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Dermal substitutes for full-thickness wounds in a one-stage grafting model

HENRY J. C. DE VRIES, MD[a]; JAN R. MEKKES, MD[a]; ESTHER MIDDELKOOP, PhD[a]; WOUTER L. J. HINRICHS, PhD[b]; CHARLES R. H. WILDEVUUR, MD, PhD[b]; WIETE WESTERHOF, MD, PhD[a]

We tested different biodegradable matrix materials as dermal substitutes in a porcine wound model. Matrixes were covered with a split-skin mesh graft and protected with a microporous, semipermeable membrane, which prevents blister formation, wound infection and provides ultimate healing conditions. Evaluation parameters were as follows: epithelization, dermal reconstitution, wound contraction, and cosmetic and functional aspect. A microfibrillar matrix of nondenatured collagen gave the best result, with immediate fibroblast ingrowth and epidermal outgrowth. Slight inflammatory reaction and minimal wound contraction were observed. Application of a split-skin mesh graft, in combination with this collagen matrix, generated a thicker dermal layer than did a split-skin mesh graft directly applied on a wound bed. However, the histologic dermal architecture was less optimal than one obtained with a full-thickness punch graft method. Other matrixes caused inflammatory reactions, interfering with epithelization and dermal reconstitution. We conclude that a nondenatured collagen matrix, in combination with a split-skin mesh graft, can provide a substitute dermis in a full-thickness wound. This combination is preferable to a split-skin mesh graft directly applied on the wound bed. With our microporous semipermeable membrane, the combined use of a dermal substitute and a split-skin mesh graft can be applied in a single-stage operation. **(WOUND REP REG 1993;1:244-52.)**

Normally, ingrowth of epithelium over full-thickness skin defects takes place from the wound edges at a rate of about 1 to 2 mm/day. Various grafting techniques have been developed to accelerate wound healing for larger skin defects. Autologous full-thickness grafts consisting of both epidermis and dermis provide the best functional and cosmetic results. The more dermis that is transferred with a skin graft, the more rapid regeneration occurs, with less contraction and scarring.[1] The use of full-thickness grafts is intrinsically restricted by the defects created at the donor sites. For small skin defects, the application of full-thickness *pinch* grafts is the treatment of choice.[2] We prefer a modification of this technique, the full-thickness *punch* graft method, and consider this currently the best treatment for venous leg ulcers, where donor skin is available in sufficient amounts.[3]

For efficient wound closure of larger skin defects, such as large burns, split-skin mesh grafts are commonly used. Scarring and wound contraction make this technique inferior to full-thickness grafting, especially in deep dermal defects. This also applies to grafts consisting of cultured pure epidermal sheets.[4-8] The need for both dermal and epidermal components in the healing of full-thickness wounds has been recognized, and both the importance of dermal control in the regulation of epidermal proliferation and the epidermal modulation of dermal elements is crucial in wound healing.[9-11]

Efforts have been made to develop a cultured full-thickness skin equivalent.[12,13] Culturing of the dermal and epidermal component requires at least 3 weeks. A sophisticated laboratory with highly trained personnel is necessary. Consequently, this technique is not applicable in every center.

The use of an artificial biodegradable dermal matrix together with a thin split-skin mesh graft might combine satisfactory dermal reconstitution with the possibility to treat large skin defects.

The first attempt to substitute dermis with an artificial matrix was carried out in 1962 by Chardack et

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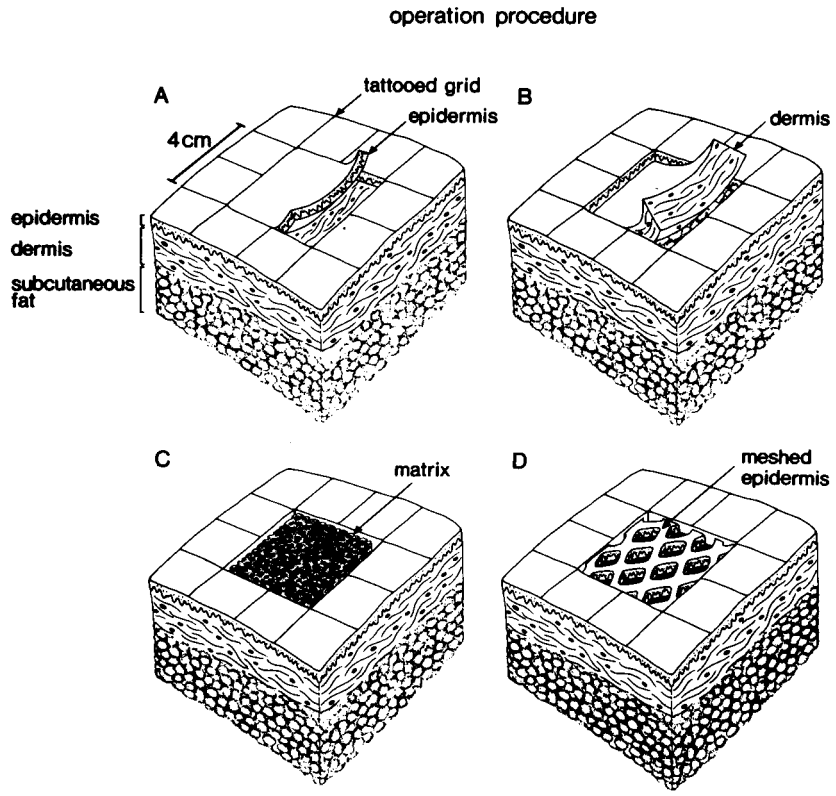

