

Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin

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Communicated by Robert W. Mann, August 10, 1988

ABSTRACT Regeneration of the dermis does not occur spontaneously in the adult mammal. The epidermis is regenerated spontaneously provided there is a dermal substrate over which it can migrate. Certain highly porous, crosslinked collagen-glycosaminoglycan copolymers have induced partial morphogenesis of skin when seeded with dermal and epidermal cells and then grafted on standard, full-thickness skin wounds in the adult guinea pig. A mature epidermis and a nearly physiological dermis, which lacked hair follicles but was demonstrably different from scar, were regenerated over areas as large as 16 cm². These chemical analogs of extracellular matrices were morphogenetically active provided that the average pore diameter ranged between 20 and 125 μm, the resistance to degradation by collagenase exceeded a critical limit, and the density of autologous dermal and epidermal cells inoculated therein was >5 × 10⁴ cells per cm² of wound area. Unseeded copolymers with physical structures that were within these limits delayed the onset of wound contraction by about 10 days but did not eventually prevent it. Seeded copolymers not only delayed contraction but eventually arrested and reversed it while new skin was being regenerated. The data identify a model extracellular matrix that acts as if it were an insoluble growth factor with narrowly specified physicochemical structure, functioning as a transient basal lamina during morphogenesis of skin.

Throughout development, extracellular matrices (ECMs) are continuously being remodeled—i.e., synthesized, degraded, and resynthesized (1–5). Healing of a deep skin wound also requires remodeling of an ECM—the basal lamina (basement membrane) between the epidermis and the dermis (2). ECMs are largely insoluble and nondiffusible, and they confer stiffness and strength to multicellular systems (1, 2). During remodeling, the ECM necessarily suffers degradation of macromolecular chains, a process that dramatically reduces the insolubility of the ECM and impairs its role as mechanical reinforcement of a multicellular system undergoing development. It is not clear just how the resistance of the ECM to degradation affects its role during morphogenesis.

In physical terms, ECMs can be described as macromolecular networks that are covalently crosslinked and are highly swollen in extracellular fluid. Accordingly, the physical structure of an ECM can be characterized initially by specifying the volume fraction of macromolecular components (swelling ratio), the average diameter of pores in the highly swollen network, the density of crosslinks tying chains to each other, and the degree of crystallinity present. This model leads to questions such as the following ones. Is it necessary for a developmentally active ECM to persist as an undegraded, crosslinked macromolecular network (and, therefore, remain insoluble and nondiffusible) over a critical time scale? Is it necessary for such an ECM to contain pores of a critical size? We have answered these questions in a

preliminary way by use of well-defined collagen-glycosaminoglycan (CG) graft copolymers that are simple chemical analogs of ECM. A series of CG copolymers was prepared in a way that morphogenetic effects due to the degradation rate of the copolymers in collagenase could be studied in isolation from effects due to their average pore diameter. Other structural features, including the swelling ratio, the degree of crystallinity, and the chemical composition were not changed through the current series of copolymers.

The chemical analogs of ECM were fabricated into membranes and used to study skin morphogenesis in the standard wound-healing model of Billingham and Medawar (6, 7). In this model the complete dermis and epidermis are excised over a rectangular area from the back of a rodent, and the striated muscle underneath is thereby exposed, serving as a reproducible wound bed. When left ungrafted, this wound bed contracts at a well-known rate until opposite wound edges touch and a linear scar is formed. Dermis is not regenerated in the ungrafted Billingham–Medawar skin-wound model (6, 7). It has been shown that a membrane fabricated from at least one member of the CG copolymer series, which was relatively resistant to collagenase (8) and was highly porous (9), delayed significantly the onset of wound contraction when grafted on this animal model (9). Furthermore, when seeded with autologous dermal and epidermal cells, this CG membrane graft induced partial regeneration of the dermis in the same model (10, 11). In the current study, we found that there were severe restrictions to the physical structure of cell-seeded CG copolymers that induced morphogenesis. Only copolymers with critically adjusted pore size and degradation rate delayed the kinetics of wound contraction remarkably before undergoing degradation. When seeded with a minimal density of autologous epidermal and dermal cells, members of this group of contraction-inhibiting copolymers were capable of arresting contraction and inducing synthesis of a dermis and an epidermis over the area of the wound. The data support the conclusion that these cell-seeded simple chemical analogs of ECM induced morphogenesis of skin provided that their macromolecular network structure was adjusted within highly specific limits.

