing

Zbigniew Ruszczak*

INNOCOLL GmbH, Saal, Germany
Dermatology, UMDNJ, New Jersey Medical School, Newark, NJ, USA
European Research Center, Saal a.d. Donau, Germany

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Abstract

Dermal substitution and wound healing are areas of medicine in which there have been many recent advances, but neither the commercially available products nor the products currently described in experimental studies are able to fully substitute for natural living skin. There is an overall consensus that to heal wounds, the substitution of connective tissue matrix, the main component of each wound, is necessary.

Both artificial and natural polymers have been used to reconstitute dermis. Nowadays, collagen has been discovered again. Collagen is a natural substrate for cellular attachment, growth and differentiation, and promotes cellular proliferation and differentiation.

Once dermis reconstruction is done, the covering of the wound surface with both in vitro expanded epidermis and autologous split-skin transplants is significantly easier and has an improved chance of success. Nowadays, many commercial and experimental products have been introduced to improve cutaneous wound healing. This review discusses some of both acellular and cell-containing products used in the treatment of skin wounds.

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1. Introduction

The best way to heal a wound is to close it surgically, according to long-known standards, and to do this as quickly as possible after injury. This procedure is, however, limited to those wounds and to those anatomical localizations that allow both excision and adaptation of wound borders and to close the wound per primam. In large-surface and deep wounds in which the primary wound closure is not possible or not practicable, it is important to keep the wound free of infection, to reduce or eliminate pain, to eliminate all potential factors inhibiting the natural healing properties (i.e., dead tissue in burns) and to replace or substitute as much as possible missing tissue.

The process of wound repair involves the timed and balanced activity of inflammatory, vascular, connective tissue and epithelial cells. All of these components need an extracellular matrix to facilitate the healing process. To minimize scar formation and to accelerate healing time, different techniques of skin substitution have been introduced in the last decades [1]. Autologous skin grafting is still a gold standard. However, in cases in which skin grafts are used, a new wound is created on the donor side. Thus, there is a need to eliminate a “new” wound to close the “old” one, and to close as many tissue defects as possible without the risk of large area infection, necrosis, tissue hypertrophy and contraction, as well as deformation of wound borders.

The evolution of biologic and synthetic wound dressings began with the recognition that any skin wound requires a barrier protection to prevent infection and desiccation and cell guidance by dermal elements to maximize healing.

Any successful artificial skin or skin-like material should replace all of the functions of skin and therefore consist of a dermal portion and an epidermal portion.

To achieve this goal, different scaffold materials have been developed, each with different physical properties and each associated with a specific and unique host response when implanted. Scaffold materials can be either synthetic or naturally occurring. Synthetic materials such as poly(l)-lactic acid) and poly(glycolic acid) have received considerable attention for tissue engineering applications and have shown promise in preclinical animal studies and some early human clinical trials. Synthetic materials have predictable and reproducible mechanical and physical properties (e.g., tensile strength and pore size) and can be manufactured with great precision. However, synthetic materials tend to elicit a foreign material type of response in the host, specifically, a fibrous connective tissue deposition leading to formation of dense scars and fibrosis. Therefore, naturally occurring materials such as hyaluronic acid and purified collagen have been investigated as alternatives to synthetic scaffolds.

Currently, bilayer concept of wound coverage in which both epidermal and dermal analogs are used is widely accepted [2]. The outer layer of such construct has to have a barrier function to protect the wound not only from bacterial contamination, fluid loss, but also, overheating and accumulation of tissue fluids. Dermal elements are important for cell guidance during granulation tissue formation, re-modeling and re-epithelialization. Dermal element may vary from an interface for healing (temporary substitute) to incorporation of dermal elements (permanent substitutes). It is responsible for restoring normal tissue architecture and for the prevention of scars.

The increasing survival of large-surface or deep wounds as well as massive burns, where available skin for autografting is very limited, leads to development of a “permanent” skin substitute. The increasing emphasis on rehabilitation and “quality of skin cover” has further accelerated this field. A skin substitute which has the properties of a dermis is the marker for gauging a “permanent” substitute [2–4].

Currently, the following scenario seems to be widely accepted: “temporary” dressings—material designed to be placed on a fresh wound (superficial
or partial thickness) and left until healed, (b) “semi-permanent”—material remaining attached to the excised wound, and eventually replaced by autogeneous skin grafts, and (c) “permanent” incorporation of an epidermal analog, dermal analog or both as a permanent replacement [2].

Diverse biologic and synthetic skin substitutions are currently available. Most of them have been reviewed previously [1,2]. Summarizing, naturally occurring tissue-derived materials are: cutaneous allografts, cutaneous xenografts and amniotic membranes. The group of skin substitutes contains synthetic-based bilaminate matrices (Dermagraft® or OrCel®), collagen-containing engineered composites (TransCyte®, Biobrane®, Integra®) and collagen-based natural analogues like de-epithelialized allograft (Alloderm®). Additionally, co-culture-derived tissue constructs have been proposed. One of the most advanced products of this kind is Apligraf®, a bilayer human-tissue-like product containing living dermal fibroblasts and epidermal keratinocytes within the type I collagen matrix. Diverse cell culture models have also been introduced, leading to products based on cultured autologous keratinocytes, fibroblast-seeded dermal analogs, collagen–glycosaminoglycan (GAG) matrices, hyaluronic acid or polyglycolic acid meshes seeded with autogenic epidermal analogs.

In this review, skin substitutes, which can replace either dermal portion or both dermal and epidermal component, will be discussed.

Substitutes utilizing only epidermal component, and used for superficial wound treatment (e.g., in vitro reconstructed epidermal sheets), have been extensively discussed by us previously [1–3].