Figure 1. Operative procedure: **A,** split-skin graft is harvested in the wound area; **B,** full-thickness wound is created; **C,** dermis is replaced by a porous matrix; and **D,** the matrix is covered with a split-skin mesh graft.

Table 1. Types of matrixes

Treatment	No. of tests	No. of animals	Manufacturer	Thickness (mm)	Degradation time (days)	Pore size (μm)
Nondenatured collagen type I	7	5	Bournonville Pharma, Almere, The Netherlands	1.0	14-20	≈100
Reconstituted collagen type I	3	3	OPG Biomaterials, Utrecht, The Netherlands	1.0	–	≈100
Polyether urethane	3	3	University of Twente, Enschede, The Netherlands	2.0	≈28	≈75
Polyglactin	3	3	Johnson & Johnson, Amersfoort, The Netherlands	1.5	60-90	≈250
Punch biopsy	6	5				
Split-skin graft	4	4				

al.[14] They tested a formalinized porous polyvinyl alcohol sponge covered with a silicone rubber sheet on full-thickness defects on the backs of pigs. The sponge was invaded by granulation tissue within a few days, but a severe inflammatory reaction with giant cell formation and fibrosis occurred in the end. In the early 1970s, Yannas et al.[15] designed a skin substitute consisting of an outer layer of silicone film, covering an inner layer of porous biodegradable bovine collagen, cross-linked with chondroitin-6-sulphate. When this skin substitute was placed on freshly excised wounds of a burn patient, new dermis reformed within the matrix.[16,17] After 2 weeks, a second operation was necessary in which the outer silicone layer was peeled off and a mesh split-skin graft was applied on the granulating wound bed. Because we have developed a microporous polyether urethane membrane (Exkin; Utermöhlen, Utrecht, The Netherlands),[18] which lim-

its wound infection and creates optimal healing circumstances, mesh split-skin grafts can now directly be applied on a biodegradable dermal matrix in one operation session. In this single-stage treatment model we tested different porous biodegradable matrixes as substituents of dermis.

METHODS

We started with four readily obtained porous "dermal" matrix materials (Table 1): (1) microfibrillar nondenatured bovine collagen (Colgen; Bournonville Pharma, Almere, The Netherlands), (2) microfibrillar glutaraldehyde reconstituted sheep collagen (OPG Biomaterials, Utrecht, The Netherlands), (3) a woven structure of polyglactin (Vicryl; Johnson & Johnson, Amersfoort, The Netherlands), and (4) a polyether urethane matrix (University of Twente, Enschede, The Netherlands).

A novel semipermeable bilayered polyether urethane membrane (Exkin; Utermöhlen, Utrecht, The Netherlands), was used to cover the wounds. Its top layer is microporous, pore size 0.3 to 1.0 μm (thickness 5 μm) to prevent bacterial penetration. Simultaneously it limits evaporative water loss but allows effective wound drainage. The bottom layer has a pore size of 50 to 200 μm (thickness 150 μm), and adheres immediately and firmly to the wound surface. This is achieved by capillary suction forces through the pores and rapid binding of fibrin to its anchoring structures.[18]

Operation procedure

This study was approved by the Animal Use Committee from the University of Amsterdam. On arrival, female domestic New Yorkshire pigs weighing 20 kg were washed and placed in quarantine for 7 days. In this period the animals could get used to the new environment and be submitted to a daily health inspection.