MATERIALS AND METHODS

Preparation of CG Copolymers. A white coprecipitate of type I collagen from bovine hide and of chondroitin 6-sulfate from shark cartilage in 0.05 M acetic acid (pH 3.2) was converted into a highly porous white membrane with a fractional pore volume of *ca.* 0.99 by freeze-drying as described elsewhere in detail (8, 12, 13). Control of the average pore diameter was achieved by adjusting the initial shelf temperature in the range from –90° to –5°C, respectively (13). Subsequent exposure of the dry solid to 105°C and a vacuum of 50 torr (1 torr = 133 Pa) over 24 hr introduced

covalent crosslinks between the polypeptide chains of collagen without denaturing it to gelatin (9, 14, 15). The porous CG sheet was knife-coated with a thin layer of viscous poly(dimethyl siloxane) prepolymer (Silastic medical A, Dow Corning, Midland, MI) to control the loss of moisture from the wound bed as well as to prevent bacterial contamination. After polymerization of the poly(dimethyl siloxane) prepolymer to a 0.025-mm-thick elastomeric layer, the bilayer membrane was immersed in a bath containing 0.25% aqueous glutaraldehyde in 0.05 M acetic acid, and the collagenous component of the CG copolymer underwent therein additional covalent crosslinking (12). Increase of the residence time in the glutaraldehyde bath from 0 to 24 hr yielded CG copolymers varying widely in their crosslink density, as reflected in values of \bar{M}_c , the average molecular weight between crosslinks (12), which ranged from *ca.* 60 kDa to *ca.* 12 kDa, respectively. It has been shown that the degradation rate of CG copolymers that are either implanted subcutaneously in rodents (9) or exposed to collagenase *in vitro* (16) increases as \bar{M}_c increases (9). After the CG copolymers were rinsed in deionized water over 24 hr, the glutaraldehyde concentration in the rinse water was found to be <5 ppm with the analytical reagent 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald, Aldrich) (17). No histological or other evidence of toxicity due to exposure of the CG copolymers to glutaraldehyde during preparation was detected over several years of their use as grafts or implants in animals and humans (9–11). Sterile procedures were followed in the preparation of membranes.

Characterization of CG Copolymers. The average pore diameter was determined either with a scanning electron microscope (13) or by embedding the porous membrane in methyl methacrylate, sectioning, staining with toluidine blue, and viewing with an image analyzer. The glycosaminoglycan content was determined by hexosamine assay (12) and averaged $2.6 \pm 0.3\%$ (wt/wt; dry basis) through the copolymer series. Small-angle x-ray-scattering studies showed that the collagen fibers became highly swollen and disaggregated and lost their banding below pH 4.25 ± 0.30 (18). Exposure to glutaraldehyde before the final rinsing and buffering to pH 7 prevented the recovery of the banding pattern prior to grafting (18). Nevertheless, infrared spectroscopic measurements (19) showed that the triple helical structure of collagen was intact at the time of grafting. The degradation rate of CG copolymers in collagenase was determined by a colorimetric procedure after incubation of the finely comminuted polymer for 5 hr at 37°C in a stirred 0.08% solution of bacterial collagenase from *Clostridium histolyticum* (GIBCO) in a buffer (pH 7.4) (20). Previous studies have shown that the use of bacterial collagenase as an *in vitro* empirical screening device provides an order-ranking of collagen implants according to degradation rate that agrees with *in vivo* observations (9, 16). Assay results were expressed in enzyme units, which were calculated by using a standard solution of 0.002 M L-leucine. By definition, one enzyme unit liberates amino acids from collagen that are equivalent in ninhydrin color to 1.0 μ mol of L-leucine in 5 hr at pH 7.4 and at 37°C (20).

Seeding of Polymers with Cells. Certain CG copolymers were seeded with autologous, uncultured dermal and epidermal cell suspensions that were enriched in basal cells (10). The procedure used for cell extraction depends on careful control of the standard trypsinization procedure of Medawar (21) as elaborated by Prunieras and coworkers (22, 23), who reported a basal cell content of $71 \pm 4\%$ for these cell preparations. In our work, a 0.2-mm-thick layer of skin was harvested from the guinea pig dorsum by use of a dermatome, and at least 10×10^6 viable cells per cm^2 of biopsy were obtained. The desired density of cells inside the copolymer layer was arrived at by centrifugation. Seeding was accomplished by equilibrating cell-free CG membranes (with the

silicone layer attached) for 30 min in DMEM enriched with 10% (vol/vol) fetal bovine serum (Hazelton Research, Lenexa, KS) and then placing the membranes in a polycarbonate holder specially designed to maintain the centrifugal force vector perpendicular to the plane of the membrane. The cell suspension was pipetted on top of the porous CG copolymer layer and centrifuged for 15 min at $100 \times g$. Scanning electron microscopy of the seeded membrane showed that the majority of cells had been positioned near the interface between the silicone layer and the CG layer.

