

An Article From:

SCIENCE & MEDICINE

MAY/JUNE 2000

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VOLUME 7, NUMBER 3

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Of greater concern, irregularities in the process of informed patient consent were alleged to have occurred. The two issues together were of sufficient gravity to warrant formal investigations by the FDA and the Recombinant DNA Advisory Committee of the NIH. These investigations as well as direct congressional scrutiny turned up additional problems.

Specifically, it became clear that other therapy-related deaths had occurred during gene therapy trials for coronary artery disease, carried out by separate groups at Cornell University in New York and St. Elizabeth's Hospital in Boston. Apparently favorable clinical results had already focused great attention on these trials, but initial enthusiasm was tempered substantially by the surprising disclosure that the deaths of patients may not have been reported to the FDA or NIH as is required.

In addition, in both of these cases as well as in the University of Pennsylvania case, investigators have links to corporate entities with interests in the outcomes, suggesting that fundamental issues of conflict of interest might have been operative. Clearly, the unfortunate death of the patient in Philadelphia has served as a nidus for an examination of issues far beyond the initial concerns of safety and oversight.

Beneath the headlines related to these events are many ironies and some verities that bear consideration. The first truth is that therapy-related deaths are an inevitable outcome of any experimental therapy. Consideration of just about any modern therapeutic agent from a historical perspective will demonstrate such events, even in the case of therapeutic agents currently offering the most exciting clinical results, such as anti-tumor monoclonal antibodies. That particular events have led to deaths during gene therapy does not belie the inevitability of such deaths.

Gene therapy has been unique among new medical fields in the

scrutiny that has attended its development since inception. It is fair to say that no other field in medical history has proceeded under such rigorous oversight, and on that basis, it must be concluded that errors did not happen because of a lack of scrutiny. More accurately, confusion and lack of coordination among the overseers was more relevant. None of the serious issues raised during the recent investigations is unique to gene therapy or to its practitioners.

Perhaps most unfortunately, wide publicity of these events has obscured developments of greater significance. In recent months, the first clear evidence of clinical benefit afforded by gene therapy has been published by three different groups conducting clinical trials for adenosine deaminase deficiency, hemophilia, and atherosclerotic heart disease. Each of these trials was a phase I study, designed only to determine treatment-related toxicity. Such studies usually use lower doses of vector than would be required to elicit a therapeutic effect, so achieving therapeutic benefits is especially noteworthy.

In each of these positive trials, the fundamental logic of gene therapy intervention was the same as in earlier attempts to treat the same diseases. That is to say, no advances have occurred in understanding the disease pathophysiology or the genes involved.

What has happened is that advances in gene therapy technology achieved first in animals have been incorporated into the design of these trials, as Orkin and Motulsky recommended. Successful outcomes predicated on new vector designs highlight the concept that the difference between clinical benefit and its lack may be ascribed to the most basic aspects of gene delivery technology.

Gene therapy at the moment is rife with ironies. The most scrutinized of all medical fields is faulted for inadequate scrutiny; a delivery

vector is the basis for gene therapy's nadir and almost simultaneously for its first glimmer of hope; a gene therapy protocol provokes a death while other protocols offer the promise of extending life.

Whereas the passage of time will provide perspective and context for gene therapists, recent events may make their scientific lives more difficult in the meantime. At the moment when the gene therapy field should rejoice in its first successes, it is instead the subject of withering censure.

Ironies notwithstanding, the path forward for the field is clear. Increased scrutiny of human clinical trials has resulted in the design of protocols with more clearly measurable end points. In addition to obligatory phase I safety and toxicity indices, interval end points have evolved that validate basic vector efficiency and clinical effects.

On this basis, trial-derived data can be exploited more directly in the design of advanced-generation vectors: delivery agents that have been engineered to address the biology relevant to achieving effective gene delivery. More stringent trials in conjunction with the evolving science of "vectorology" will provide a mutually reinforcing dynamic that will advance the field toward achieving the full promise that its technology has long been recognized to possess.

