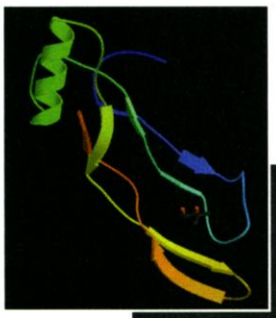
NEWS

Tissue Engineers Build New Bone

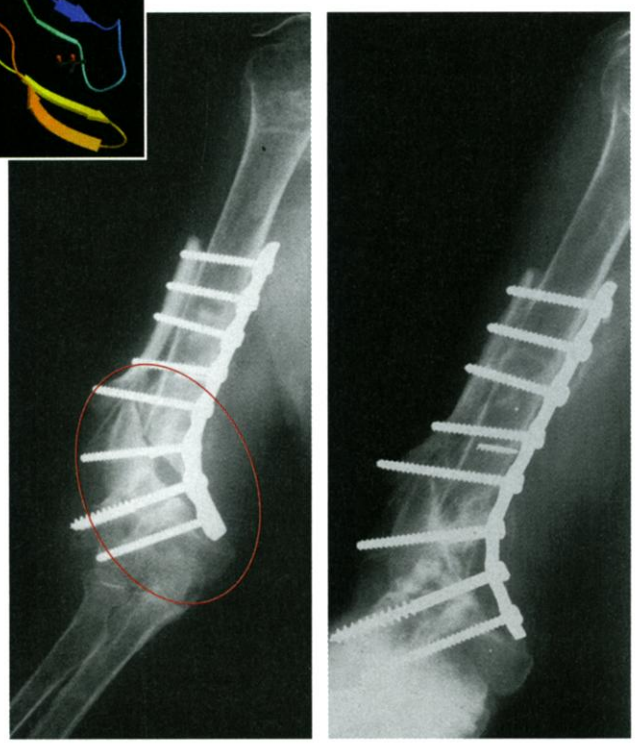
Bone repair may be one of the first major applications of tissue engineering; efforts to encourage the growth of new bone using novel matrices, bone morphogenic proteins, gene therapy, and stem cells are all showing promise

Mending broken or damaged bones is a hit-or-miss business. Orthopedic surgeons have become adept at manipulating, pinning, and immobilizing fractures, giving the body's natural bone-healing processes an opportunity to knit the broken pieces together. In recent decades, they have also learned to graft bone from elsewhere in the body to repair major damage from accidents or disease: Every year doctors in the United States alone perform about 450,000 surgical bone grafts. But some fractures simply refuse to heal, and bone grafting adds to the pain of recovery. At times, this procedure can't even be attempted because "in many patients the quality and quantity of bones you can harvest is not sufficient," says Scott Bruder, a bone tissue engineering expert at DePuy, a Johnson & Johnson company based in Raynham, Massachusetts. Now, however, many researchers believe bone repair is entering a new era that could make painful grafts and unmended bones a thing of the past.



In several clinical trials now under way or nearing launch, researchers are testing novel ways to replace damaged bone. Research teams, primarily in the United States and Europe, are implanting biomaterials laced with molecular signals designed to trigger the body's own repair mechanisms. They are also culturing a class of bone marrow stem cells—versatile cells that can develop into bone, cartilage, and other tissues—and transplanting them into the damaged area. And they are attempting to repair damage by gene therapy, transfusing cells carrying genes that produce key bone-repair proteins.

These trials mark the latest wave of progress in the burgeoning field of tissue engineering, in which researchers are trying to grow replacement tissues to repair damaged organs such as livers, hearts, and bones. Although the field is still maturing, tissue engineers working with bone are beginning to pull ahead of the pack. "Tissue engineering has made great strides," says Steven Goldstein, who directs orthopedic research at the University of Michigan, Ann Arbor, "but lots of tissues are not ready for prime time." That's not the case with bone, says Goldstein: "There has been more success in bone than anyplace else." Adds David Mooney, a tissue engineer at the University of Michigan, Ann Arbor, "If you compare it to the challenge of engineering a complete internal organ, bone is thought to be realizable in a much nearer time scale." Tissues such as the kidney and lung consist of numerous cell types that must be arranged in the proper three-dimensional structure and coaxed to express particular genes at different times. Structural tissues such as bone and cartilage are not as complex, Mooney notes. Goldstein adds that because the body naturally replaces, or "remodels," old bone with new, all that is needed is to get this regenerative process up and running smoothly. "If you can kick off repair, the normal process of remodeling helps you quite a bit," Goldstein says.



Encouraging signal. A break in the humerus of a 91-year-old woman that had not healed in 13 years (*left*) is filled with new bone 8 months after treatment with a signaling molecule called BMP-7 (*right*). A similar signaling molecule called BMP-2 (*inset*) is also proving effective in aiding repair of recalcitrant fractures.

That promise has sparked intense commercial interest in bone engineering. Companies ranging from biotech start-ups to traditional orthopedic powerhouses are jumping into the field. And although most of their efforts remain in the research stage, one company, Stryker Biotech in Hopkinton, Massachusetts, already has a product. It has applied to the Food and Drug Administration (FDA) for approval to market a collagen matrix composite infused with a natural protein that signals bone marrow cells to turn on the process of bone regeneration. Indeed, the commercial stakes are so high that some researchers are worried that patent claims, and a reluctance to test competing technologies in combination, could delay progress in the field.