Grafting Procedures. White female adult Hartley guinea pigs weighing about 400 ± 50 g were grafted and bandaged as described elsewhere in detail (9). The excision protocol used followed closely the standard mammalian free-skin-grafting model described by Billingham and Medawar (6, 7). The skin was excised down to, but not including, the *panniculus carnosus*, the layer of muscle responsible for skin-twitching movements in rodents. An anatomically well-defined wound bed, usually measuring 1.5 cm by 3.0 cm but as high as 5.0 cm by 6.0 cm in some animals, was thereby prepared. The test group of animals was grafted with a CG copolymer membrane coated with poly(dimethyl siloxane) prepolymer. Certain grafts were seeded with cells. Control wounds were treated only with a Xeroform gauze (ungrafted) or were autografted (6, 7). The wound area was calculated from photographs by using measurements taken from edges of cut dermis and not from the advancing edge of epithelial cell sheets, as described (6, 7, 9). From these data we estimated the wound half-life, $t_{1/2}$, or time required for contraction of the wound area to half the original.

Morphological Studies. One year after the surgical procedures, biopsy specimens were obtained from grafted sites, from scars resulting from closure of ungrafted full-thickness wounds, and from site-matched normal skin. Specimens were finely minced, fixed in 4% glutaraldehyde, postfixated in osmium tetroxide, and embedded in an Epon/Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL JSM 100 transmission electron microscope. Small-angle light-scattering measurements of thin (histological) sections were made with a laser beam (24).

RESULTS

Kinetics of Wound Contraction. The wound half-life, $t_{1/2} = 8 \pm 1$ days, observed in our present study with ungrafted wound controls, compares well with $t_{1/2}$ values of 7 days, which we estimated from independently obtained data with guinea pigs (25). Ungrafted wounds eventually contracted fully, forming linear scars (6, 7). Wounds covered with full-thickness autografts showed contraction only to about 80% of the original area by about day 30 and subsequently expanded over the next 6 months to an area slightly larger than the original because of the growth of the animal (6).

Control of the mean pore diameter, \bar{d} , of CG copolymers was a very effective procedure for delaying the onset of wound contraction (Fig. 1). A series of cell-free copolymers was prepared with \bar{d} values varying systematically from less than *ca.* 1 μ m to *ca.* 800 μ m by appropriate control of freeze-drying conditions. All copolymers in this series were exposed to 0.25% glutaraldehyde in 0.05 M acetic acid for 24 hr at 23°C. Collagenase degradation assays of copolymers in this series gave values of the degradation rate R that averaged 11 ± 4.7 enzyme units. A maximum increase in $t_{1/2}$ to 27 ± 2 days occurred when the mean pore diameter ranged between 20 ± 4 μ m and 125 ± 35 μ m (Fig. 1).

The degradation rate of CG copolymers affected the kinetics of contraction independently from the mean pore diameter (Fig. 2). A series of cell-free copolymers was prepared with degradation rate values varying from 2.6 ± 0.6 enzyme units to *ca.* 200 enzyme units by control of the glutaraldehyde crosslinking step. All copolymers in this

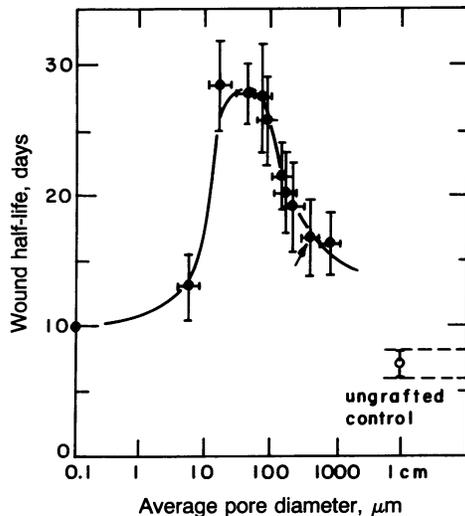


FIG. 1. Variation of the skin wound half-life (the time required for a wound to contract to 50% of the original area) with the average pore diameter of CG copolymer used as the graft. The arrow marks the average pore diameter of the copolymer series used to obtain the data in Fig. 2.

