sumably is due to an increase in the actin concentration in these areas. However, this pattern may also be related to an active constriction of putative microfilaments leading to a condensation of actin which is reflected as increased fluorescence. In either case it seems unlikely that the increased fluorescence is secondary to changes in morphology, since redistribution of intense staining precedes bending of the neural plate in these areas. Furthermore, ultrastructurally an increase in apical microfilaments during neurulation has been well documented (*1–4*) and increased fluorescence in this region is consistent with this observation.

Increased fluorescence in the neural groove of later-stage embryos suggests that this region may be a focal point for contractile activity, but may also represent overlap of apical cell areas that are close together in the groove. The intense fluorescence in overlying ectoderm cells suggests an active role for this tissue in the process of neurulation as proposed by Schroeder (*9*). This localization of fluorescence may also be related to the role these cells play in bridging the gap between folds and for making initial contact. This phenomenon is an active process, and ectoderm cells extend numerous filopodia to initiate contact between opposing sides. Perhaps contractile proteins play a role in this process as they do in fibroblast migration.

Thus we have shown that the pattern of actin distribution is consistent with the hypothesized role of this contractile protein during mouse neurulation. Our results provide new evidence that a redistribution or reorganization of actin in cranial neural folds occurs at the time that folds initiate the change from a biconvex morphology to a biconcave tube-like structure. This changing pattern appears to be related to the alterations in cranial neural fold morphology that are more complex in mammalian embryos than in avian or amphibian embryos. However, whether or not this changing pattern of fluorescence coincides with the presence of microfilaments remains to be determined.

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Wound Tissue Can Utilize a Polymeric Template to Synthesize a Functional Extension of Skin

Abstract. *Prompt and long-term closure of full-thickness skin wounds in guinea pigs and humans is achieved by applying a bilayer polymeric membrane. The membrane comprises a top layer of a silicone elastomer and a bottom layer of a porous cross-linked network of collagen and glycosaminoglycan. The bottom layer can be seeded with a small number of autologous basal cells before grafting. No immunosuppression is used and infection, exudation, and rejection are absent. Host tissue utilizes the sterile membrane as a culture medium to synthesize neoepidermal and neodermal tissue. A functional extension of skin over the entire wound area is formed in about 4 weeks.*

Excessive fluid loss and massive infection are two major problems immediately threatening the life of an individual who has suffered extensive skin loss. This is the case, for example, with victims of deep burns extending over more than 50 percent of the body. Current treatment emphasizes fluid resuscitation and prompt closure of wounds with autografts, cadaver skin, or pig skin following the excision of dead tissue (*1*). Failure to achieve closure of burn wounds within 3 to 7 days after injury significantly increases the probability that the patient will die (*1*).

We have developed a bilayer polymeric membrane that promptly and aseptically closes skin wounds in animals and humans while serving as a template for the construction of a functional extension of the skin. Our design has evolved over the past 11 years from studies of guinea pigs (*2*). The top layer of the membrane is a silicone elastomer and the bottom layer is a highly porous, covalently cross-linked network of bovine hide collagen and glycosaminoglycan (GAG) (Fig. 1) (*3*). We have achieved reproducible conditions under which autologous basal cells, seeded into the