A 2 × 2 cm grid was tattooed on both flanks (Figure 1) 3 to 7 days before surgery. The hair was clipped from the skin extending from the forelegs to the hind legs across the back and flanks. Immediately before the procedure the pigs were sedated with azaperon (Stressnil; Janssen, Gent, Belgium), administered intravenously. They were anaesthetized with a mixture of halothane, oxygen, and nitrous oxide with a face mask. The procedure was carried out under sterile conditions. Incisions of controlled depth (1 mm) were filled with sterile antiseptic tattooing paste (Foliac tattooing paste; Rocol, Leeds, Great Britain).

After 3 days, again under general anesthesia, six 4 × 4 cm split-skin grafts (0.15 mm thickness) were obtained from outlined areas with a Brown electric dermatome (Zimmer, Maarssen, The Netherlands). Split-skin grafts were expanded by a meshing device up to a 1:3 ratio. The superficial donor site wounds were re-excised with the electric dermatome to a depth of approximately 2.5 mm. Thus, six full-thickness wounds were created, surrounded by a 2 × 2 cm grid (Figure 1). All dermal matrixes were soaked in saline solution before implantation. Matrixes were positioned into the wounds. Split-skin mesh grafts were applied over the matrixes and fixed with 6 Vicryl 3-0 sutures. All wounds were covered with the polyether urethane top layer. Finally, the wounds were protected against mechanical trauma with a covering hydrophillic gauze layer and fixed with adhesive tape (Mefix; Mölynlycke Health Care AB, Mölynlycke, Sweden) and elastic stockings (Tubigrip; Seton Healthcare Group P/C, Oldham, United Kingdom). Bacterial cultures were obtained from the wounds after 1 week to exclude infected wounds. Wounds were inspected and photographed each week, from week 2 to week 6. Each week a 4 mm punch biopsy specimen was taken. The biopsy specimens were fixed in alcoholic formalin, processed by routine histologic procedures, and embedded in paraffin. Serial sections were cut at 6 to 8 μm thickness for routine observations (hematoxylin and eosin staining). For immunohistochemical observations, a human monoclonal antibody against laminin, cross-reacting with porcine tissue was used.

Evaluation methods

Wound evaluation and the evaluation of the microscopical slides were executed by three independent arbitrators. Dermal matrixes were examined on the following selection criteria.

Percentage of split-skin mesh graft that could survive on top of the matrix was estimated visually after 1 week. At this time point it was easy to distinguish whether the grafts were viable and attached to the underlying wound bed or had become necrotic and detached from the underlying surface. Speed of epithelization was surveyed weekly, whereas several qualitative parameters of epithelialization were evaluated after 6 weeks. These parameters were as follows: the number of cell layers, terminal differentiation, and quality of the basement membrane, visualized with an anti-laminin antibody. Laminin is present in all basement membranes and plays a role in the specific attachment of keratinocytes to the basal lamina.[19,20]

Inflammatory reactions directed against the dermal matrix were studied with regard to type, severity, and persistence of the inflammatory infiltrate. On day 7,