series were freeze-dried under virtually identical conditions. Pore-size determination for these polymers gave \bar{d} values that averaged $450 \pm 100 \mu\text{m}$. Based on an arbitrary level of $t_{1/2} \geq 15$ days (twice the value for the ungrafted control), we estimated from Fig. 2 an upper cut-off point of 140 ± 25 enzyme units. Above this limiting value, $t_{1/2}$ rapidly decreased, approaching the value for the ungrafted control. The sensitivity limit of the assay was *ca.* 1 enzyme unit.

Morphogenetic activity was shown by CG copolymers that not only possessed values of the mean-pore diameter and enzymatic degradation rate within the critical limits prescribed by Figs. 1 and 2, but also were seeded with dermal and basal epidermal cells prior to grafting. When grafted with CG copolymers that had been seeded with at least 5×10^4 cells per cm^2 of graft area, the wounds initially displayed contraction kinetics that were roughly similar to those of cell-free, contraction-inhibiting CG grafts (Fig. 3). The onset of contraction occurred on day 10 ± 1 . The contraction rate then reached a maximum and eventually slowed down until the wound perimeter showed almost no change between days 35 and 50. Thereafter, the wound perimeter expanded at a rate significantly in excess of the normal growth rate of the animal until it asymptotically reached $72 \pm 5\%$ of the original area (Fig. 3). Neoepidermal confluence was usually detected

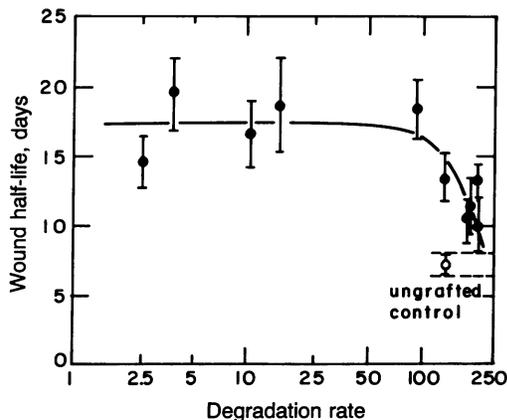


FIG. 2. Variation of the skin wound half-life with degradation rate R of CG copolymer used as the graft. The degradation rate is in empirical units, which are defined in the text.

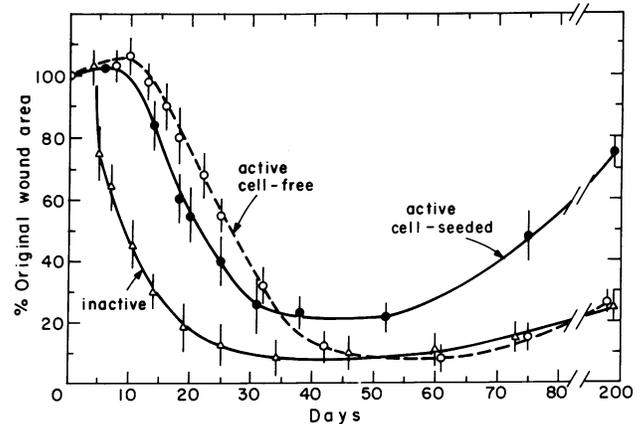


FIG. 3. Change in the original wound area with time observed when full-thickness guinea pig skin wounds were grafted with inactive CG copolymers (Δ), active cell-free CG copolymers (\circ), and active cell-seeded CG copolymers (\bullet).

at about 2 weeks or less after grafting as formation of a thin shiny surface comprising layers of *stratum corneum*, which could be removed and identified microscopically. Light microscopic studies also showed that formation of a new dermal layer proceeded underneath the neoepidermis over a period of several weeks. After about 90 days, the wound perimeter, itself consisting of a thin layer of scar tissue, eventually circumscribed an area of tissue (up to 16 cm^2 with the largest grafts) that grossly appeared identical in color, texture, and touch to intact skin outside the wound perimeter, with the exception that the new skin was entirely hairless (Fig. 4). The gross appearances of the scarred line perimeter and of the tissue area within were similar to corresponding features of the autograft.