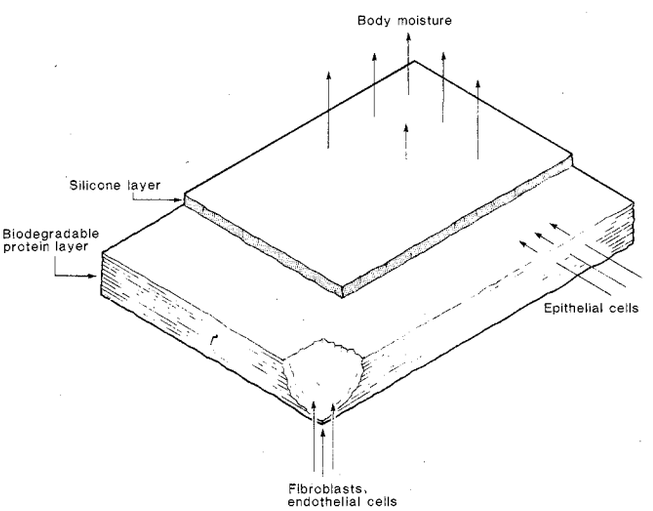

Fig. 1. Schematic representation of a standard version of the noncellular bilayer polymeric membrane (stage 1). The moisture flux through the layers ranges from 1 to 10 mg/cm^2 per hour. Fibroblasts and endothelial cells migrate into the bottom layer while epithelial cell sheets advance from the wound edge in the plane of the membrane, between the two layers. The top layer is a conventional medical-grade silicone which undergoes cross-linking at room temperature following exposure to ambient moisture. The bottom layer is a cross-linked network of collagen and chondroitin 6-sulfate, a GAG (bound GAG content 8.2 ± 0.8 percent by weight, average molecular weight between cross-links 12,750 ± 3,300, pore volume fraction 96 ± 2 percent, mean pore size 50 ± 20 μm). The collagen-GAG layer undergoes biodegradation at a controlled rate and is replaced by neodermal tissue while the silicone layer is spontaneously ejected following formation of a confluent neoepidermal layer under it. Stage 2 membranes are identical except that autologous epidermal (basal) cells are seeded into the membranes before grafting.

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membrane before grafting, synthesize mature neoepidermal tissue in vivo while mesenchymal cells from the wound bed synthesize a neodermal tissue that differs from conventional scar tissue. These conditions are obtained by controlling several physicochemical and biochemical parameters of the bottom layer, including the average molecular weight between covalent cross-links, the ratio of collagen to GAG, the pore structure, and the density of the autologous epidermal cells seeded before grafting (*4–7*).

Autografts provide prompt and long-term wound closure and leave minimal scarring. However, the patient's intact skin is often in short supply, and the operation to obtain it is undesirable. Homografts, obtained from cadavers and used immediately or after preservation in a skin bank (*8*), are also in short supply and, unless immunosuppressive agents are used, frequently are rejected early. However, the use of immunosuppressive agents increases the risk of infection. Heterografts, obtained from animals—especially pigs—are available commercially and are widely used to achieve short-term wound closure. Normally they are removed between the third and ninth day following application. A number of natural and synthetic polymer membranes have been employed in the treatment of burns but their use has not prevented infection (*2*). Recently, a culture of autologous epidermal cells was grafted into full-thickness skin wounds in humans 5 weeks after harvesting (*9*). A reconstituted collagen lattice populated by cultured autologous fibroblasts and epidermal cells has been grafted onto rats at least 2 weeks after harvesting (*10*). The latter two procedures require lengthy in vitro culturing of tissue prior to grafting and thus appear to require treatment of the patient with temporary wound closures.

Stage 1 bilayer membranes (Fig. 1) are prepared by the procedures described elsewhere by Yannas and colleagues (*3–5*). These cell-free, suturable membranes, potentially available in large quantity, can be stored indefinitely in the sterile state and can be used minutes after removal from their container. Stage 1 membranes have maintained 3 by 1.5 cm full-thickness skin wounds free of infection and exudation in more than 140 guinea pigs over the past 4 years. No immunosuppression was used. There was no evidence of inflammation after a 4-day period following the grafting operation and no evidence of rejection over the entire observation period. By comparison, guinea pigs usually reject homografts between 16 and 22 days after grafting. Wounds covered with stage 1 membranes began contracting 10 days after they were inflicted, whereas contraction began after just 2 days in animals that did not receive grafts. The wounds contracted to half their original area in 11 days in the animals without grafts and in 25 days in the animals with grafts. Autografts show much less contraction.

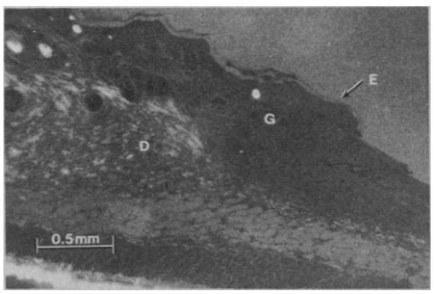

Fig. 2. Photomicrograph of a cross section of guinea pig wound edge 26 days after grafting with stage 1 membrane. The preparation is viewed between partly crossed polarizers. The epidermal edge (*E*) has advanced over the collagen-GAG layer (*G*), which is apposed to the birefringent dermis (*D*). The silicone layer covering was removed to facilitate histological processing of the specimen (stain: hematoxylin and eosin).