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Designing tissue substitutes requires a fundamental understanding of cell-scaffold interactions, particularly how the three-dimensional organization of scaffolds modulates cellular adhesion, migration, proliferation, and differentiation as well as extracellular matrix remodeling. This knowledge allows tissue engineers to regulate wound healing and tissue remodeling as well as to optimize the design of analogs that replace damaged tissues and organs.

Significant effort has been devoted to producing biocompatible scaffolds with defined pore sizes that help ensure proper cell-cell contacts and cell-matrix interactions and that preserve cellular function. Ioannis Yanniss's laboratory at the Massachusetts Institute of Technology determined the average pore diameters (skin 20 to 125 μm , nerve 5 to 10 μm) and orientations (skin random, nerve axial) that are critical for optimizing tissue ingrowth into collagen-glycosaminoglycan sponges.

Lisa Freed and her colleagues, also at MIT, showed that 12 μm diameter polyglycolic acid fibers processed into nonwoven meshes that were 96 or 97% porous facilitated proliferation of chondrocytes and production of sulfated glycosaminoglycans and type II collagen. These polyglycolic acid meshes also promoted formation of neocartilage with specific, reproducible three-dimensional shapes and histologic features closely resembling those of native cartilage.

Although sponges, foams, and matrices with defined porosities are useful for the fabrication of relatively large porous three-dimensional analogs of the extracellular matrix, their usefulness in forming a basal lamina is limited. The basal lamina is a thin membranous layer of connective tissue that underlies

cells in many organs and tissues, and it performs several important functions.

In the glomerulus of the kidney, for example, the basal lamina separates endothelial cells from podocytes and acts as a selectively permeable barrier to plasma molecules. During both organogenesis and wound healing, the basal lamina acts as a template for controlling cell migration and differentiation. In the skin, the basal lamina acts as an adhesive membrane that anchors the epithelial cell layer (epidermal keratinocytes) to the underlying mesenchymal tissue (dermis). It selectively separates the fibroblasts in the dermis from the epidermal keratinocytes while allowing innervation of the epidermis and movement of immune cells into and out of the epidermis.

As in most tissues, the basal lamina in the skin is not a simple flat plane of connective tissue. Rather, it conforms to a series of ridges and invaginations known as rete ridges and papillary projections. My colleagues and I at the Shriners Hospital for Children and Massachusetts General Hospital recently used a microfabrication process to produce novel analogs of the basal lamina with precisely engineered topographic features. A test pattern was first designed with features comparable in size to those of native basal lamina structures. A positive master was produced by using a laser to machine the geometric specifications into a polyimide chip. Negative replicas

of the chip were made of polydimethylsiloxane silicone elastomer, and those were used as templates for the production of thin ($\sim 21 \mu\text{m}$) membranes composed of either a collagen-glycosaminoglycan copolymer or gelatin.

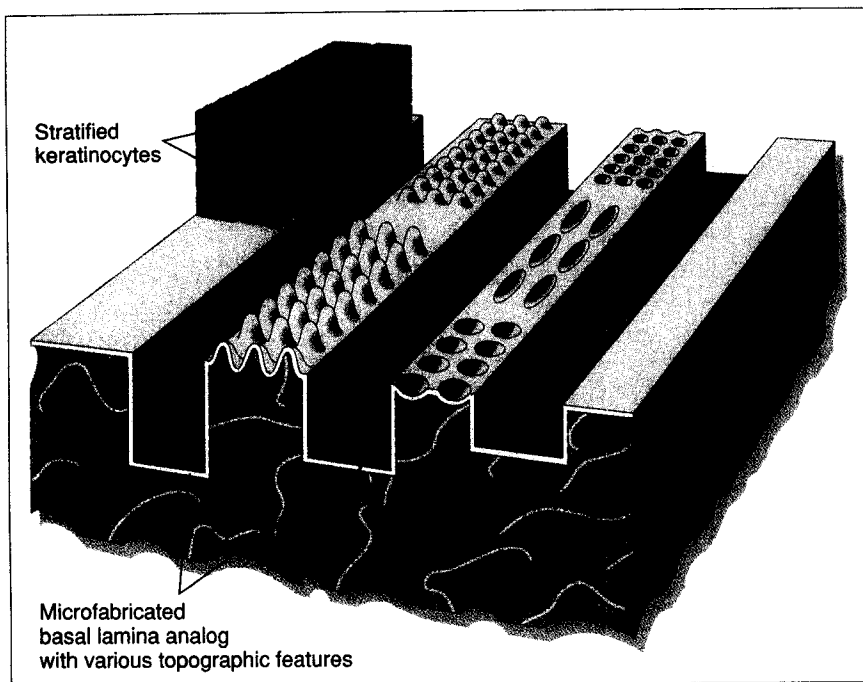
The membranes had a complex topography of ridges and channels that recapitulated the features of the master chip. To demonstrate their utility, these membranes were laminated to sponges made of type I collagen, and their surfaces were seeded with cultured human epidermal keratinocytes to form a skin equivalent.