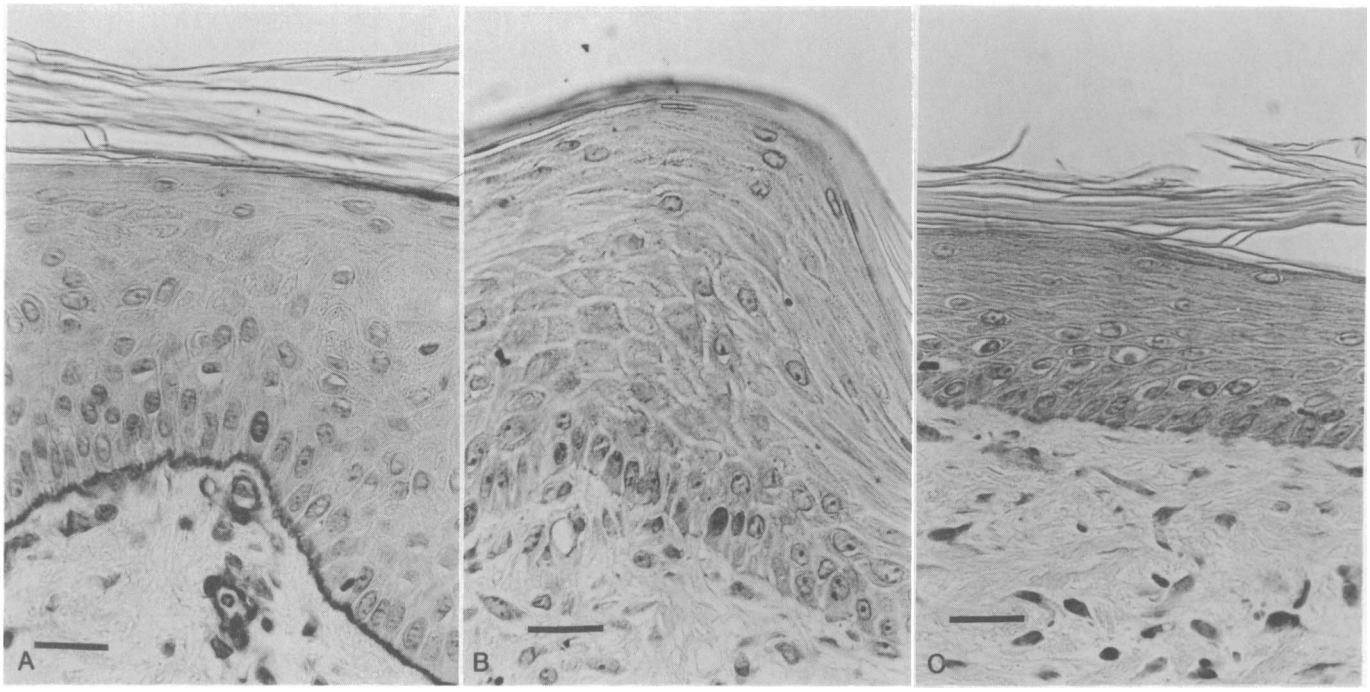

Figure 2. Basement membrane regeneration after 6 weeks, as visualized by anti-laminin staining. **A,** Nondenatured collagen matrix. Normal terminal keratinocyte differentiation and a continuous laminin layer are present. **B,** Polyglactin matrix. Abnormal keratinocyte differentiation with parakeratotic cornification can be noted. No laminin is present in the basal layer. **C,** Polyether urethane matrix. A thin epidermal layer has formed and the laminin layer is discontinuous. (Original magnification ×760; *scale bar* indicates 25 μm.)

Table 2. Split-skin mesh graft take and speed of complete epithelization

Treatment modality	No.	Take	Epithelization (weeks)	Laminin in basement membrane
Split-skin graft	4	Fully	2	Present
Nondenatured collagen	7	Fully	2	Present
Polyether urethane	3	Fully	3	Discontinuous
Reconstituted collagen	3	Partly	4	Present
Polyglactin	3	Poor	5	Absent

Fully, Take of at least 90% of the split-skin mesh graft; *Partly*, take of between 50% and 90% of the split-skin mesh graft; *Poor*, take of less than 50% of the split-skin mesh graft.

swabs for bacterial cultures were taken from each wound. Wounds that were contaminated were excluded from further observation.

Dermal reconstitution was evaluated after 6 weeks. Thickness of the dermis was measured with a microscope with a built-in scale. Collagen fiber maturation was visualized with a polarization microscope (mature collagen fibers cause double refraction of polarized light, granulation tissue does not). The amount of fibroblasts and endothelial cells in the dermis was estimated.

Cosmetic and functional aspect were also evaluated after 6 weeks. An important parameter was wound contraction. The contraction was defined as maximal approximation of opposite wound margins and expressed as a percentage of decrease of the initial wound. Contraction was corrected for local expansion of the skin (expansion of the tattooed grid around the wounds) caused by growth of the animal. Other cosmetic parameters were as follows: skin color, skin smoothness, skin fold thickness, and the skin level of the regenerated skin.

Reference wound healing methods and matrix requirements

As a reference for optimal dermal and epidermal regeneration, we used a full-thickness punch graft method. Wound contraction and speed of epithelization were compared with the conventional split-skin mesh graft method (without a dermal matrix).

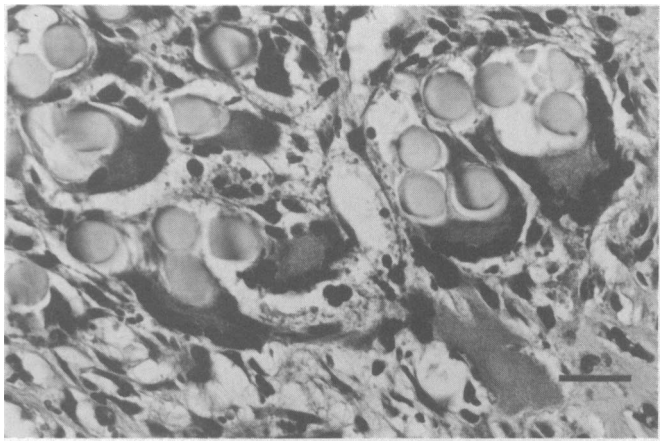

Figure 3. Giant cell formation around polyglactin fibers at week 2. (Hematoxylin and eosin staining, original magnification 760×; *scale bar* indicates 25 μm.)