2. Skin-derived matrices: substitution of dermal component

2.1. Allogeneic skin-based matrix

One of the main differences between the cultured epidermal sheet and a split-thickness autograft is the lack of the dermal structure from the cultured autograftable sheets. Absence of dermis is perceived as the major cause for a lower percentage of graft takes and higher fragility and blistering after epidermal sheet transplantation, compared to split-thickness autograft.

As early as the 1940s, human allogenic skin has been used as a matrix to help manage acute, mostly burn- or trauma-dependent wounds. These allotransplants initially had the function of a temporary biological wound dressing and served the purpose of preparation of the wound bed for the actual transplantation, or grafting, of autologous skin.

After early debridement of deep and extensive burns, temporary closure of the wound may be achieved using cadaver allograft matrix. Instead of completely removing cadaver skin before final wound closure, an excision of allogeneic epidermis with a dermatome has been applied in order to only maintain the allogeneic dermis as an implanted, collagen-rich three-dimensional matrix [5]. Since nonliving dermis alone may not be rejected [6], autologous cultured epidermal sheets can be grafted onto it to enhance healing. Indeed, cultured epidermal sheets grafted onto such dermal collagen-network-rich matrices display early rete ridge development and anchoring fibrils regeneration, in addition to a 95% graft take [7].

Knowing that de-vitalization of allografts reduces their antigenicity, the use of allogeneic cadaver collagen-rich skin matrix as a biological dressing is now widely accepted. The preservation of such allograft matrices can be performed by different techniques, such as freeze-drying [8], glutaraldehyde fixation [9] or glycerolization [10]. Cryopreservation of homografts with glycerol is the most popular method of cadaver skin matrix processing [11]. Moreover, preservation can reduce the risk of virus transmission from skin-derived matrix grafting, providing time to rid the donor skin of pathogens. In order to provide sufficient cadaver skin instantly accessible for the burn patient, skin banks, such as the larger European institution—Euro Skin Bank in Beverwijk, The Netherlands—have been well developed through the years [12]. However, allogeneic skin banking has a significantly higher cost compared to xenogeneic skin banking and biological dressings [13].

2.2. Acellular dermal component

An alternative to the removal of allogeneic epidermis from cadaver skin to maintain allogeneic dermis attached on the wound is the transplantation of acellular allograft dermal matrix. This acellular der-
mal matrix is produced from fresh human cadaver skin by a controlled process that removes the epidermis and the cells from the dermis without altering the structure of the extracellular matrix and the basement membrane complex [14]. Transplantation of this matrix on deep burns and its coverage with thin and widely meshed autografts allows a high percentage of take of both the dermal matrix and the autograft and avoids the undesirable scarring and contracture that usually results from the wide meshed grafts. Acellular dermal matrix produced from fresh porcine skin has also been developed using a similar process in order to compensate for the lack of cadaver skin availability [15].

2.3. Xenogenic dermal matrix

Xenotransplants—per definition, material coming from genetically and evolutionary different species—in this particular case of non-human origin were used in the treatment of tissue defects in burns for the first time in the 1960s.

Tissues of animal origin have been used for thousands of years to cover extensive wounds. While it has become evident in our century that xenograft achieves only temporary wound coverage, its unlimited availability under well-controlled conditions still makes animal skin a favorable wound covering. Porcine skin is the most common source of xenograft because of its high similarity to human skin. Sterility is an essential concern with xenogeneic tissues transplanted on wounds. Ionizing radiation appears to be the most suitable method to guarantee this sterility and for application in mass production. In addition, irradiation coupled with freeze-drying seems to decrease the antigenic properties of the pigskin graft and increase its potential to inhibit bacterial growth [16]. Thus, pigskin is a well-suited temporary dressing for the coverage of second-degree burns, especially after early excision, and usually promotes scar-free healing with an average healing period of about 10 days. In addition, pigskin provides a suitable overlay to cover widely meshed (1:8 to 1:12) autografts [17]. Since freeze-drying and irradiation are expensive, a low-cost alternative preservation technique was successfully developed using 98% glycerin as antiseptic followed by storage at room temperature for 20–300 days [18].

2.4. Xenogenic collagen-based scaffolds

In order to cover wounds with a dermal matrix to favor graft take of cultured epidermal sheets as well as prevent rejection of xenogeneic tissues, efforts have been made to develop non-immunogeneic artificial dermal matrices. Such dermal components must promote the prompt coverage of the largest excised full-thickness wounds, control fluid loss and prevent infections. Recent advances in the technology of in vitro tissue reconstruction have made it possible to approach these requirements. As previously discussed, devitalization of allogeneic or xenogeneic dermis is a first and interesting approach to produce a dermal matrix.

Natural polymers such as fibrin [19,20], hyaluronic acid [21,22], fibrinogen [23] and collagen [1,3,24–26] have been recently tested in different matrix systems for local drug delivery and wound healing.

Collagen is unique in possessing different levels of structural order: primary, secondary, tertiary and quaternary [27]. In vivo, collagen molecules form fibers having a specific internal and structural orientation and strengthened together by two types of covalent cross-linking: intramolecular and intermolecular. Intermolecular cross-linking is essential to form macromolecular fibers and, consequently, for its mechanical stability and other physical properties.

