



REGENERATION

The secrets of healing without scars

Skin regeneration is an imperfect process that is impeded by a host of factors. Working out the part played by each could lead to fresh approaches to treating burns and scars.

BY CASSANDRA WILLYARD

The body's largest organ might seem barely more than cellular wrapping paper, but skin has roles that range from fending off microorganisms to regulating body temperature. It also has a considerable flaw: severely damaged skin can heal, but it can't regenerate. Instead, it forms scars. These marks are not just cosmetic defects. Scar tissue can inhibit a person's movement and, because it lacks sweat glands, prevent the body from cooling off. Although scars seem to be thicker than normal skin, the tissue is actually weaker.

Scarring seems to be an inevitable part of being human. But three decades ago, it became clear that the youngest patients don't scar.

When Michael Harrison, a paediatric surgeon at the University of California, San Francisco, began to perform the first ever surgeries on fetuses, he noticed something curious about the babies who survived. Incisions he had made in them in the womb seemed to heal without scarring.

Harrison asked Michael Longaker, a postdoctoral researcher in his laboratory, to investigate the phenomenon. Longaker was sceptical. Because his boss was the only physician who was performing fetal surgeries, he says, "My first reaction was, 'Gosh, that doesn't seem like a big health-care problem because you're the only one making [fetal] wounds.'" But it didn't take long for Longaker to understand the potential implications: by

deciphering what drives this *in utero* healing, he might discover ways to prompt scar-free healing outside the womb. "My reluctant one year in the lab became four," Longaker says. "I became obsessed with scarring."

Longaker, now a plastic surgeon with a focus on regenerative medicine at Stanford University in California, has not yet unravelled the mystery completely. Nor have other researchers. Although many studies have provided valuable insight into how scarring occurs, they have yielded few clinically useful treatments. "There's been some improvement," says Stephen Badylak, deputy director of the McGowan Institute for Regenerative Medicine at the University of Pittsburgh in Pennsylvania. But it's still far from the expectations raised by

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the hype of the work that began in the 1980s.

Yet many researchers are cautiously optimistic that a better understanding of the mechanisms that lead to scarring will pave the way for innovative strategies for reducing the formation of scar tissue. In September, the US Food and Drug Administration approved the first treatment to involve a 'spray-on' skin, and numerous other skin-healing products are in clinical trials. The field of skin regeneration is moving in a different direction, Badylak says. Rather than growing skin in Petri dishes in the lab, and then transplanting it onto people, researchers are using the body as a bioreactor and encouraging skin to do what it did during fetal development — regenerate. They want to find out more about how scarring occurs, as well as how it might be stopped.

EVOLUTIONARY ADVANTAGE

Cut the skin and it will bleed. And then it will heal. Initially, a clot forms to staunch blood flow, which kicks off a massive inflammatory response. Immune cells flood the region to clear bacteria and debris, while cells called keratinocytes in skin's outer layer divide rapidly in a race to close the wound and prevent infection. Next, the wound begins to fill. Spindle-shaped cells known as fibroblasts migrate to the damaged area and churn out collagen and other proteins that provide tissue with structure. Within three weeks of the injury occurring, the wound has healed.

But such speedy healing has a major downside. These quick repairs often result in scars, particularly when the wound is deep. In healthy skin, collagen fibres form a lattice. But during wound healing, fibroblasts lay down collagen fibres parallel to each other, which creates tissue that is stiff and weak. That's because evolution has selected speed over perfection: before the discovery of antibiotics, slow healing would probably have meant acquiring an infection or experiencing prolonged bleeding. "It's really a matter of survival versus aesthetics," says Jeff Biernaskie, a stem-cell biologist at the University of Calgary in Alberta, Canada.

When such repairs to skin are small, they don't pose much of a problem. But large scars can be life-changing. Scar tissue "doesn't have the stretch and the mobility and the range of motion that normal skin does," says Angela Gibson, a burn surgeon who studies wound healing at the University of Wisconsin School of Medicine and Public Health in Madison. That can be especially problematic when scars cover joints. Imagine, Gibson says, not being able to hold a fork or to raise your arms to wash your hair.

But scarring might not be inevitable. Fetal skin begins to scar only late in gestation, which suggests that human skin possesses at least some regenerative capabilities. All researchers have to do is to work out how to unlock them.

FANTASTIC FIBROBLASTS

Fetal wounds are not the only wounds that are resistant to scarring. **Thomas Leung,**

a dermatologist at the Perelman School of Medicine at the University of Pennsylvania in Philadelphia, noticed that older people often develop thinner scars than do younger adults. To understand why, Leung turned to mice. He and his colleagues compared wound healing in young and old mice by punching holes in the rodents' ears'. In one-month-old animals, such wounds healed with a thick scar and never closed fully — similar to earring holes in people, Leung says. In 18-month-old mice, which are roughly equivalent to 65-year-old people, healing took longer, but the holes closed completely, and with less scarring. The same observations held for wounds on the backs of the mice.