The time to reach neoepidermal confluence as well as the kinetics of wound contraction were strongly affected by the density of cells inoculated in the CG copolymer at the time of grafting. Table 1 shows that an increase from 5×10^5 to 1×10^6 cells per cm^2 did not significantly change the time for neoepidermal confluence from the shortest value recorded in our study, 12.0 ± 2 days ($n = 5$). However, decrease in the cell density to 5×10^4 per cm^2 led to the significantly increased time for neoepidermal confluence of 20.5 ± 11 days. Wounds grafted with unseeded contraction-inhibiting copolymers did not show epidermal confluence and, after a delay in onset of contraction, eventually closed completely (Fig. 3).

Morphological Studies. A brief comparative morphological study of 1-year-old guinea pig skin, scar, and newly synthe-

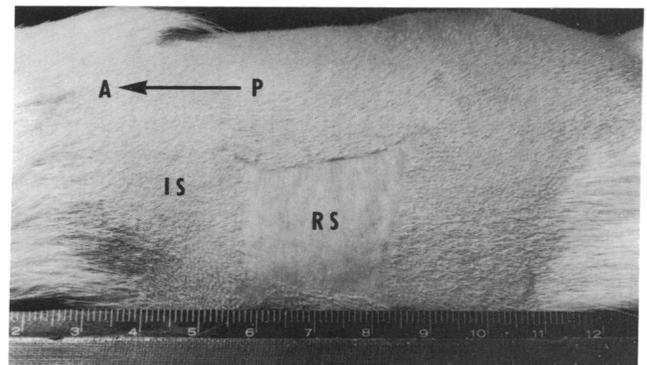


FIG. 4. Regenerated guinea pig skin (RS), obtained by grafting a $3 \text{ cm} \times 3 \text{ cm}$ full-thickness wound with an active cell-seeded CG copolymer, occupies an area of *ca.* 6.5 cm^2 and has no hair follicles, whereas intact skin (IS) does. The anterior-posterior (AP) axis of the live animal (155 days after grafting) is shown. The scale is marked in cm.

Table 1. Effect of cell density of CG copolymer grafts on epidermal confluence

Cells, no. per cm ² graft area	Days to confluence
0	No confluence
5×10^4 ($\pm 10\%$)	20.5 ± 11
2×10^5	14.5 ± 3
5×10^5	12.6 ± 2
1×10^6	12.0 ± 2

sized skin by electron microscopy appears in Figs. 5–7. Partly regenerated skin (Fig. 5B) was remarkably similar to normal guinea pig skin (Fig. 5A) in most respects. The epidermis of regenerated skin was variably hyperplastic, and all cell layers were normal in maturation sequence and relative proportion (Fig. 6B). Keratohyaline granules of the neoepidermis were generally larger and more irregular in contour than those of the normal granular cell layer (Fig. 6C and D). Langerhans cells and melanocytes (Fig. 5B), normal constituents of the epidermis, were observed in new skin, as were a well-formed rete ridge pattern and interdigitations with dermal papillae (Fig. 6B). In contrast, scar showed marked thinning or atrophy of the epidermis as well as loss of rete ridges and associated dermal papillae. The dermis of regenerated skin exhibited elastic fibers but, unlike the thinned and fragmented fibers seen in scar (Fig. 7A), they exhibited a delicate particulate structure (Fig. 7B). The collagen fibers forming the dermis in scar were highly oriented in the plane of the epidermis and were interspersed with elongated fibroblasts (Fig. 7A), whereas in regenerated skin the fibroblasts were not elongated nor were the collagen fibers highly oriented in the plane of the epidermis. Small unmyelinated nerve fibers were present within dermal papillae in close approximation to the epidermis in both normal and regenerated skin (Fig. 5A and B); these were not prominent features of scar tissue. However, neither regenerated skin nor scar had hair follicles or other skin appendages. Small-angle light-scattering measurements quantita-