Collagen is a natural substrate for cellular attachment, growth and differentiation in its native state. In addition to its desirable structural properties, collagen has functional properties. Certain sequences of the collagen fibrils are chemotactic and promote cellular proliferation and differentiation. Collagen provides considerable strength in its natural polymeric state. The source of collagen either purified from animal sources or as an integral component of a more complex extracellular matrix, and its treatment prior to use are important variables in the design of tissue-engineered devices. Biomaterials made of collagen offers several different advantages: They are biocompatible and nontoxic to tissues (including neural and brain tissue) and have well-documented structural, physical, chemical, biological and immunological properties. Additionally, mechanical and—to some extent—immunologic properties of collagen scaffolds can be influenced by modification of matrix properties (porosity, density) or by different chemical treatment affecting its degradation rate [28,29]. Several methods of cross-linking
and sterilization can be utilized to alter (i.e., usually decrease) the rate of in vivo degradation or to change the mechanical properties of collagen [30]. These methods include glutaraldehyde treatment, carbodiimide treatment, dye-mediated photoxidation, exposure to polyepoxy compounds and glycerol treatment. In addition, collagen has a defined and well-established regulatory pathway in many countries worldwide.

Different approaches to utilize animal-derived collagen for tissue substitution have been developed in the past 20 years: (a) the collagen gel, made of a mixture of fibroblasts and bovine collagen, (b) the collagen sponge based upon the production of a lyophilized collagen matrix in which fibroblasts are cultured and migrate, (c) the synthetic mesh composed of a nylon or a polyglactic acid mesh on which fibroblasts are cultured, (d) the collagen membrane used alone or with reconstructed epidermal sheet, and (e) the in vitro reconstructed skin-like products based on collagen matrix [1].

These dermal matrices can be grafted onto deep wounds after early excision and may promote the reconstruction of a new dermis suitable to support the graft of autologous cultured epidermal sheets. Only a few of these products have been approved for dermal wound healing and are commercially available.

One of them approved in the US and well documented in the literature is SkinTemp (Biocore, Topeka, KS). It is a type I bovine spongy-like collagen matrix that may provide a safe, readily available approach to secondary intention healing in patients whom immediate reconstruction is contraindicated and who needs a long-term biological dressing that stimulates wound healing [31,32].

The use of collagen sponges or pads either plain or containing antimicrobial drug has been reported in successful regeneration of dermal component and acceleration of wound healing. Especially, the use of drug containing collagen sponges was found beneficial in both partial-thickness and full-thickness burn wounds. Such products approved and commercially available in Europe and many countries outside the US [e.g., Collatamp®-G, Collatamp®-EG (Innocoll GmbH, Germany, and Syntacoll AG, Switzerland), Sulmycin®-Implant, Schering-Plough, USA, or Septocoll® (Biomet Merck, Germany)] speed up granulation tissue formation and epithelialization, and additionally protect the recovering tissue from potential infection or re-infection [33,34]. In the US, collagen/nylon mash derivates such as SkinTemp® (see above), which belong to a family of diverse animal type I collagen-based products (including gel and powder), have been widely introduced.

3. Immunology and biocompatibility of xenogenic collagen material

The presence of an immune response to collagen or any other biomaterial must be viewed in the context of its clinical performance. The immune response to xenotransplants includes both natural and induced humoral components, while a humoral response to allotransplants is generally seen only after sensitization.

The level of natural antibodies that react with organ xenotransplants increases proportionally with the phylogenetic distance between the xenogenic species involved. In organ transplantation, the presence of such antibodies leads to hyperacute rejection, which occurs within minutes to hours after revascularization, and, consequently, to the loss of the transplanted tissue. This negative phenomenon can be avoided if an acellular and avascular tissue or a purified connective tissue matrix made from a natural biologic polymer such as collagen is used [35–37].

The major target for immunologic rejection of allogeneic skin grafts is the epidermis or the vasculature. The dermis, especially the dermal collagen, is accepted by the host without eliciting an immune response. Studies in several species have demonstrated that the antibody response to collagen is preliminarily against three groups of antigenic determinants: helical, terminal and central. Recent data on T-cell reactivity to bovine-derived collagen in man suggests that the immune response to collagen is linked to major histocompatibility complex genes. The collagen component of allogeneic skin grafts is not the source of sensitization or the target of rejection [36].

Collagen is a naturally occurring, highly conserved protein that is ubiquitous among mammalian species and accounts for approximately 30% of all body proteins. Since it is one of the first proteins synthesized during embryogenesis and then during organogenesis, its homology between species is very high. Bovine and porcine type I collagen provide a readily available
source of scaffold material for numerous applications and have been shown to be very compatible with human systems.

The traditional and still widely used method of collagen extraction from tissues such as skin, tendons and ligaments is solubilization, and then reconstitution into injectable low-osmotic gels, fibrils and pads.

Literature data collected during pre-application tests showed that approximately 3% of the population develops hypersensitivity to the initial skin challenge with injectable collagen. Since most of these reactions occur within the first 72 h, it indicates that a pre-sensitization to bovine collagen, presumably due to dietary exposure, exists. In addition, about 1% subsequently treated with these injectable bovine collagens will develop localized hypersensitivity responses. In addition, antibodies in sera of the patients treated with bovine collagen implants or injections are specific for bovine collagen and do not cross-react with human collagen. These antibodies bind to bovine dermal collagen implants but not to surrounding host dermal components [37,38]. This immunologic reaction is a localized inflammatory response that resolves as the implant is resorbed. Clinical studies have also shown that antibodies to several types of human collagen do not display disease specificity and appear to represent a secondary response to tissue injury. In addition, the appearance of antibodies to collagen has not been shown to correlate with the activity of autoimmune disease in either animal or human models.

Whereas an immune response to xenogenic collagen has been demonstrated in both animal and human models, the data clearly demonstrate that immunity per se is not associated with significant adverse sequelae in vivo [39]. The presence of antibodies to xenogenic collagen is an epiphenomenon and not an indicator for rejection of the implant. Empiric observation based on the widespread use of xenogenic collagen and collagen-derived products for more than 50 years indicates that, in the case of these highly purified or native xenogenic collagens, no danger of acute or latent immunologic reaction occurs. Thus, appropriately purified xenogenic collagen has little or no significant immunogenicity and no discernible threat of inducing a systemic autoimmune disease.