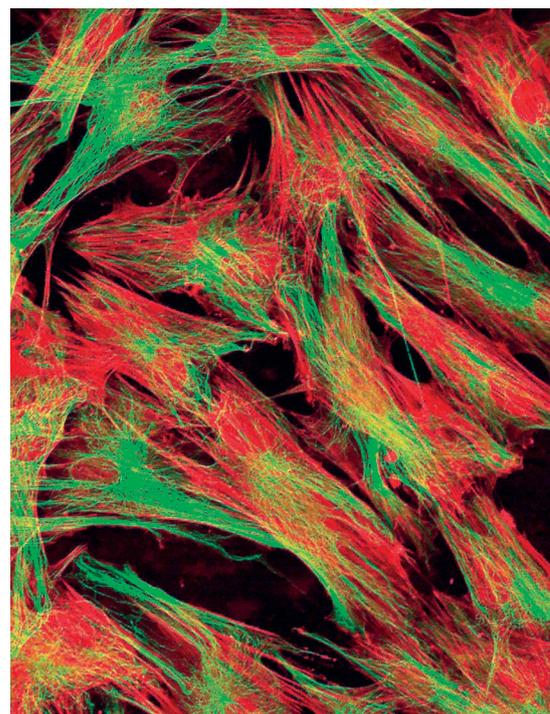
Leung and his colleagues wondered whether a component of the blood of young mice promotes scar formation. To test the idea, they joined together old and young mice, giving them a shared circulatory system through a surgical technique called parabiosis. The team found that exposure to the blood of young animals caused wounds in elderly mice to scar¹. Further experiments revealed the probable culprit: *Cxcl12*, a gene that encodes a protein called stromal cell-derived factor 1 (SDF1). When the team knocked out SDF1, even wounds in young animals healed with minimal scarring. This discovery suggests a route towards scar-free wound healing in people: suppressing the activity of *CXCL12*.

In fact, there's already a drug on the market that interferes with the SDF1 pathway — plerixafor. The drug is used to mobilize stem cells from bone marrow in people with certain types of cancer. Leung and his colleagues hope to test whether plerixafor can minimize the recurrence of keloids — thick, raised scars that tend to keep growing — in a clinical trial. The team is also looking at how SDF1 promotes initial scar formation.

Scarring is a complex process, and SDF1 is only part of the story. Fibroblasts are another prominent player. These cells have long been blamed for scar tissue. "We've had this assumption that fibroblasts are all the same," Biernaskie says. But research in the past five years has revealed that fibroblasts comprise a diverse group of cells, and that some seem to have a larger role in scar formation than do others.

In 2015, Longaker and his colleagues conducted an inventory of the fibroblasts on the skin of a mouse's back². When they created a wound on the back, they found that only one of two lineages of fibroblast — expressing homeobox protein engrailed-1 — was responsible for the formation of most scar tissue. And when the team disabled those cells in mice, wounds healed more slowly but also formed less scar tissue, similar to what happened in mice that lack SDF1. Longaker thinks that if he and

"The really cool part is that once you get a hair follicle, it kind of normalizes the skin."



Fluorescence micrograph of human skin fibroblasts.

other researchers can find a way to identify and block the same fibroblasts in people, it might be possible to prompt wound healing to follow a more regenerative pathway. "I would be disappointed if we're not doing something like that in humans in the next five to seven years," he says.

Although some fibroblasts are clear drivers of scar formation, other research suggests that fibroblasts also contribute to regenerative healing. About a decade ago, George Cotsarelis, a dermatologist at the Perelman School of Medicine, and his colleagues were trying to develop a mouse model to understand the role of stem cells in hair follicles. Scientists had long thought that when an adult hair follicle is lost, it is gone for ever. But then the team noticed something odd: when they made a large wound on the back of a genetically normal mouse, hair regrew in the middle of the wound³.

Even more strangely, skin around hair follicles seemed to be normal, and a layer of fat formed beneath — something that doesn't usually occur under scar tissue. In 2017, a team led by Cotsarelis showed in mice that new hair follicles secrete growth factors called bone morphogenetic proteins (BMPs) that can transform fibroblasts into fat cells⁴. "The really cool part," Costarelis says, is that "once you get a hair follicle, it kind of normalizes the skin".

Human fibroblasts also seem able to make the leap from fibroblast to fat. When the team took such cells from a keloid scar and exposed them to a BMP, or placed them near a BMP-secreting hair follicle, they too turned into fat cells. These findings suggest that it might be possible to prod injured skin towards regeneration rather than scar formation. But translating the work into a treatment protocol poses considerable difficulties, Cotsarelis says. Skin



Reindeer antler velvet has regenerative properties.

regeneration will require the right signals to be delivered at the right time, and at the right dose. For example, “When hair follicles form, their spacing is determined by gradients of growth factors,” he says. Altering those gradients, even slightly, might alter the follicle pattern or even function. “Precision is really required,” he says.

