"Matrix Algebra" Heals Life's Wounds

Recent work shows that interactions between growth factors and the extracellular matrix are of central importance in the process that brings wounds to a close

Keystone, Colorado—WOUND HEALING HAS been the subject of medical "experiments" for thousands of years. Mud packs, honey poultices, fire, dry and moist dressings, all have been used to treat wounds. Often, of course, no treatment at all works just fine—and the wound heals on its own. But how? In spite of millennia of experimentation, that question has remained unanswered until recently.

Only in the last 5 years have researchers begun to work out the underlying molecular signals that direct the process of wound healing. One of the essential findings has been that a handful of growth factors play key roles in every step—from the response immediately following an injury to bringing the healing process to an orderly close. But a central mystery has remained: What mechanisms control the growth factors themselves, turning them off and on with the exquisite timing needed for a wound to be rejoined?

In the last 18 months a surprising answer has emerged to this tough question: the extracellular matrix. This complex, cross-linked structure of proteins and polysaccharides, which organizes the geometry of normal tissues, was long thought to be little more than an inert framework. But it turns out that the matrix is far more than a scaffold. By modifying the action of growth factors (among other activities) it is proving to be an essential component of wound healing. Indeed, the latest results suggest that the dynamic relationship between growth factors and the matrix lies at the heart of wound healing.

When the organizers of a recent Keystone conference on growth factors and wound healing started planning 18 months ago, they hoped to catalyze the field by bringing together researchers who work on growth factors with those who work on the extracellular matrix. But the area is moving so fast that "by the time we got around to the meeting, that was already happening," says Michael Pierschbacher, a biochemist at Telios Pharmaceuticals, Inc. in La Jolla, California, who helped plan the meeting.

It became clear at a number of sessions at the Keystone meeting that what Pierschbacher calls the "delicate balance" between growth factors and the matrix is evident in every step of healing. It has long been known that, following a cut or scrape, a protein called fibrinogen, circulating in the bloodstream, forms a fibrin clot, sealing damaged blood vessels. Fibronectin, another protein in the clot, links up to the matrix, forming a bridge between clot and surrounding tissue.

But the matrix does far more than simply stabilize the clot. Alterations in the matrix caused by injury appear to initiate cellular responses—including the release of growth factors. Before wounding, for example, basic fibroblast growth factor (bFGF) is tightly bound to the matrix outside normal skin and muscle cells, and probes for the bFGF receptor on the cells' surfaces or for its messenger RNA inside them come up negative.

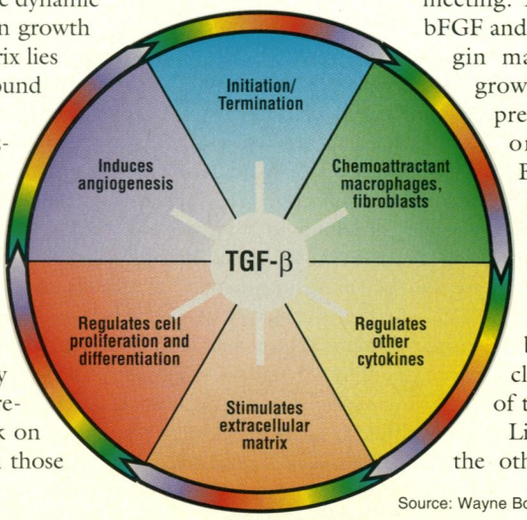
Soon after any major insult, such as a mechanical or chemical injury, however, this picture changes dramatically, Andrew Baird of the Whittier Institute in La Jolla told several hundred attendees during the first session of the meeting. The matrix releases bFGF and signals cells to begin manufacturing that growth factor and expressing its receptor on their surface. Baird noted that knowledge of when and where growth factors are expressed during wound healing will be one key to the clinical applications of the protein factors. Like bFGF, most of the other growth factors produced during wound healing cause a variety of cell types to proliferate. As a result, unless the growth factors are confined to the injured area, they could stimulate cell division where it's not needed—and could be harmful. Once again, it's the matrix that choreographs the process; molecules like collagen, fibronectin, and heparin, which make up the matrix, bind growth factors tightly and prevent them from traveling. Says Pierschbacher. "Without this binding, wound healing would be a systemic response, not the local one it needs to be."