4. Tissue-engineered allogenic and xenogenic skin substitutes

The ideal xenogenic material used for dermal substitution should have the following clinical properties: (a) be hemostatic and possess good adherence to any wound bed (including cartilage and bone surfaces), fully cover the wound surface without any dead spaces; (b) adhere immediately to the wound borders; (c) cover the whole wound area and protects it against infectious agents and against the loss of water and tissue fluids; (d) cover the wound area, reducing or eliminating pain; (e) lack any specific inflammation-stimulatory agents, and not produce any foreign body reaction, granuloma formation, or acute or chronic immunological rejection; (f) serve as a natural matrix for host granulation tissue formation, coordinate fibroblasts proliferation and angiogenesis with early tubular formation and capillary development; (g) serve as a natural surface, promoting host epithelial cells proliferation, re-epithelization and development of basal membrane structures and a stable connection between the new, developed connective tissue and the new, proliferated epidermis; (h) promote a normal epidermal differentiation and enhance the maturation of epidermis, which covers the healing wound (natural collagen matrix); (i) due to (a)–(g), protect against the contracture of wound borders and against typical scar formation; and (j) be fully transparent and allow excellent clinical observation of the wound area and of the healing process.

4.1. Acellular matrices for dermal substitution

The engineering of skin tissue and the development of a skin substitute has been studied from a variety of approaches. The acellular collagen–chondroitin sulfate material proposed by Burke et al. and Yannas et al. [40–48] represented one of the first attempts at engineering a dermal component to substitute the volume of missing tissue. Bell et al. [49–54] proposed a bilayered model of skin using contracted collagen lattices containing living dermal fibroblasts covered in a second-step procedure with in vitro reconstructed epidermal sheets.

Native collagen and native collagen containing products have been proposed for covering superficial wounds, for tissue augmentation or as hemostatics in
visceral surgery. The practical use of soluble collagen for wound healing is limited due to problems with storage stability and the time required to prepare enriched collagen solutions. Traditional collagen pads or vlieses manufactured from solubilized collagen material are not suitable for these purposes because of their high compression after application onto the wound surface and their lack of transparency. This last phenomenon is of great importance because of the possibility of permanent visual control of the wound during each healing phase.

Early 1994, our group proposed a treatment for superficial and deep wounds and for tissue substitution in the form of a “composite graft” [55–59]. This method was a two-step procedure based on xenogenous collagen implantation for dermis substitution and reconstructed keratinocyte allografts for surface covering. Transplanted basal keratinocytes supplied keratinocyte-derived signaling molecules and growth factors, which actively helped to restore a dermo-epidermal exchange pathway and stimulate healing. The collagen material was a ready-to-use, mechanically stable, in vivo non-contractable, primarily free of any non-biologic and synthetic components, nonpyrogenic, biologically and immunologically neutral and long-term preservable membrane. This material could be used both as wound dressing and as an implant for healing chronic, acute and surgical superficial, partial- or full-thickness wounds.

This collagen membrane, originally developed and manufactured by Innocoll (CollatampFascie®, INNOCOLL, Germany) is currently available as sheets ranged from 36.0 × 18.0 mm to 300.0 × 200.0 mm. CollatampFascie® (either bovine or equine origin) has been approved in Europe (CE-mark) as a wound covering material, wound implant and tissue substitute.

Recently, a novel collagen spongy matrix containing oxidized regenerated cellulose (ORC) named Promogran® has been introduced to both US and EU market [60]. Promogran® has been designed to treat exuding wounds including diabetic, venous and pressure ulcers. The matrix is composed of 45% ORC and 55% collagen. ORC/collagen matrix binds to metalloproteases in chronic wound exudate, without altering the activity of essential tissue growth factors and creates a milieu for moist wound healing [60]. Since metallo-proteases may be elevated in chronic wounds and contribute to degradation of important extracellular matrix proteins and inactivate growth factors, their binding into the ORC/collagen matrix may have positive effect on physiological wound healing process [61].

It has been found that Promogran® significantly increases the healing ratio of diabetic foot ulcers compared to traditional moistened gauze procedure, especially in ulcers less than 6 months in duration [62].

Both products described above are a “single layer” construct and may require additional moisture control barrier to complete the dressing.

An original method of Burke et al.’s and Yannas et al.’s artificial skin [40–48] is now called Integra® Regeneration Template and commercialized [63]. Burke et al.’s and Yannas et al.’s artificial skin was a bilayer membrane composed of a dermal portion that consists of a porous lattice of fibers of a cross-linked bovine collagen and GAG composite and an epidermal layer of synthetic polysiloxane polymer (silicone). The GAG that is used is chondroitin-6-sulfate; the degradation rate of the collagen–GAG sponge is controlled by glutaraldehyde-induced cross-links. The collagen–GAG dermal layer functions as a biodegradable template that induces organized regeneration of dermal tissue (neodermis) by the body and the infiltration of fibroblasts, macrophages, lymphocytes and endothelial cells that form a neovascular network. As healing progresses, native collagen is deposited by the fibroblasts, and the collagen portion of artificial skin is biodegraded over approximately 30 days.

Serial biopsies, ranging from 7 days to 2 years after the application of the artificial skin, demonstrated that an intact dermis was achieved with re-growth of apparently normal papillary and reticular dermis. No scar formation appeared in the biopsies of patients examined [63,64].