A MORE PERFECT MODEL

The mice in which most research on wound healing is performed differ from people in important ways. Their skin is loose, whereas that of humans is tight. Furthermore, mouse wounds heal by contraction: such wounds pull together rather than filling in. “I don’t know how you can even begin to think you could test something there and then translate it to humans,” Gibson says.

In search of a better model, in 2009, Ashley Seifert, a developmental and regenerative biologist at the University of Kentucky in Lexington, travelled to Kenya and began to study African spiny mice (*Acomys kempfi* and *Acomys percivali*) — species with a unique defence mechanism. Because their skin tears easily, these mice can escape the jaws of predators. Seifert expected to find that such mice had speedy wound-repair processes or ways of preventing infection. But what he and his colleagues found was much more intriguing: spiny mouse wounds heal relatively scar free⁵.

The spiny mouse is one of only a few mammalian models of skin regeneration. But such mice provide a comparative framework. Seifert can punch a hole in the ear of a spiny

mouse, which regenerates, and another in the ear of a conventional lab mouse, which does not, and then evaluate how the healing process differs. His team is now beginning to define those differences.

Some seem to involve the immune system. Researchers tend to view inflammation as an impediment to regenerative healing. Accordingly, the difference between scar formation in adults and the fetus might be that adults mount a strong inflammatory response after injury whereas a fetus does not. But a connection between inflammation and regeneration has been difficult to establish. Efforts to prevent scar formation by suppressing inflammation haven’t panned out, Seifert says. And he and his colleagues have found, at least in spiny mice, that inflammation does not preclude regenerative healing. In the wild, these mice mount a strong inflammatory response yet still manage to regenerate skin.

“We know that too much inflammation is bad. And we know that no inflammation isn’t helpful either,” Seifert says. In 2017, he and his colleagues showed that macrophages, immune cells that are a key orchestrator of inflammation that is typically associated with scarring, are also required for regenerative healing in spiny mice⁶. Now, the team is trying to determine which factors might tip macrophages and other immune cells away from scarring pathways and towards regeneration.

A much larger mammal — reindeer (*Rangifer tarandus*) — is also providing insight into the regenerative potential of skin. Both male and female animals sprout new antlers each year. The downy velvet that covers the antlers as they grow is remarkably similar to human skin — thick with blood vessels, hair follicles and sebaceous glands. But it differs in one important way. “If we wound the velvet, it regenerates perfectly,” Biernaskie says. “It’s really a beautiful and powerful model for skin healing.”

That capacity for regeneration seems to be inherent to the velvet. Biernaskie and his colleagues are now comparing changes in gene expression during wound healing in two anatomical areas of reindeer — skin on their backs, which doesn’t regenerate, and antler velvet, which does. They hope that the comparison will help them to better understand the signals that prompt velvet to regenerate, and perhaps lead them to treatments that promote regeneration and prevent scarring. “We could start to develop cocktails of drugs where we could mimic those signals,” Biernaskie says.

BENCH TO BEDSIDE

Skin regeneration is still a distant goal, but several companies are working to bring wound-healing therapies to market. The

spray-on skin system approved by the Food and Drug Administration earlier this year, and marketed as ReCell by biotechnology company Avita Medical in Valencia, California, is an example of an early success.

To prepare the treatment, surgeons remove a piece of skin about the size of a postage stamp from the patient and douse it with an enzyme that liberates skin’s component cells: fibroblasts, keratinocytes and pigment-producing melanocytes. These cells are then loaded into a nozzled syringe and sprayed onto the patient’s wound. People with burns who require skin grafts typically receive pieces of skin that are harvested from unaffected parts of their bodies. Surgeons take only the top layers of skin to create these grafts, which are known as split-thickness grafts. One clinical trial showed that in people with second-degree burns, which affect both skin’s epidermal and dermal layers, the ReCell therapy works as well as do conventional grafts, but requires much less donor skin⁷. Although split-thickness grafts can be cut into a mesh that covers an area about three times their size, ReCell can treat skin wounds that are 80 times larger than the donor piece of skin. ReCell can also be combined with meshed grafts to treat deeper burns.

Gibson is testing an alternative treatment for burns, a skin substitute called StrataGraft. It comprises two layers of collagen: a bottom layer that is seeded with human fibroblasts and a top layer that is seeded with cells that give rise to keratinocytes. The therapy originated at the University of Wisconsin, but is now being developed by Mallinckrodt Pharmaceuticals in Staines-upon-Thames, UK. One of the first clinical trials of StrataGraft, published in 2011, showed that it did not induce an acute immune response⁸, and the substitute is now being tested in a phase III trial.

Such therapies could be a boon for people with burns. Other companies are working on treatments for tricky-to-heal wounds, such as ulcers in people with diabetes or bedsores. “The market size is just gigantic,” Badylak says. But the main goal of these treatments is to promote better healing, rather than to prompt skin to regenerate. Achieving that next step — scar-free healing — is “a tall order to fill”, Gibson says. However, she is optimistic that if clinicians who treat skin wounds collaborate closely with researchers who are working to understand scarring, the problem can be solved. “That’s when the science will move forward,” she says. ■

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