Microscopic observation of the interface between a stage 1 graft and the wound bed reveals copious synthesis of tissue. There is a well-vascularized neodermis containing collagen fiber bundles, the morphology of which resembles that of normal dermis. Control studies (*6, 7*) suggest that most of the original layer of collagen and GAG undergoes biodegradation in 3 to 4 weeks. Newly synthesized tissue is probably responsible for the development of clinically significant peel strengths of about 9 N/m (9 g/cm) as early as 24 hours following grafting, with a maximum of about 45 N/m (45 g/cm) after 10 days (the peel strength is determined by measuring the force necessary to separate the graft from the wound bed at a 90° angle). Epidermal migration from the wound edge consistently occurs at the interface between the silicone (top) layer and the collagen-GAG (bottom) layer of the graft (Fig. 2). The collagen-GAG layer of a stage 1 graft in guinea pigs with 3 by 1.5 cm wounds is completely covered with epithelial cells after 30 to 40 days. The silicone layer of the graft is spontaneously ejected at about the same time, revealing a scar.

Stage 2 membranes are prepared by seeding the cell-free (stage 1) membranes with uncultured autologous basal cells before grafting. A small area of guinea pig skin is harvested, washed with buffered saline, and dissociated with trypsin in phosphate-buffered saline (p*H* 7.2) for 40 minutes at 37°C (*11, 12*). A suspension of basal cells is prepared by discarding the top epidermal layer, placing the bottom layer in Eagle's minimum essential medium (Dulbecco's modification) containing 10 percent fetal calf serum, vortexing the medium, separating the suspended basal cells from the pieces of dermal tissue by filtration, and centrifuging the cell suspension until the desired density of viable cells is attained (*11*).

We use various procedures to seed the collagen-GAG layer with the basal cell suspension in vitro. In one procedure, the cells are inoculated with a hypodermic syringe into the collagen-GAG layer. In another procedure the cells are driven into the porous membrane by mild centrifugation. This is accomplished by lining the centrifuge bucket with a membrane holder designed to maintain the plane of membrane normal to the centrifugal force vector and the collagen-GAG

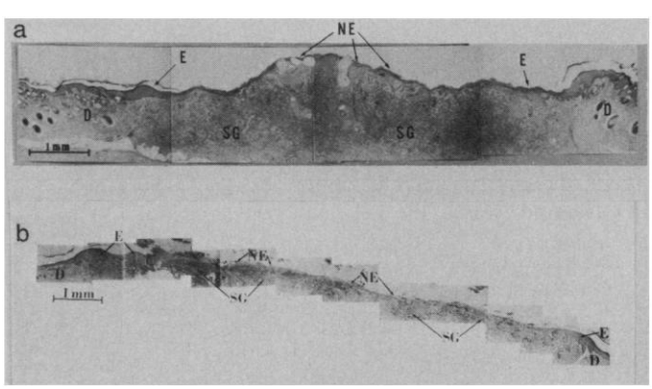

Fig. 3. (a) Composite photomicrograph of entire cross section of a guinea pig skin wound 12 days after grafting with a bilayer membrane (*SG*) that was seeded with autologous basal cells by centrifugation at 50*g* for 15 minutes. A continuous keratinized sheet of neoepidermal cells (*NE*) is forming near the center of the wound, which measured 1.5 cm between edges. Epidermal sheets (*E*) are advancing from the edges (*D*, dermis) toward the interior. The silicone layer was removed to facilitate preparation of this hematoxylin and eosin–stained section. Both stratified cells and keratohyaline granules are evident. (b) Composite photomicrograph showing formation of keratinized neoepidermal cell sheets as in (a), except that the membrane was seeded by centrifugation at 500*g* for 10 minutes.

layer facing that vector, pipetting the cell suspension into the membrane holder, and operating the centrifuge at 50*g* for 15 minutes or at 500*g* for 10 minutes. In general, it is possible to graft stage 2 membranes onto guinea pigs less than 4 hours after harvesting the basal cells from the animals. Promptness of wound closure following injury, an essential component of our design, is thereby achieved. The time required for the seeding step can probably be shortened further.