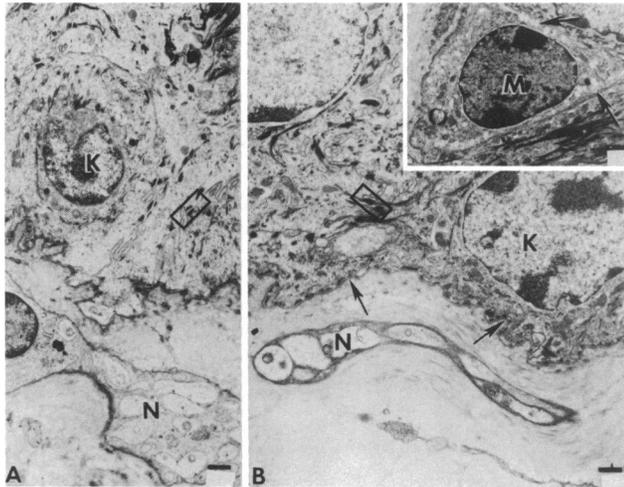


FIG. 5. (A) Normal guinea pig skin. The epidermis is formed by keratinocytes (K) joined by junctional complexes (desmosomes—enclosed by rectangle). Note the characteristic unmyelinated nerve fiber (N) within the superficial dermis. ($\times 3250$). (B) Regenerated skin 14 months after grafting. The neoepidermis is formed by keratinocytes (K) that are identical to intact control skin in A. Desmosomes (enclosed) and basal lamina (arrows) are well-formed and normal in appearance. Melanocytes (M in *Inset*) containing characteristic melanosomes (arrows in *Inset*) also repopulate the neoepidermis, where they normally function in pigment production and donation. A dermal nerve fiber (N), similar to those of normal skin, is also observed ($\times 5400$; *Inset*, $\times 3250$). Reprinted with permission from ref. 26 (copyright CRC Press, Inc., Boca Raton, FL). (Bar = 1 μm .)

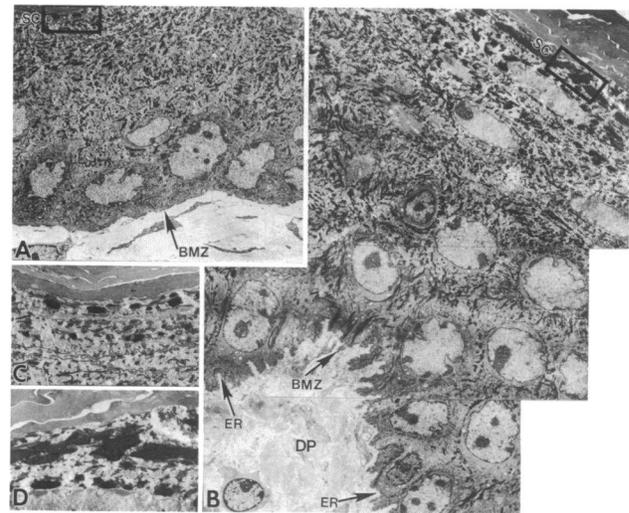


FIG. 6. Comparison of the ultrastructure of full-thickness normal guinea pig epidermis (A) and regenerated epidermis 1 year after grafting (B) (identical magnifications). The photographic montage in B demonstrates an epidermis that is thicker and contains many more nucleated cell layers than in normal skin (A). In addition, in contrast to normal epidermis (A and C), keratohyaline granules (enclosed by rectangle) beneath the stratum corneum (SC) of reconstituted epidermis are large and show irregular contours (D). DP, dermal papilla; BMZ, basement membrane zone; ER, epidermal rete ridges. (A and B, $\times 1200$; C and D, $\times 2500$.)

tively confirmed the presence of low orientation of collagen fibers in normal dermis, moderate orientation in regenerated dermis, and high orientation in scar (24).

DISCUSSION

The contraction-inhibiting activity of CG copolymers depends on their persistence as insoluble networks over a critical period. The observation that wound contraction is delayed if the degradation rate of the copolymer is lower than a critical

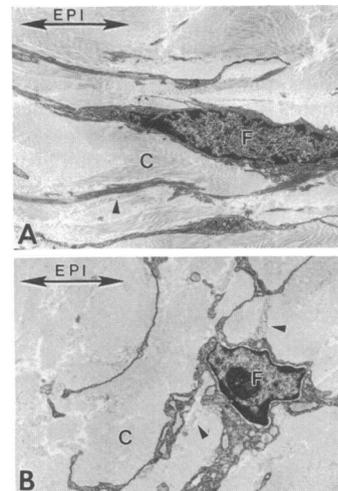


FIG. 7. Comparison of collagen fiber and fibroblast orientation in scar (A) and regenerated dermis (B) after 1 year. In scar, fibroblasts (F), collagen bundles (C), and thinned and fragmented elastic fibers (arrowhead) are aligned in parallel with the horizontal axis of the overlying epidermal layer (EPI, double arrow). In regenerated dermis, fibroblasts and collagen bundles are randomly aligned with respect to the epidermal axis, a configuration also observed in normal dermis. In addition, elastic fibers (arrowheads in B) are delicate and nonfragmented, suggesting relatively recent synthesis. ($\times 3500$.)