RESULTS

Most matrix materials were malleable and easy to handle, except for the polyglactin pads, which were rigid. Nondenatured collagen and reconstituted collagen turned into a gel-like substance in contact with saline solution. The protective top layer of polyether urethane attached firmly to the open wound surfaces. Between the split-skin mesh graft and the punch biopsy sites, wound exudate leaked through the polyether urethane top layer. Blister formation did not occur.

Take of split-skin mesh graft and speed of epithelization

On top of the nondenatured collagen and the polyether urethane substitutes, the split-skin mesh graft took fully (over 90%; respectively, $n = 7$ and $n = 3$) (Table 2). Epithelization of the nondenatured collagen matrix and the reference wound treated with a split-skin mesh graft only was completed in 2 weeks (respectively, $n = 6$ and $n = 4$). Epithelization of the polyether urethane was completed in 3 weeks. On the reconstituted collagen matrix, the split-skin graft took only partly (50% to 70%, $n = 3$), and epithelization was completed in 4 weeks. Poor split-skin graft take occurred on the polyglactin pad (<50%, $n = 3$).

Epidermal histologic characteristics

Wounds treated with nondenatured (Figure 2, *A*) and reconstituted collagen showed a basement membrane that appeared normal when stained with an anti-laminin monoclonal antibody. Wounds treated with polyglactin (Figure 2, *B*) and polyether urethane (Figure 2, *C*) showed discontinuous or absent staining of laminin in the basement membranes and caused parakeratotic cornification.

Inflammatory reactions

No bacterial infection was experienced in the evaluated wounds. The nondenatured collagen matrix ($n = 7$) gave little influx of macrophages and some lymphocytes (Table 3). A small amount of inflammatory cells, grouped around capillaries, remained for the duration of the experiment. No infiltration of granulocytes or granulomatous tissue occurred.

Reconstituted collagen ($n = 3$), polyether urethane ($n = 3$) and polyglactin ($n = 3$) all elicited a foreign body reaction. The inflammatory infiltrate consisted of large amounts of macrophages and multinuclear giant cells formed around the implanted materials (Figure 3). A granulomatous process persisted up to 6 weeks in all wounds treated with these three materials.

Dermal reconstitution

The punch graft reference method ($n = 6$) showed newly formed collagen bundles after 3 weeks. At week 6, wavelike collagen bundles were formed in a neodermis of normal thickness. With the split-skin graft reference method ($n = 4$), little dermis was reconstituted (Figure 4, *A*). Moreover, features of scar tissue–like thick collagen bundles and few capillaries were noticeable.

Of all tested dermal matrixes, only nondenatured collagen ($n = 7$) gave rise to dermal tissue reconstitution (Figure 4, *B*). Newly formed collagen bundles started to appear from week 2 on. After 6 weeks, the dermis consisted of thin, mature collagen fibers, a normal amount of fibroblasts, and a normal appearing capillary network. When the mean dermal thickness reconstituted by the non-cross-linked dermal matrix (3.0 mm ± 0.7, $n = 7$) was compared with the dermal thickness regenerated with the split-skin mesh graft method (1.7 mm ± 0.5, $n = 4$), a statistically significant difference was noted ($p < 0.01$, Student's t test).

In contrast, reconstituted collagen, polyether urethane, and polyglactin proved to be unsuitable as dermal substitutes. Hypergranulation persisted for the duration of the experiment without maturation of collagen fibers, and no neodermis regenerated.

Wound contraction and cosmetic and functional aspect

The skin of the wound treated with full-thickness punch grafts felt firm and supple and was in the same