The keratinocytes formed a translucent, hydrophobic epidermis with gross topographic features that correlated with the microfabricated surface of the membrane. Histological analyses showed the epidermal layer to be composed of differentiated and stratified keratinocytes that conformed to the surface of the skin equivalent, forming rete ridge-like structures comparable in dimension to those in natural skin.

The basal lamina analogs in this study served as membranes that supported attachment and growth of keratinocytes in complex topographies and prevented keratinocytes from passing through while allowing diffusion of nutrients. Compared to natural basal lamina (50 to 340 nm), the analogs were relatively thick; future efforts will be devoted to producing thinner membranes.

Another difference between the analog and natural basal lamina is composition. Although the exact composition varies between tissues, all basement membrane structures are believed to contain type IV collagen, laminin, fibronectin, and heparan sulfate, whereas the analogs were produced with type I collagen or gelatin. Preliminary data

"Tissue Engineering" is edited by Jeffrey R. Morgan and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School.



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showed that similar membranes could be produced with material containing the natural components.

Interestingly, the topography of the membrane influenced the differentiation of the keratinocytes, as stratification was enhanced in the deeper channels. This finding suggests that the topographical microenvironment influences keratinocyte differentiation and proliferation by affecting the spatial arrangement of cell-matrix contacts or cell-cell contacts or both.

In human palmar epidermis, 80% of proliferating basal and suprabasal cells are located in the deeper rete ridges. Expression of $\alpha_2\beta_1$ integrin, a marker of epidermal stem cells, also varies with topography. High expression is found in patches of basal cells located on the tips of dermal papillae (foreskin and scalp) or at the bottom of the deep rete ridges (palm). A microfabricated membrane may be a useful tool to investigate the relationship between the dimensions of topographic features and the proliferation and differentiation of basal epidermal keratinocytes.

Microfabricated membranes may also be useful for examining whether topographic features of

the skin contribute to its mechanical stability. In areas of the skin exposed to considerable friction, such as the plantar and palmar surfaces, the dermal papillae and epidermal ridges are longer and more numerous. This suggests that enhancement of the interface between the epidermis and the dermis provides additional mechanical stability.

By testing the properties of skin equivalents that have rete ridges and dermal papillae of various geometries, it should be possible to establish a quantitative link between topography and mechanical properties. Moreover, these studies can be extended to the molecular level by examining the distribution and numbers of known adhesive proteins such as integrins and hemidesmosomes.

Tissue-engineered skin equivalents have clinical applications in the treatment of severe burns and chronic ulcers. Steven Boyce's laboratory at the Shriners Hospital for Children in Cincinnati produced a composite skin substitute by laminating the surface of a flat collagen-glycosaminoglycan sponge. Fibroblasts were seeded onto the porous sponge, and a flat epidermal layer

was cultured on the laminated surface. In clinical studies, these skin substitutes have had some success in the treatment of full-thickness burns. Organogenesis, Inc., of Canton, Massachusetts, has produced a skin equivalent by culturing a flat epidermal layer on the surface of a fibroblast-populated collagen gel. This skin equivalent has been approved for the treatment of venous leg ulcers.