The superficial silicone layer of Integra® Dermal Regeneration Template is imbedded with monofilament nylon sutures to easily distinguish it from the collagen dermal layer. This pseudo epidermal layer must be eventually removed by the surgeon and is usually replaced by thin epidermal autografts during the second step of transplantation. At present, Integra® Dermal Regeneration Template is approved in the US only for the postexcisional treatment of life-threatening full-thickness or deep partial-thickness thermal injury where sufficient autograft is not available at the time of excision or not desirable due to the physiological condition of the patient.
Another bilayer skin substitute used mostly for severe burns is Biobrane® (Bertec Pharmaceuticals, Morgantown, WV, USA). Biobrane® is a biosynthetic wound dressing constructed from a silicon film with a nylon fabric partially imbedded into the film. The fabric presents to the wound bed a complex 3-D structure of trifilament thread to which collagen has been chemically bound and cross-linked [65]. Blood/plasma clots in the nylon matrix, thereby firmly adhering the dressing to the wound until epithelialization occurs. Biobrane® was introduced in 1979 for commercial use in the treatment of burn wounds and donor sites and has several advantages. These include adherence, safety, control of evaporative water loss, flexibility, durability, bacterial barrier, ease of application and removal, availability, hemostatic properties and cost-effectiveness. In comparison with pig skin and skin allografts, Biobrane showed superior wound adherence. The product has been found to significantly reduce local wound pain, speed up the healing process and significantly prevent bacterial colonization of the wound surface [2,65].

### 4.2. Matrices containing living skin-derived cells

The living cell-containing, bilayered product designed to speed up both partial-thickness or full-thickness wounds including diabetic leg ulcers has also been recently approved both in the US and in Europe.

TransCyte® (Smith & Nephew, Largo, FL, USA) was the first human-based, bioengineered temporary skin substitute for the treatment of excised full-thickness and partial-thickness burns approved by the U.S. Food and Drug Administration in 1997 [66]. TransCyte® consists of a nylon membrane and newborn human keratinocyte cells cultured under aseptic conditions in vitro on a nylon mesh. Prior to cell growth, this nylon mesh is coated with porcine dermal collagen and bonded to a polymer membrane (silicone). This membrane provides a transparent synthetic epidermis when the product is applied to the burn.

As fibroblasts proliferate within the nylon mesh during the manufacturing process, they secrete human dermal collagen, matrix proteins and growth factors. Following freezing, no cellular metabolic activity remains; however, the tissue matrix and bound growth factors are left intact. The human fibroblast-derived temporary skin substitute provides a temporary protective barrier. TransCyte® is transparent and allows direct visual monitoring of the wound bed.

TransCyte® is indicated for use as a temporary wound covering for surgically excised full-thickness and deep partial-thickness thermal burn wounds in patients who require such a covering prior to autograft placement. This product is also indicated for the treatment of mid-dermal to indeterminate depth burn wounds that typically require debridement and that may be expected to heal without autografting.

TransCyte® contains essential human structural and provisional matrix proteins, glycosaminoglycans and growth factors known to facilitate wound healing. The outer layer, synthetic epidermal layer, is biocompatible and protects the wound surface from environmental insults. It is semi-permeable to allow fluid and gas exchange. The inner layer, bio-engineered human dermal matrix, adheres quickly to the wound surface. It contains essential structural proteins (types I, III and V collagen), provisional matrix proteins (fibronectin, tenascin, SPARC), glycosaminoglycans (versican, decorin) and growth factors (TGF-B1, KGF, VEGF, IGF-1). In partial-thickness wounds, the patient’s epithelial cells can proliferate and migrate across the wound, resulting in rapid wound healing.

TransCyte® must be stored frozen between −70 and −20 °C and defrozen directly before use [66].

Recently, a novel bilayer skin substitute—OrCel®—developed by Ortec® (Ortec International, New York, NY, USA) and containing living allogenic human cells have been approved in the US [67].

OrCel® is a bilayered cellular matrix in which human allogeneic epidermal keratinocytes and dermal fibroblasts have been cultured in two separate layers into a type I bovine collagen sponge. Donor dermal fibroblasts are cultured on and within the porous sponge side of the collagen matrix while keratinocytes, from the same donor, are cultured on the coated, non-porous side of the collagen matrix.

OrCel® serves as an absorbable biocompatible matrix that provides a favorable environment for host cell migration and has been shown to contain the following cell-expressed cytokines and growth factors: FGF-1 (bFGF), NGF, GM-CSF, IL-1a, IL-1b, IL-6, HGF, KGF-1 (FGF-7), M-CSF, PDGF-AB, TGF-a, TGF-b1, TGF-b2 and VEGF. OrCel® is not intended to be a human skin replacement and does not contain Langerhans cells, melanocytes, macrophages, lympho-
cytes, blood vessels or hair follicles. DNA analysis performed on two OrCel®-treated donor site patient tissue samples showed no trace of allogeneic cell DNA after 2 or 3 weeks, respectively [67].

Another advanced bioengineered skin equivalent based on xenogenic type I collagen (of bovine origin) was Organogenesis’ main product, Apligraf® [68–70]. It is a commercialized form of “living skin equivalent,” an original idea proposed by Bell et al. [50–52]. This preparation, earlier also known as Graftskin™ [71,72], is made by separating out the cells (keratinocytes and fibroblasts) from normally discarded infant human foreskin. The lower layer (dermis) consists of a collagen matrix formed by purified bovine type I collagen mixed with a suspension of dermal fibroblasts when the collagen matrix is believed to be condensed. This reduction of the volume of collagen approximately 30-fold within days and the formation of dense collagen lattices serve later as the dermal component. In the second step, human dermal keratinocytes are seeded on such a collagen matrix, forming an epidermis-like structure. The Apligraf® obtained the Premarket Approval Application (PMA) from the FDA for use in the treatment of chronic venous leg ulcers and diabetic foot ulcers. In what was said to be the first controlled study of tissue therapy in acute wounds, 20 patients with acute split-thickness donor site wounds were evaluated; this product took clinically and proved a safe, pain-free and effective form of tissue therapy [71]. Since it behaves similar to autograft, its use can avoid the creation of a donor site wound. A 3-in.-diameter (ca. 76.2 mm) discs and a 4 × 8-in. (ca. 101.6 × 203.2 mm) sheets have been available recently.