limit (Fig. 2) is most simply interpreted by postulating that the activity of the macromolecular network partly depends on its resistance to rapid solubilization. Alternatively, the data show that CG copolymers do not delay contraction if grafted in a rapidly degradable form. The requirement of a minimum average pore diameter for observation of contraction-inhibiting activity (Fig. 1) can be explained most simply in terms of adequately large pore channels for migration of seeded cells. The observed requirement for a maximum average pore diameter, above which the contraction-inhibiting activity of CG copolymers nearly vanishes, probably reflects a requirement for maintenance of a minimal specific surface (cm^2/g) for the insoluble matrix. The specific surface of the copolymers decreases greatly with increase in average pore diameter. The probable importance of specific surface suggests that a critical density of binding sites on the surface of pore channels of CG copolymers is required for a sufficient number of cell-copolymer interactions to occur. The combined data (Figs. 1 and 2) identify a singular developmentally active factor—an insoluble chemical analog of the ECM with well-defined physicochemical structure.

Inhibition of onset of wound contraction can be interpreted most simply in terms of a specific interaction between the model ECM and the myofibroblast (27) or a precursor cell. Wound contraction must take place by transfer of mechanical stresses over the scale of the wound. Such transfer occurs if the myofibroblast population is modeled as an uninterrupted three-dimensional network, bonded either by direct intercellular junctions or by cell-ECM-cell bonds, that extends over the entire domain of the wound (28). According to this mechanism, the copolymer with critically adjusted pore size and degradation rate (Figs. 1 and 2) may delay the onset of contraction quite simply by inhibiting differentiation of mesenchymal cells to myofibroblasts. Preliminary ultrastructural data support this mechanism (29). Results of another study to be reported elsewhere suggest strongly that the regenerated epidermis derives from the seeded cells rather than from epithelial cells migrating from the wound edge.

Regeneration of the dermis does not occur spontaneously in the adult mammal (6, 7). The epidermis regenerates spontaneously provided there is a dermal substrate over which it can migrate (6, 7). Our study shows that a new dermis and a new epidermis can be simultaneously regenerated in a fully excised skin wound provided that the muscle wound bed has been grafted with a highly specific model ECM inoculated with autologous skin cells. Although the regenerated skin is only partly a replica of normal skin, it is significantly different from scar; therefore, its synthesis constitutes a morphogenetic event. Clearly, the observed morphogenesis is partly under the control of the model ECM identified in this study. Acting as if it were a transient basal lamina, this model ECM probably mediates the specific interaction between epithelial and mesenchymal cells seeded into it and, thereby, induces normal tissue differentiation and development in an adult mammalian model where such processes do not spontaneously occur (30).

One of the unseeded CG copolymers that induced morphogenesis of skin in this study has been successfully used as a graft to treat major burns in humans in a multicenter randomized clinical trial (10, 31, 32). Furthermore, a series of unseeded CG copolymers with systematically varying structures have induced functional regeneration of the rat sciatic nerve when they were used to bridge large gaps (10 mm and 15 mm) in the transected nerve (33). The available evidence suggests strongly, therefore, that these copolymers, acting as a temporarily insoluble growth factor, may directly control crucial differentiation steps in a variety of developmental processes.

We thank E. C. Hay, K. R. Hitchcock, K. Holbrook, V. Ingram, T. F. Linsenmayer, R. W. Mann, M. Nimni, and B. P. Toole for

useful discussions. E. Chen, M. A. Dionne-Bentz, A. Ferdman, and S. Flynn-Izatt obtained useful background data. This research was supported in part by National Institutes of Health Grant GM23946 and Department of Defense Grant DAMD17-87-C-7130, and in part by the office of the Associate Provost of Massachusetts Institute of Technology.