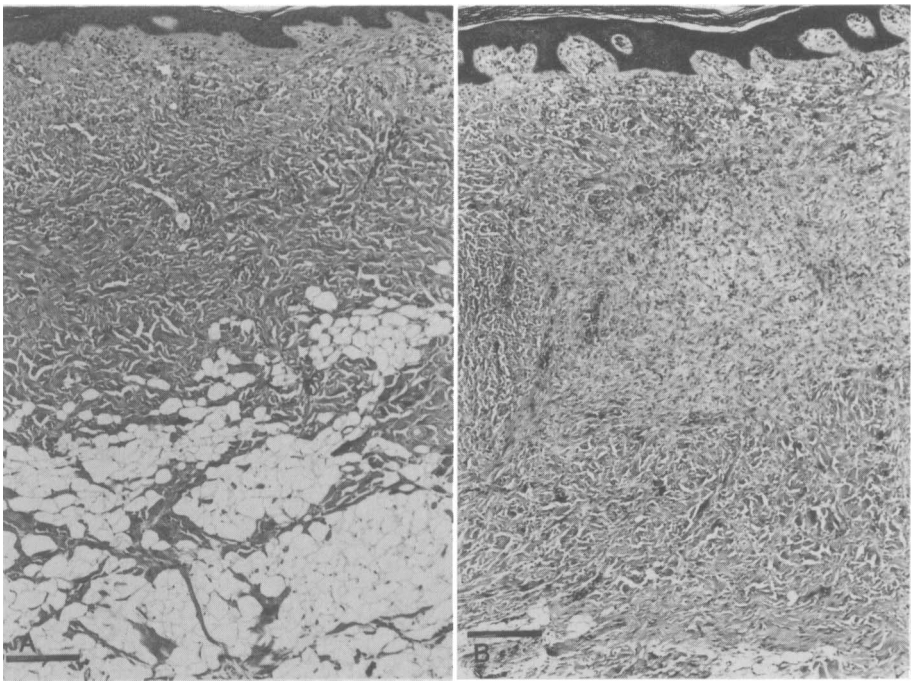

Figure 4. Dermal reconstitution after 6 weeks. **A,** Cross section of the split-skin mesh graft treated wound. Sparse amount of cells are visible in a thin dermal layer. **B,** Cross section of the wound treated with non-cross-linked collagen together with split-skin mesh grafts. Significantly more dermis has reformed. The dermal layer is rich in cells. (Hematoxylin and eosin staining, original magnification 190×; *scale bar* indicates 100 μm.)

Table 3. Foreign body reaction and dermal reconstitution at 6 weeks after the operation

Treatment modality	No.	Foreign body reaction	Dermal reconstitution	Dermal thickness (mm)*
Split-skin graft	4	No	Yes	1.7 ± 0.5†‡
Punch biopsy	6	No	Yes	3.1 ± 1.0†
Nondenatured collagen	7	No	Yes	3.0 ± 0.7‡
Reconstituted collagen	3	Yes	No	Hypergranulative tissue
Polyether urethane	3	Yes	No	Hypergranulative tissue
Polyglactin	3	Yes	No	Hypergranulative tissue

*Mean dermal thickness ± standard deviation of the mean.
†$p < 0.05$, Student's t test.
‡$p < 0.01$, Student's t test.

level as the surrounding hide (Table 4). However, a disfiguring cobble stone aspect, caused by the remainders of the full-thickness biopsy specimens, makes this method cosmetically dissatisfying (Figure 5, *A*). Wounds treated with the conventional split-skin mesh graft method reformed a thin skin, which was not in level with the surrounding hide. A mesh pattern remained visible in the regenerated skin. The best cosmetic result was achieved with nondenatured collagen (Figure 5, *B*). The upper skin layer was smooth and had the same color as the unaffected skin, yet it lacked the firmness and thickness of normal pig hide.

Minor contraction was observed with nondenatured collagen matrix (21% ± 3%, $n = 7$) (Table 4). Polyether urethane (Figure 5, *C*) and reconstituted collagen matrixes caused severe wound contraction (respectively, 40% ± 15%, $p < 0.02$, and 42% ± 12%, $p < 0.01$, n = 3; p values when compared with nondenatured collagen). All polyether urethane (Figure 5, *C*), reconstituted collagen and polyglactin matrixes caused indurated scars.

DISCUSSION

The aim of these studies was to develop a single-step skin grafting procedure that gives rise to a normal dermal architecture. The use of the Exkin membrane as a protective layer made it possible to conduct a