Unfortunately, the distribution of the product by Novartis (Basel, Switzerland) has been suspended in September 2002 [68]. Organogenesis announced a new distribution agreement with a US-based company [73].

5. Cell–matrix interaction in vivo

There are only a few publications that describe behaviour of xenogenic collagen after implantation into human skin wounds. The possible antigenicity of a xenogenic collagen used for dermis substitution has been discussed above. Chvapil et al. [74,75] and Davies [76,77] described as early as the 1970s that collagen is a practically universal biological molecule that exhibits only very slight differences from species to species and possesses only minimal (if any) antigenic properties to humans.

In a series of in vitro experiments using human epidermal keratinocytes, human dermal fibroblasts and human dermal microvascular endothelial cells no morphological, biochemical or immunological alterations of human cells were observed after short- and long-term in vitro cultivation on the surface of xenogenous collagen membranes [55–59].

Experimental and clinical studies have shown that both a sponge and a film consisting of xenogenic collagen, which was applied to the injured surface of the skin, did not cause any foreign-body reaction, nor any immune rejection reaction or sensitization [23,38].

Studies performed recently also demonstrated that two forms of commercially available “artificial skin” (Integra® Dermal Regeneration Template [64] or Apligraf® (Graftskin) [69]) do not possess immunogenic potential to the human host.

The in situ behaviour of a native, highly purified type I collagen products in form of both sponges and membranes during healing of large-surface and deep tissue defects has been recently described in details [16,31,32,34]. Such products fulfill almost all requirements of an ideal dermal substitute as discussed above. These xenogenous products are free of any bacterial and viral contamination, especially TSE, are not expensive and have a clear EU and FDA regulatory pathway.

The xenogenous collagen did not promote any extensive inflammatory reactions or immunologic rejection. The take of collagen implants and tissue remodelling was complete and quick, allowing them to be even immediately combine with autologous thin split-skin grafting as well as with full-skin grafting. The implanted collagen sheet enhanced the initial adhesion of keratinocytes allotransplants, supporting biological activity of the cells [55].

The newly developed skin manifested good mechanical and biological stability, an absence of edema and blistering, and a complete closing of wound contours [55].

The fully healed skin showed pattern similar to normal skin (with the exception of adnexa) and not that of a scar tissue [55].
Our previous studies demonstrated that the applied xenogenic collagen matrix was infiltrated in the first 2–4 days by a non-specific inflammatory infiltrate consisting of neutrophil granulocytes and macrophages. Significant penetration of the collagen material by the host’s activated fibroblasts and endothelial cells (neo-angiogenesis) were documented as early as the fourth day. These observations correspond to previous data which interpreted the penetration of xenogenic collagen by granulation tissue as an “functional integration” [40–43, 77–81].

In healthy skin, epidermal basal membrane serves as an anchor and sticks the epidermis to the dermis by linking cytoskeleton of keratinocytes with collagen bundles of the dermis. Development of a basement membrane is not possible without fully developed or completely reconstructed and functionally oriented dermis.

In wound healing, the early development of basement membrane has important implications for the stability of the regenerated skin. Delayed or incomplete development of basement membrane will result in blistering and loss of epidermis.

If collagen matrix has been implanted, the reconstruction of basement membrane (lamina densa, Collagen IV) was observed as early as day 7 of wound healing; a mature basement membrane zone was found between the third and fourth week after collagen matrix implantation. The presence of anchor fibrils (Collagen VII) was found in biopsies taken as early as 7 days after collagen implantation. Complete intercellular desmosomes were observed for the first time at day 14 of wound healing (day 10 of epithelialization). The expression of desmosomal proteins corresponds in the fourth week to normal skin [55].

These results indicate a significantly faster development of basement membrane than had been described after transplantation of in vitro reconstructed epithelial grafts without the use of a collagen-based dermal component. Woodley et al. [82], who used in vitro cultivated keratinocyte allotransplants for the treatment of chronic wounds, reported incipient development of lamina densa only after 6 weeks, and its completion not until 5 months from the date of keratinocyte transplantation. Langdon et al. [85], who utilized allogenic dermis with cultivated epidermis autografts for the treatment of chronic wounds, observed the first formation of a lamina densa after 7 weeks, and its completion not before week 14.

The most recent studies also demonstrate that the remodelling of the neodermis occurs more rapidly if native and un-cross-linked collagen products are used. The data suggest that glutaraldehyde cross-linking of collagen matrices may decrease its bioavailability and that delayed degradation of cross-linked collagen may result clinically in reduces engraftment of such matrices [86, 87].

Also, the blistering phenomenon frequently described in the literature did not occur if dermal component has been substituted prior or together with the epidermal portion.

Dermal component reconstructed by membranous or porous collagen matrices may be easily combined with split-skin grafts to speed up final wound closure. Our clinical observations (unpublished data) showed that split-skin grafts may be placed on the top of collagen membrane as soon as after 4 days after such membrane has been implanted into exceeded wound bed preparation.

Collagen matrices may also serve as a carrier and as an on-side donor for recombinant cytokines and growth factors or, if necessary, antibiotics or other medicaments. Moreover, collagen membranes have been successfully used to speed-up the healing and re-epithelialization of split-skin donor sites showing a benefit over other currently used methods [88–90]. The implantation of collagen-based dermis substitutes protect against the contracture of wound borders and against typical scar formation. Both bovine and equine-based collagen matrices, which can be used to substitute missing dermis, are currently approved for this purpose in the European Community.