- Hay, E. D. (1983) *Mod. Cell Biol.* **2**, 509–548.
- Loomis, W. F. (1986) *Developmental Biology* (Macmillan, New York), pp. 116–124.
- Smith, L. T., Holbrook, K. A. & Byers, P. H. (1982) *J. Invest. Dermatol.* **79**, 93s–104s.
- Blanck, C. E. & Hitchcock, K. R. (1987) *J. Cell Biol.* **105**, 139 (abstr.).
- Nimni, M. E. (1983) *Semin. Arthritis Rheum.* **13**, 1–86.
- Billingham, R. E. & Medawar, P. B. (1955) *J. Exp. Biol.* **28**, 385–402.
- Billingham, R. E. & Medawar, P. B. (1955) *J. Anat.* **89**, 114–123.
- Yannas, I. V., Burke, J. F., Huang, C. & Gordon, P. L. (1975) *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **16**, 209–214.
- Yannas, I. V. (1981) in *The Surgical Wound*, ed. Dineen, P. (Lea & Febiger, New York), pp. 171–190.
- Yannas, I. V., Burke, J. F., Orgill, D. P. & Skrabut, E. M. (1982) *Science* **215**, 174–176.
- Yannas, I. V., Orgill, D. P., Skrabut, E. M. & Burke, J. F. (1984) in *Polymeric Materials and Artificial Organs*, ed. Gebelein, C. G. (Am. Chem. Soc., Washington, DC), pp. 191–197.
- Yannas, I. V., Burke, J. F., Gordon, P. L., Huang, C. & Rubenstein, R. H. (1980) *J. Biomed. Mater. Res.* **14**, 65–81.
- Dagalakis, N., Flink, J., Stasikelis, P., Burke, J. F. & Yannas, I. V. (1980) *J. Biomed. Mater. Res.* **14**, 511–528.
- Yannas, I. V. & Tobolsky, A. V. (1967) *Nature (London)* **215**, 509–510.
- Yannas, I. V. (1972) *Rev. Macromol. Chem.* **C7**, 49–104.
- Yannas, I. V., Burke, J. F., Huang, C. & Gordon, P. L. (1975) *J. Biomed. Mater. Res.* **9**, 623–628.
- Dickinson, R. G. & Jacobsen, N. W. (1970) *Chem. Commun.* 1719–1720.
- Sylvester, M. F., Yannas, I. V., Salzman, E. W. & Forbes, M. J. (1988) *Thromb. Res.*, in press.
- Gordon, P. L., Huang, C., Lord, R. C. & Yannas, I. V. (1974) *Macromolecules* **7**, 954–956.
- Mandl, I., MacLennan, J. D. & Howes, E. L. (1953) *J. Clin. Invest.* **32**, 1323.
- Medawar, P. B. (1941) *Nature (London)* **148**, 783.
- Regnier, M., Delescluse, C. & Prunieras, M. (1973) *Acta Derm.-Venereol.* **53**, 241–247.
- Bourin, M. C., Delescluse, C., Furstemberger, G., Marks, F., Schweizer, J., Klein-Zanto, A. J. P. & Prunieras, M. (1982) *Carcinogenesis (London)* **3**, 671–676.
- Ferdman, A. & Yannas, I. V. (1987) *Trans. Soc. Biomater.* **10**, 207 (abstr.).
- Grillo, H. C., Watts, G. T. & Gross, J. (1958) *Ann. Surg.* **148**, 145–152.
- Yannas, I. V. (1988) in *Collagen: Biochemistry, Biotechnology and Molecular Biology*, ed. Nimni, M. (CRC, Boca Raton, FL), Vol. 3, p. 107.
- Gabbiani, G., Ryan, G. B. & Majno, G. (1971) *Experientia* **27**, 549–550.
- Yannas, I. V. (1988) in *Collagen: Biochemistry, Biotechnology and Molecular Biology*, ed. Nimni, M. E. (CRC, Boca Raton, FL), Vol. 3, pp. 87–115.
- Yannas, I. V. (1988) in *Cutaneous Development, Aging and Repair*, eds. Abatangelo, G. & Davidson, J. M. (Liviana Press, Padova, Italy), in press.
- Yannas, I. V., Lee, E., Skrabut, E. M., Orgill, D. P. & Murphy, G. F. (1987) *J. Cell Biol.* **105**, 223 (abstr.).
- Burke, J. F., Yannas, I. V., Quinby, W. C., Jr., Bondoc, C. C. & Jung, W. K. (1981) *Ann. Surg.* **194**, 413–428.
- Heimbach, D., Lutermaier, A., Burke, J., Cram, A., Herndon, D., Hunt, J., Jordan, M., McManus, W., Solem, L., Warden, G. & Zawacki, B. (1988) *Ann. Surg.* **208**, 313–320.
- Yannas, I. V., Krarup, C., Chang, A., Norregaard, T. V., Zervas, N. T. & Sethi, R. (1987) *Soc. Neurosci. Abstr.* **13**, 1043.