Following in vitro seeding with uncultured autologous basal cells, stage 2 membranes can be grafted onto full-thickness skin wounds. In the sterile environment of the wound closure, the basal cells rapidly reach confluence, and in less than 14 days form sheets of keratinized epidermis between the top and bottom layers of the membrane (Fig. 3, a and b). Repeated observations have left no doubt that the neoepidermal sheet nucleates and grows from the seeded basal cells rather than originating at the wound edge. Neodermal tissue synthesis also occurs in these seeded membranes and there is no evidence of conventional scar formation. New and apparently functional skin is generated in less than 4 weeks.

The polymeric membranes have also been used successfully on humans. After primary excision of dead tissue (*13*), burn victims received stage 1 membranes as large as 15 by 25 cm (*14*). After being placed on the wound bed, the grafts were carefully sutured under slight tension, avoiding wrinkling of the thin membrane. The subjects, 5- to 60-year-old males and females, received grafts over more than 50 percent of their body surface area. No immunosuppression was employed. Graft take approached 100 percent, providing continuous physiological closure without infection or rejection. Whenever the graft was next to intact epidermis the epidermal edge migrated between the two layers of the membrane over a distance of a few millimeters. Up to 46 days later, the silicone layer was removed from the vascularized artificial dermis and the wound was closed with a thin (0.1 mm) autoepidermal graft. Neither the donor site nor the grafted area showed significant contraction or scarring (*14*). Following removal of split-thickness (0.25 to 0.375 mm) autografts, which include a fraction of the dermis, the donor sites are usually significantly scarred.

We conclude that the noncellular polymeric membrane (stage 1) performs at a level superior to that attained with pig skin or cadaver skin. In fact, when eventually covered with thin autoepidermal grafts, stage 1 membranes appear to equal the autograft in clinical performance. Furthermore, stage 2 grafts provide a means for closing the largest full-thickness skin wounds without delay and without requiring autologous epidermal grafts. Perhaps appropriately designed biodegradable templates can be used to regenerate segments of other tissues or organs which have become lost or dysfunctional due to disease or trauma.

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Tumor Metastasis Is Not Due to Adaptation of Cells to a New Organ Environment

Abstract. *Murine B16 melanoma cells were adapted for lung survival and growth by allowing them to attach to Bio-Carrier beads and injecting the beads intravenously into normal mice. The beads lodged mechanically in the microcirculation of the lung. When the melanoma cells had grown into visible tumors from the arrested beads, the tumors were removed and the cells were dispersed, cultured to remove normal cells, and reattached to new beads. The process was repeated nine times. Previously another B16 subline was injected intravenously as a suspension of separate tumor cells. Those cells that survived and colonized the lungs were harvested, cultured, and injected again. This selection process was also repeated nine times. Only the subline that was injected in suspension was more metastatic than the parental line, indicating that metastasis involves selection of preexistent metastatic cells and is not an adaptive process by which all cells gradually acquire the ability to grow at particular organ sites.*

In tumor metastasis, malignant cells spread from primary sites to nearby and distant secondary sites (*1*). Often, the malignant cells spread preferentially to specific secondary organ sites (*2*). This suggests that unique characteristics of these sites may be important in determining patterns of metastasis. As early as the 1880's, Paget (*3*) proposed that both the secondary tumor environment (the "soil") and properties of the metastatic cells (the "seed") were important in the establishment of distant metastases. In an attempt to determine whether tumor cells gradually adapt to particular environments or are selected for their ability to grow at a given site, Klein (*4*) sequentially converted a solid tumor cell line to grow in peritoneal cavity ascites fluid. She found that the ascites-grown tumor cells preferentially spread to lung tissue and proposed that they had existed in the original tumor as a specialized subpopulation. Since Klein's experiments, several investigators have succeeded in obtaining highly metastatic tumor cell lines by sequentially harvesting metastases, culturing their cells in vitro,

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