6. Non-collagen-based products that enhance dermal regeneration

At the end of this review, some interesting and, in part, commercially available products manufactured
without collagen matrix will be discussed. This kind of development can be seen as an answer to some—partially non-scientific—discussions about safety of animal-derived collagen products.

During the 1990s, intensive development has been made on biocompatible materials delivering living cells into the wound bed to stimulate host wound environment and enhance healing ratio.

Smith & Nephew proposed Dermagraft®, a product containing cryopreserved human fibroblast-derived dermal substitute composed of fibroblasts, extracellular matrix and a bioabsorbable scaffold. Although this product does not contain collagen, it has been approved as dermal substitute [91]. Dermagraft® is manufactured from human fibroblast cells derived from newborn foreskin. During the manufacturing process, the human fibroblasts are seeded onto a bioabsorbable polyglactin mesh scaffold. The fibroblasts proliferate to fill the interstices of this scaffold and secrete human dermal collagen, matrix proteins, growth factors and cytokines to create a three-dimensional human dermal substitute containing metabolically active, living cells. Dermagraft® does not contain macrophages, lymphocytes, blood vessels or hair follicles. The product indicated for use in the treatment of full-thickness diabetic foot ulcers greater than 6 weeks in duration, which extend through the dermis, but without tendon, muscle, joint capsule or bone exposure. Dermagraft® should be used in conjunction with standard wound care regimens and in patients that have adequate blood supply to the involved foot.

Clinical study demonstrated that patients treated with Dermagraft® were 1.7 times more likely to heal than the control group (conventional therapy included sharp debridement, saline-moistened gauze and pressure-reducing footwear) at any given time during the study ($p = 0.044$; two-sided Cox’s proportional hazards model). Chronic diabetic foot ulcers treated with Dermagraft® closed significantly faster than ulcers treated with conventional therapy alone ($p = 0.040$; two-sided log rank test) [91].

Another possibility was to utilize hyaluronic acid manufactured in the form of non-porous and porous membranes [92]. Such membranes have been used alone or as a carrier for living cells. In this case, the patient’s own cells have been cultivated in vitro and seeded on the membrane prior to clinical application. Lasergraft® autograft is an epidermal substitute consisting of autologous keratinocytes on a laser-microperforated membrane of hyaluronic acid (HYAFF®, Abano Terme, Anato Terme, Italy). Hyalograft 3-D and Laserskin (Hyaff-11) are indicated for use on diabetic foot ulcers and venous leg ulcers. Comprised entirely of a benzyl ester derivative of hyaluronic acid, they may be used as scaffolds for the cultivation of fibroblasts and keratinocytes. Results of an animal study and preliminary clinical data on Laserskin have shown durability, good take rates and low infection rates when cultured with autologous keratinocytes and autologous/allogeneic fibroblast feeder cells. Recently, this product has been described in the treatment of diabetic foot ulcers using a two-step technique. Following wound debridement, the fibroblast grafts were applied and covered with paraffin gauze. Keratinocyte grafts were then applied after approximately 7 days and covered with gauze for a further 7 days prior to inspection. Complete healing with no complication was achieved in 53 of 58 patients (91%) in an average time of $72 \pm 48.18$ days. Subsequent histology showed good integration of grafts into newly formed granulation tissue. While these preliminary results are promising, the authors acknowledged that they should be followed up with a randomised, controlled clinical trial [92].

Recently, products designed to enhance healing of difficult to treat dermal and mucosal wounds have been designed using fibrin glue as a carrier for living cells [93]. These products are based on a two-component fibrin glue technology (Baxter International, USA) in which autologous keratinocytes have been incorporated prior to the application. BioTissue, Freiburg, Germany, has originally developed the product (BioSeed®-S and BioSeed®-M).

BioSeed®-S is an “Autologous Skin Graft” for treating poorly healing wounds. For BioSeed®-S treatment, a small piece of the patient’s own skin has to be removed. The skin cells are isolated and grown in the BioTissue laboratories. After about 2–3 weeks, the cells in fibrin adhesive (biological tissue glue) are applied to the patient’s wound. The fibrin adhesive fixes the cells to the wound and allows better in-growth. BioSeed®-S is also very simple for the doctor to handle—the gel-like skin graft is applied to the wound using a syringe. The special feature of BioSeed®-S is that the cells are still capable of dividing, which means
that they continue to increase in number after grafting and thus close the wound up [93].

BioSeed®-M is an autologous oral mucosa replacement, which is used in oral surgery and for dental treatment. For instance, BioSeed®-M is employed in oral surgery following accidents and oral cancer or in the context of implant treatment. The doctor takes a small piece of mucosa from the patient’s mouth; the mucosal cells are grown in the laboratory and, together with a carrier foil, are transplanted back into the mouth after about three weeks. This involves placing the newly cultured oral mucosa cells directly onto the wound surface in the oral cavity, where they form a natural oral mucosal structure. This saves the patient from the alternative, which is the painful removal and grafting of his or her own mucosa onto the hard palate. More than 70 patients were successfully treated with BioSeed®-M in the Clinic for Dental and Oral Surgery, University of Freiburg, Germany. Better functional and cosmetic results were achieved than by the conventional method [93].

Unfortunately, BioTissue Technologies filed a declaration of bankruptcy on July 1, 2003. It is not known if this interesting technology will be further developed [93].