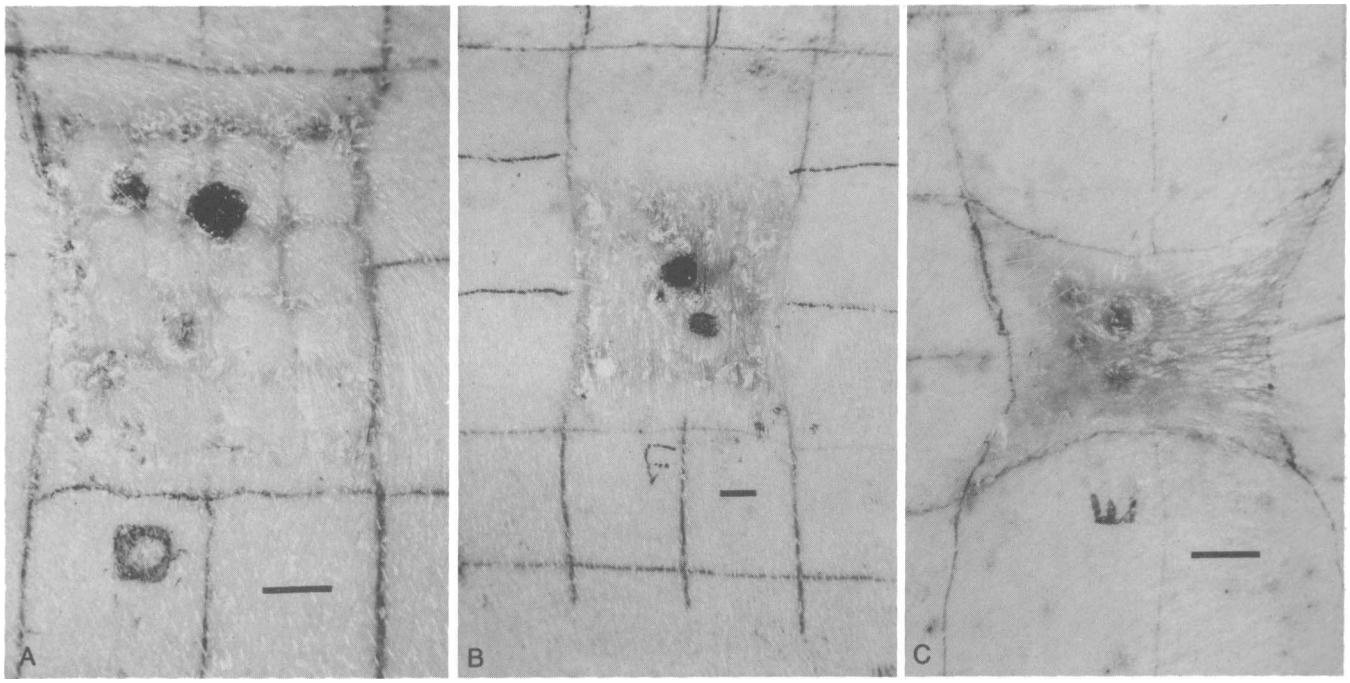

Figure 5. Cosmetic aspects of the wounds after 6 weeks. **A,** Cobble stone aspect of the full-thickness punch biopsy method. **B,** Split-skin mesh graft combined with nondenatured collagen dermal substitute. **C,** Split-skin mesh graft combined with a polyether urethane dermal substitute. Severe wound contaction has deformed the tattooed grid. Circular scabs are caused by biopsy specimens taken for histologic examination. (*Scale bar* indicates 1.0 cm).

Table 4. Cosmetic result at 6 weeks after the operation

Treatment modality	No.	Wound contraction*		Smoothness†	Color‡	Skin fold thickness§	Skin level‖	Scar tissue formation
Split-skin graft	4	19% ± 7%		+	+	−	−	No
Punch biopsy	6	24% ± 5%		0	+	+	0	No
Nondenatured collagen	7	21% ± 3%	¶	+	+	0	0	No
Reconstituted collagen	3	42% ± 12%	#	−	0	0	0	Yes
Polyether urethane	3	40% ± 15%		0	0	−	0	Yes
Polyglactin	3	24% ± 6%		−	−	0	+	Yes

*Wound contraction: mean percentage of decrease of the initial wound ± standard deviation of the mean.
† +, Like normal skin; *0*, partly normal, partly irregular skin; −, irregular skin.
‡ +, White as normal skin; *0*, pinkish; −, red like active scar tissue.
§ +, 16 to 20 mm; *0*, 14 to 16 mm or 20 to 22 mm; −, >22 mm or < 14 mm.
‖ Compared with the surrounding skin: +, elevated; *0*, in level; −, depressed.
¶ $p < 0.01$, Student's t test.
# $p < 0.02$, Student's t test.

single-stage operation. In contrast, the skin substitute of Yannas et al.[15] requires a second operation to remove the protective silicone layer and apply split-skin mesh grafts. The results show that our idea is feasible. The split-skin mesh graft could grow immediately over the nondenatured collagen matrix. At the same time, mesenchymal cells from the wound bed infiltrated the matrix and replaced it gradually with neodermal tissue that differed from the scar tissue seen with the conventional split-skin mesh graft method.