Recent studies show that the matrix functions as a kind of combined highway and road sign system, guiding rapidly moving cells toward the target zone. During healing, cells migrate toward the wound area, drawn by a variety of chemical signals. For this movement to occur, the interaction between cells and the matrix must be precisely controlled. This requires altering the profile of cell surface receptors so that the cell first breaks free from its initial position in the matrix, then moves along the matrix toward the wound, and eventually anchors itself again.

That process is exemplified by the epidermal cells called keratinocytes, which are normally anchored to a basement membrane. During wound healing, however, keratinocytes break free and migrate toward the site of injury. At the Keystone meeting, Frederick Grinnell of the University of Texas Southwestern Medical Center in Dallas described his evidence that this dramatic movement begins with changes in the cells' integrins, a family of dimeric receptors in the cell membrane that are highly specific for binding to components of the matrix.

The keratinoctyes are activated in a two-step process, says Grinnell: removal from the basement membrane, perhaps during the injury, and the subsequent arrival of as yet undetermined growth factors. The activated keratinocytes express "matured" integrins that enable them to recognize and attach to fibronectin molecules in the matrix. Just the right kinds of integrin subunits have to be in place, says Grinnell, otherwise keratinocyte migration is impossible: "Like walking through deep mud or over ice—there's either too much traction or too little."

But the cellular choreography of healing



Healing cycle. *Transforming growth factor-beta (TGF-beta) is a central player in many of the steps of wound healing, from the immediate initial response to the termination of the process.*

Source: Wayne Border/Illustration: Diana DeFrancesco

Growth Factors in the Clinic: Slow Going

In the last few years researchers have discovered that a small group of growth factors plays a key role in wound healing. Some of these factors are already in clinical trials, where they could be particularly helpful for slow-healing wounds or those that don't heal at all, a problem that plagues diabetics, cancer patients, and those confined to bed for long stretches. Care for chronic wounds, many of which require daily treatment, costs up to $4 billion a year in the United States according to a Boston University School of Medicine study.

Cutting-edge research, a crying medical need, and a huge market. Sounds like a surefire prescription for success, right? Well, think again. "Given all the work in this area, the clinical trials on growth factors and wound repair are a bit disappointing," says Richard Clark, a dermatologist at the State University of New York in Stony Brook and one of the organizers of a recent Keystone symposium on wound repair. And, despite heavy investment in research by biotechnology firms across the country, no new products in this area are near final approval by the Food and Drug Administration (FDA).

One reason clinical trials are lagging is that they have, for the most part, aimed at finding a single growth factor that would move the healing process along quickly. Although it may still be possible to find such a "magic bullet" for specific clinical situations, several new approaches could hold greater promise. One is a "cocktail" of growth factors and other molecules that may mimic the natural healing process better than any single substance. The other is concentrating on the quality—rather than the speed—of the healing process. Yet another twist may be the application of growth factor *antagonists* in situations where it seems that an excess of growth factors, rather than a deficiency, is the culprit.

The current round of clinical work was largely touched off by the finding that transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EDGF) speed up wound healing and boost wound repair in tissue culture and in animal models. More than a dozen trials are now under way in human beings, many of which are testing acidic and basic FGF, PDGF and TGF-beta on patients with nonhealing dermal ulcers such as long-term pressure sores or leg ulcers resulting from diabetes.

A key assumption behind these trials is that slow-healing wounds lack some crucial growth factor or don't have the right mix of factors. Recent work by biochemist Gregory Schultz at the University of Florida School of Medicine supports that idea. Schultz's team added fluid taken from chronic wounds to cultures of two types of cells that divide rapidly during tissue repair: endothelial cells and fibroblasts. The fluid shut down DNA synthesis, and the cells shriveled up, sent out long processes, and even lost their attachment to the culture dish. Fluid from healing mastectomy wounds, on the other hand, increased DNA synthesis fifteenfold after 2 days. Levels of TGF-alpha from the mastectomy wound fluids were found to be double or triple those in the nonhealing wound fluid.

In spite of such suggestive evidence, simply applying one or another of these growth factors to chronic wounds has not had the substantial effects required for FDA approval. Partly as a result, some investigators are now pursuing the "cocktail" approach, which is already being tested in Phase III clinical trials. David Knighton, a surgeon at the University of Minnesota Hospital and Clinics and his colleagues are treating leg ulcers of people with diabetes using a platelet-derived mix of growth factors, proteases (enzymes that cleave proteins), and cell-adhesion molecules. Applications of the mixture, accompanied by aggressive medical and surgical care, have completely healed wounds that had been open for years and resisted other treatments.