7. New developments and perspectives

The use of an appropriate biologically active matrix to speed up the healing of missing tissue becomes a standard in skin reconstruction [94,95]. But what are the sources of new cell population in healing wound? Many cells derive from adjacent tissue (local pre-existing cell population), but there are increasing evidences that both circulating (marrow-derived) and pre-existing (organ-specific) stem cells can evolve tissue regeneration. These cells have surface markers of progenitor cells and a board differentiation capacity. Transfer studies demonstrated that mesenchymal precursors can populate several tissues, and that vasculogenesis can arise from circulating endothelial progenitor cells such [96].

The availability of such cell types may be rate-limiting for dermal wound healing. Moreover, stem cells are important targets for the local application of growth factors and for gene therapy and participate in the natural healing processes occurring during the wound organization and remodeling. The CD-34-positive cell population and its subpopulations (i.e., fibrocytes) have been demonstrated to participate into rapid new vessel and matrix formation. They secrete chemokines, hematopoetic growth factors and fibrogenic cytokines. Moreover, this progenitor cells have been found to express each of the surface components that are required for antigen presentation, including class II MHC, co-stimulatory signaling molecules and different adhesion molecules [97–99].

The application of autologous (or allogenic) stem cells alone or together with some specific growth factors utilized in an appropriate three-dimensional matrix may help to initiate, control and, if necessary, also terminate the de novo reconstruction of missing skin.

Recently, the State Drug Administration of China approved the production and marketing of a domestically developed artificial skin. Produced by Shandong-based Greenleaf Pharmaceutical, the artificial skin is said to be the first of its kind in the world.

A Shandong Greenleaf spokesman said the artificial skin, which is manufactured using a special cream in conjunction with living marine material, would be used in the treatment of patients with skin injuries such as scalds, burns and scars [100]. No other information about the matrix or active ingredients as well as clinical benefit of this product is currently available.

8. Conclusion

There are many different approaches to speed up the healing of dermal wounds.

In the last decade, the main interest has been on the development of an in vitro reconstructed skin that can be transplanted directly to the wound bed and permanently replace the missing tissue. Neither the commercially available products nor the products currently described in experimental studies are able to fully substitute the natural living skin [101–105]. A big effort has, however, been achieved in the substitution of the main component of each wound, the missing connective tissue matrix creating human dermis. Once dermis is reconstructed, the covering of the wound surface with both in vitro expanded epidermis and autologous split-skin transplants is significantly easier and has much better chance to succeed.
Collagen is a long-time known protein. It has been extensively studied in the 1970s and early 1980s. Beginning late 1980s, artificial polymers seemed to push collagen into the corner of medical history. However, artificial polymers—despite their programming biodegradability, good mechanical properties and (relatively) easy manufacturing—cannot provide some fundamental requirements such as biocompatibility, receptors necessary for cell migration and growth promotion, neo-angiogenesis support and scar-free healing [106].

The handling of collagen-based product currently used for dermal substitution is easy. However, Integra® Dermal Regeneration Template must be extensively washed before use, Apligraf® must be defrosted and pre-warmed, and CollatampFascie® needs to be rehydrated. Other products containing living cells (Transcyte®, OrCel®, Dermagraft®) require only a few simple preparation steps. Biobrane® is principally ready to use as packed and may be obtain in two forms having either a “standard” or “low” adhesion. SkinTemp® can be easily applied on superficial and partial-thickness wounds and also easy removed. This product is approved in the US as a temporal wound dressing and has to be removed from the wound within 7 days.

Clinically, all products can be applied on all types of skin wounds (including burns and split-skin donor sites). Additionally, collagen membranes used as a single agent can be used on mucous membranes. Collagen sponges and membranes used as a single agent or in combination with other implantable agents can be applied for deep tissue substitution and filling of dead spaces as well as a cover for nerves, tendons, ligaments or vessels without or, if necessary, with additional fixation (i.e., by a tissue sealant). Comparison of the effect of a collagen membrane versus a polyurethane membrane on the healing of split-thickness graft donor sites showed that the use of collagen significantly improved wound healing time and quality [88–90].

A further advantage of collagen products is the possibility to combine them with both patient-derived (autologous) and recombinant growth factors, cytokines, living cells and other stimulatory or antimicrobial agents to speed up the granulation tissue, scar-free healing and re-epithelialization. Currently, commercially manufactured collagen sponges containing immobilized antibiotic (e.g., gentamicin, Collatamp®-G, Innocoll, Germany, or Sulmycin®-Implant, Schering-Plough, USA) approved in many countries worldwide are successfully used to treat chronic wounds (leg ulcers, decubital wounds, etc.) and to treat and prevent wound infection in burns serving both as dermis substitutes and controlled drug delivery system.

It is remarkable, however, that none of engineered skin substitutes containing living cells (even those approved in the US) have been approved in Europe. Due to the lack of legal basis that regulates both manufacturing safety and the use of human-derived living cells in Europe, products like Apligraf®, Transcyte®, Dermagraft®, OrCell® or BioSeed® could not be fully introduced into the European market.

Nowadays, collagen—an old good and safe natural polymer—has been discovered again. It seems to be the best currently available matrix for tissue and organ regeneration. Collagen is generally treated as a “self” tissue by recipients into whom it is placed and is subjected to the fundamental biological processes of tissue degradation and integration into adjacent host tissues when left in its native ultrastructure. Collagen is a natural substrate for cellular attachment, growth and differentiation in its native state. In addition to its desirable structural properties, collagen has functional properties. Certain sequences of the collagen fibrils are chemotactic and promote cellular proliferation and differentiation. This is especially the case for native, insoluble and highly purified reconstituted materials, which, due to their excellent bioavailability and “natural-like” incorporation into the host tissue, seems to be in favor over chemically cross-linked collagen materials.

References


Parenteau, Cultured skin as a “smart material” for healing wounds: experience in venous ulcers, Biomaterials 17 (1996) 311–320.