Each of these skin substitutes has a flat interface between the epidermis and the dermal component. A microfabricated membrane that formed a complex interdigitating interface might improve the resistance of skin substitutes to failure caused by shear forces. Such a complex interface might also facilitate mass transport of nutrients and growth factors to the epidermis and aid in graft take. Moreover, because microfabrication can be used to produce complex patterns, it should be possible to mimic the fine lines and pore structures of native skin to form a more "natural" and cosmetically acceptable skin substitute.

Because basal lamina are an integral part of many tissues other than skin, microfabricated membranes may have other applications in tissue engineering and developmental biology. For example, it may be possible to enhance mass transport in tissue-engineered small intestine by using a microfabricated membrane that mimics the topography of the intestinal mucosa. During embryogenesis, basal laminae perform critical roles in tissue compartmentalization and organized cellular migration. With the recent isolation of embryonic stem cell lines derived from human blastocysts, microfabricated analogs of the basal lamina may be useful for exploring the roles of cell and matrix interactions in tissue morphogenesis and mammalian development.

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Mitochondrial Iron in Friedreich's Ataxia

by Jerry Kaplan

A group of neurological disorders is associated with defects in mitochondrial oxidative phosphorylation, and a separate group is caused by a genetic abnormality in which a particular nucleotide triplet is repeated many times more than is normal. The genetics and pathophysiology of Friedreich's ataxia include both elements, but with surprisingly different effects than those seen in other neurodegenerative diseases. Friedreich's ataxia is a paradigm for contemporary molecular medicine, because the responsible gene has an equivalent in other species, in which the effects of inactivating the gene can be investigated.

Friedreich's ataxia is the most frequent of the recessive ataxias, with an incidence of 1 in 40,000 among Caucasians. The disease presents most commonly as a disorder of gait and coordination, and the typical patient dies in early adulthood of hypertrophic cardiomyopathy. There is great variability in the age of onset and severity of symptoms. Friedreich's ataxia is most commonly diagnosed in children, with symptoms developing at an average age of 12 years, but cases have been reported with early onset (age 2 to 4) and late onset (age 50). Early-onset cases show a greater rate of disease progression than late-onset cases.

The neurologic and cardiac disturbances in Friedreich's ataxia have been considered to be separate consequences of a common defect. Because neurons and cardiac myocytes are sensitive to impaired energy production, the responsible defect has long been thought to lie in mitochondria, and various deficiencies in mitochondrial function have been reported in patients.

SYMPTOMS ASSOCIATED WITH FRIEDREICH'S ATAXIA

- Progressive limb and gait ataxia
- Absent tendon reflexes in legs
- Dysarthria
- Loss of position and vibration senses
- Pyramidal weakness of legs
- Cardiomyopathy

Within a family, the pattern of Friedreich's ataxia shows recessive mendelian genetics, but it has not been clear whether the disease involved one gene or many. In the past four years, there have been watershed discoveries that have illuminated the genetic basis and pathophysiology of the disorder, and these are the subject of this article.

Loss of Function of a Gene Product Is Involved

Fundamental to advances in understanding the genetic basis of Friedreich's ataxia was a clear and rigorous outline of the clinical criteria used to define the disease phenotype. The Montreal Neurological Consortium and the late English neurologist Anita Harding deserve credit for articulating the diagnostic criteria. Rigorous patient ascertainment was the essential prerequisite that permitted the use of positional cloning to map and identify the disease gene.

Positional cloning makes use of identifiable stretches of DNA called markers. An example of a marker is a tandem repeat, usually of a cytosine-adenine pair (CACACA etc.). Thousands of such markers are distributed throughout the human genome, and they can be reliably located. The closer such a marker lies to a gene, the lower is the recombination frequency. A marker that is always transmitted with a disease phenotype (or in other words that shows no recom-