Though most work has emphasized faster wound healing, researchers are beginning to realize that speed may not always be of the essence. "The other thing we need to be looking at is the quality of the healing, and what kind of scar is produced," says Glenn Pierce, associate medical director of Amgen in Thousand Oaks, California, who is working on PDGF and dermal ulcers. "Maybe we should be asking if we can regenerate tissue rather than just help it heal."

As clinical research on growth factors in wound healing moves ahead, it has become clear that growth factors aren't just wonder workers—they also have a dark side. For example, work by nephrologist Wayne Border of the University of Utah School of Medicine points to TGF-beta as the culprit in glomerulonephritis: a disorder caused by accumulation of too much extracellular matrix in the glomeruli, the kidney's filtration units. This condition is the leading cause of kidney failure in people with such diseases as lupus, diabetes, and hypertension; estimates for treatment run as high as $4 billion annually.

The trick here, says Border, may be to add molecules that stop TGF-beta in its tracks. As the amount of TGF-beta and matrix increases in the kidneys, so does the amount of decorin, a matrix molecule that binds to the growth factor and reduces its activity. Border's experiments with rats show that clinicians can mimic this process by delivering anti-TGF-beta antibodies or decorin—thereby reducing excess matrix and, in turn, the amount of swelling of the glomeruli. Indeed says Border: "At one time, everyone was thinking TGF-beta would be the wonder drug [for speeding wound healing]. Who knows? Anti-TGF-beta may end up being a wonder drug itself." ■ **P.S.**

requires more than just having the proper integrin molecules on the cell's surface, according to Erkki Ruoslahti, scientific director of the La Jolla Cancer Research Foundation; the integrins must also be present in precisely the right amounts. Too few receptors and the cell can't grasp the matrix tightly enough; too many and it can't let go. Here again, the dynamic balance between the matrix and growth factors seems to be central to the correct progression of events.

Growth factors, especially transforming growth factor-beta (TGF-beta), are known to have striking effects on the expression of integrins at the cell surface. But it's quite possible that the growth factor doesn't act directly on the gene itself. Instead, Ruoslahti hazards, growth factors act on a molecule "lurking in the cytoskeleton that responds to some change out there and goes straight to the nucleus" where it can then turn integrin genes off or on.

Cells need more than just changes in things like integrin expression to help them negotiate their way to the wound site. Like a fullback plunging into the line, the cells must sometimes cut straight through the matrix as well as move along it. As keratinocytes move toward the wound, they come into contact with matrix molecules and growth factors that stimulate them to produce collagenase, an enzyme that breaks up collagen molecules. This enables the cells to cut "through a sea of collagen and migrate underneath any scab to get to the wound bed," says Stanford dermatologist David Woodley. The response is

Wound Healing in the Womb

Babies born after undergoing fetal surgery often grow up missing something—scars from their operations. "The only way we could find the incisions on some of these babies was because the stitches were still in place," says N. Scott Adzick, a pediatric surgeon with the University of California, San Francisco's Fetal Treatment Program, the sole U.S. institution where fetal surgery is performed.

So far, Adzick, Michael Harrison, and their colleagues have operated on 28 fetuses between 18 and 28 weeks old for life-threatening problems such as congenital diaphragmatic hernia and blockage of the urinary duct. They are finding that the younger the fetus is at the time of surgery, the less likely he or she is to be born with surgical scars, especially those marking the spots where electrocardiogram leads were implanted in the skin.

In fact, healing of the skin in prenatal mammals barely resembles the process in adults. In all species examined (humans, monkeys, sheep, rabbits, mice, and pigs) the prenatal process is faster and more efficient—and produces new tissue rather than a scar. Histologically, it is almost impossible to distinguish healed tissue from its unmarked counterpart.

Now, that doesn't mean adult wound healing is bad. "We have been healing with scars for untold generations, so it must have its advantages," says Thomas Krummel, a pediatric surgeon who recently moved from the Medical College of Virginia to the Hershey Medical Center of the Penn State School of Medicine to continue his studies of fetal wound healing. In the case of adults, says Krummel, evolution has probably sacrificed aesthetics to function—quickly closing a wound to keep out infection, something that isn't so crucial for the fetus.

From the start, fetal and postnatal wound repair differ dramatically. In the adult process, inflammation, cell migration, and significant changes in the extracellular matrix are common (see main story). In the fetus, inflammation is slight, and only small numbers of fibroblasts migrate to the wound. In addition, the influence of the extracellular matrix—which researchers now see as a key to adult wound healing—is only a shadow of its postnatal counterpart.