Speed of epithelization and dermal reconstitution depend on the ultrastructure of the matrix. Cells need a supporting stroma for movement and attachment if they are to flourish.[21] Collagen appears to have a nearly

ideal surface geometry for fibroblast and keratinocyte migration. Complete epithelization within 2 weeks was observed on the nondenatured collagen matrix, suggesting that on this material epidermal cells can spread as quickly as on an ideal wound bed. This is likely to be attributed to the fibrillar structure of collagen. Our experience with a gelatin (Gelfoam; Upjohn, Kalamazoo, Mich.) dermal matrix (data not shown) is noteworthy. Although the split-skin mesh graft remained viable, epidermal cells could not migrate over the gelatin matrix. Only after 2 weeks, when most of the gelatin was degraded, did epithelization start to occur and was completed after 3 weeks. Ingrowth of granulation tissue was delayed compared with the collagen matrix. It appears that gelatin lacks the right structure for contact guidance, a phenomenon also noted by Suzuki et al.[22]

A fibrillar pattern that resembles normal dermis not only facilitates epidermal cells to spread, but also directs fibroblasts to grow in and to synthesize normal connective tissue elements.[23,24] The combination of a nondenatured dermal matrix and a split-skin mesh graft resulted in the formation of a neodermis, which was significantly thicker than the neodermis of the reference wound treated with a split-skin mesh graft only (mean dermal thickness 3.0 mm $\pm$ 0.7 mm and 1.7 mm $\pm$ 0.5 mm, respectively; $p = 0.01$, Student's t test).

Split-skin mesh grafts did not fully survive on top of the polyglactin and the reconstituted collagen matrixes. The grafts became partly necrotic as a result of a severe foreign body reaction against the implanted materials. Matrix materials that caused such a chronic inflammatory reaction also interfered with dermal reconstitution. Reconstituted collagen, cross-linked with glutaraldehyde, induced a granulomatous reaction and persistence of hypergranulation. In contact with water, aldehydes form polymers with large molecular weights.[25] These polymers are retained within the collagen structure because of their size. After implantation, continued hydrolysis induces toxicity. In a pilot study, a dermal matrix of caprolactone/morpholinedione also elicited a severe polymorphonuclear and granulomatous reaction, which caused graft rejection, probably because of toxic components (data not shown).

Polyglactin as a dermal matrix evoked a chronic inflammatory reaction in our study. This is in contrast to results with a comparable polyglactin dermal substitute called Dermagraft (Advanced Tissue Sciences, Inc., La Jolla, Calif.).[26,27] This matrix material was tested in an athymic mice model, and no serious foreign body reaction against the implanted material was reported. The discrepancy may be caused by a difference in species-specific phagocytosis of the animals used in the two studies.

The best dermal architecture was achieved with the full-thickness punch graft method. Wave-like collagen bundles and randomly arranged fibroblasts were visible between the biopsy sites. In contrast, dermis of the nondenatured collagen showed thinner collagen bundles, which is a sign of poorer dermal integrity. It is likely that the incorporation of the extra dermal fibroblasts through transplantation of punch biopsy specimens is responsible for a more normal dermal architecture.

Wound contraction and scar tissue formation were elicited when a chronic inflammatory reaction against the dermal matrix occurred. This was the case with the reconstituted collagen, polyether urethane, and the polyglactin material.

Although the best histologic result was achieved with the transplantation of full-thickness punch biopsy specimens, this method left a disfiguring cobble stone pattern in the regenerated skin (Figure 5, *A*). With the application of the conventional split-skin graft method, the mesh pattern remained visible in an indurated skin with poor integrity.

The combination of a nondenatured collagen matrix and a split-skin mesh graft improved the cosmetic result of the full-thickness defects (Figure 5, *B*). A smooth, normal-looking skin regenerated.

Our results with a nondenatured collagen dermal matrix and split-skin mesh graft covered with the Exkin membrane indicate that dermal substitution in full-thickness wounds is feasible in a single-stage treatment. However, the histologic comparison with the full-thickness punch graft method suggests that transplantation of dermal cellular components might improve the grafting method. The combination of a nondenatured collagen matrix with seeded dermal cells, in addition to a split-skin mesh graft, might contribute to a better dermal architecture and function.[28] Our direction is to use autologous cells that are harvested, seeded in a dermal substitute, and placed directly into the wound as a single-stage treatment. Our microporous polyether urethane membrane, Exkin, covering such a skin substitute creates a moist environment ideal for migration and proliferation of

seeded cells. Such studies are currently being carried out.

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