Growth factors also appear to play a different role in fetal wound healing. They exist in the fetal environment but appear to direct the process less than in adults. In the fetus, the real director of events may be hyaluronic acid, says Adzick. This substance appears wherever rapid tissue development takes place; fetal skin and amniotic fluid are loaded with it. Soon after injury to a fetus, hyaluronic acid is deposited, followed later by highly organized collagen, a key matrix component.

Wound healing in adults retains some curious echoes of the healing processes active in the womb. In adults, a burst of hyaluronic acid appears soon after injury but disappears in a day or two, to be followed by disorganized collagen that gets remodeled over the following months—that's what's called a scar in plain English. The transition from the fetal regime to adult wound healing happens gradually, and is generally complete some time during the third trimester of pregnancy.

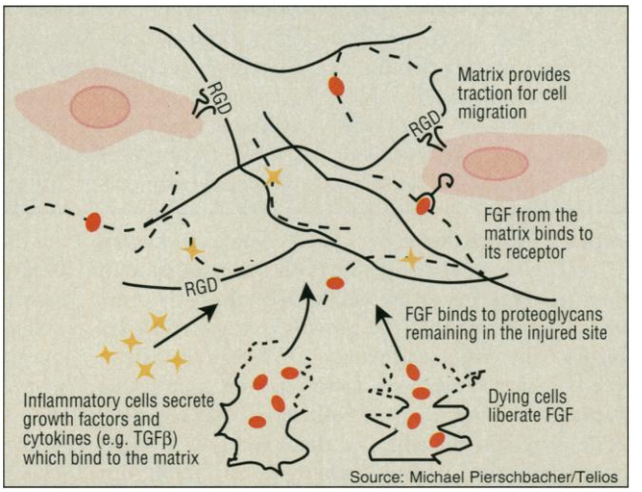
The ultimate "pie in the sky," enthuses Krummel, is to apply what is learned from fetal wound healing to the world outside the womb. In virtually every adult organ, scarring creates problems, from damage to heart valves caused by rheumatic fever to abdominal adhesions and disfiguring keloid scars on the skin. Someday, Krummel suggests, researchers may be able to manipulate adult wounds so that all of us can heal without scars. ■ **P.S.**

quite specific, Woodley adds: Keratinoctyes not in contact with the matrix make little collagenase.

Another type of cell—the fibroblast—also migrates to the wound; indeed, fibroblasts are the dominant cells in healing tissue. The potent combination of matrix molecules and growth factors also influences what happens to these cells when they arrive at their goal. Once at the injury site, the cells' response to growth factors such as fibroblast growth factor (FGF) appears to be controlled by the extracellular matrix. Grinnell has shown that fibroblasts growing on contracted, asymmetric collagen gels begin dividing when growth factor-containing serum is added to the medium, while those on relaxed, symmetric gels don't divide. This may mirror what happens after injury, when wounding has stressed the extracellular matrix, and in this "disrupted environment" FGF causes fibroblasts to proliferate, says Grinnell.

Cell proliferation is clearly important for tissue to regenerate. Yet equally important for proper wound healing is the end of the process, when the proliferation is turned off. Says Grinnell: "It's not only interesting that wounding stimulates cells to start dividing, but that it eventually comes to an orderly end, and cells that were activated return to an inactive state."

The inactivation depends partly on TGF-beta, which is abundant in most wounds. In response to that growth factor, the fibroblasts that have migrated to the injury begin making considerable quantities of collagen, which restores the damaged matrix and relieves the stress that may have initiated the cell proliferation. As the stress is relieved, the cells' responsiveness to growth factors diminishes. Eventually, fibroblasts stop dividing—even though they are still bathed by growth factors in the almost healed wound.



Help is on the way. *Migrating cells—guided by growth factors such as TGF-beta and fibroblast growth factor (FGF)—use the extracellular matrix as a highway.*

Understanding the complex interactions between matrix and growth factors that control wound healing could ultimately pay off in products that speed wound repair. Growth factors by themselves have a "checkered past" when it comes to clinical use, Pierschbacher noted at the conference. Insights into the matrix could be just what is needed to play a better, more effective game on this clinical checkerboard. "It may be that we just need to figure out how to use the natural control mechanisms—like matrix—to regulate [the growth factors'] actions."

■ **P.J. SKERRETT**

P.J. Skerrett is a free-lance science writer based in Boston.