Molecular scaffolding

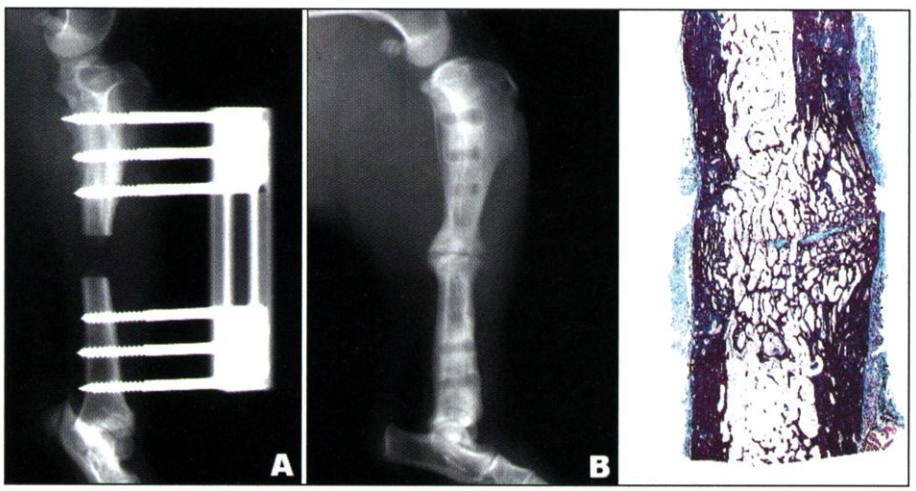
Like civil engineers building a new structure, bone engineers start by erecting scaffolding: They insert a matrix of special material into gaps in bone. This molecular scaffolding lies at the heart of all the new tissue engineering approaches.

Surgeons have used matrices made from materials such as collagen and hydroxyapatite for decades to coax the patient's own cells to colonize the damaged area and form new bone. The technique has been particularly successful in filling small divots, but it often has trouble fixing larger defects, says Mooney. So he and others have been looking for better materials. Antonios Mikos at Rice University in Houston, Michael Yazemski at the Mayo Clinic in Rochester, Minnesota, and their colleagues, for example, have been working on a plastic precursor that can be injected into the repair site, where it quickly polymerizes and hardens into a porous matrix capable of holding new bone cells. As new bone grows in, the plastic matrix breaks down into natural metabolites that are then excreted from the body. Thus far, says Yazemski, work in animals has shown that the biodegradable polymer not only sparks new bone growth over time, but also provides needed mechanical

CREDITS: (LEFT TO RIGHT) C. SCHEUFLER *ET AL.*, *J. MOL. BIOL.* **287**, 103 (1999); STRYKER BIOTECH

strength and appears fully biocompatible.

Building on such successes, tissue engineers have recently achieved more dramatic results when they give the matrix a helping hand—by seeding it with bone growth factors. The approach owes its early progress to a bit of serendipity. In 1965, Marshall Urist, an orthopedic surgeon at the University of California, Los Angeles (UCLA), was studying how minerals deposit on the collagen-based matrix on which bone naturally forms. When he implanted demineralized fragments of rabbit bone in muscle tissue, he found that new bone was created at the site. Something in the bone matrix itself, it seemed, was coaxing cells in the muscle to start producing new bone at this unusual site. That something turned out to be a class of proteins called bone morphogenic proteins (BMPs). But "it took 25 years to purify [BMPs]," says A. Hari Reddi, the director of the Center for Tissue Regeneration and Repair at the University of California, Davis.



Gene therapy. A 1.6-centimeter gap in the leg bone of a dog (*left*) is filled 6 months after treatment with plasmids containing a gene for part of parathyroid hormone (*center*). After 40 weeks, the animal's natural bone-remodeling processes were functioning at the repair site (*right*).

CREDIT: S. GOLDSTEIN/UNIVERSITY OF MICHIGAN

Reddi's lab was one of several that set out to track down these chemical signals. In the mid-1970s, Reddi and his colleagues showed that proteins in natural bone matrix first attract stem cells from the bone marrow, then spur them to proliferate and become bone-producing osteoblasts. A few years later, Reddi's group isolated the first of these proteins, which later came to be known as BMP-7. But it wasn't until 1989 that researchers at Creative Biomolecules in Hopkinton, Massachusetts, cloned the gene for BMP-7, a development that opened the door for researchers to produce a recombinant version of the protein that they could then add to matrix implants. Shortly thereafter, researchers at Genetics Institute in Cambridge, Massachusetts, cloned the gene for BMP-2—a similar cell signal.

These signaling proteins quickly proved that they could kick start the bone-regeneration process. Throughout the early 1990s, researchers at Genetics Institute and Stryker Biotech—which owned the rights to Creative Biomolecules' work with BMP-7 for orthopedic applications—completed a series of animal studies showing that their BMPs seeded on simple collagen matrices prompted rapid healing of bone defects, while similar defects remained unhealed in control animals. Stryker Biotech launched the first human clinical trial in 1992 for troublesome "nonunion" fractures that had not healed in over 9 months. According to Stryker president Jamie Kemler, the trial's results show that implants of BMP-7 on a collagen matrix generate new bone as well as, or better than, autografts of healthy bone transplanted from another part of the patient's body. The company is currently awaiting FDA approval to begin selling its matrices. Genetics Institute, too, is nearing the end of similar clinical trials with BMP-2.

But every great promise has its fine print, and this method of bone building may have limitations, too. Some researchers point out that when BMPs are released naturally by cells, mere nanogram quantities of the proteins per gram of bone matrix are enough to trigger the bone repair cascade. Yet microgram quantities of BMP per gram of matrix material—over six orders of magnitude higher—seem to be needed to produce the same effect with an artificial matrix. Although there are no known health problems associated with such high BMP concentrations, the cost may be high, potentially thousands of dollars per treatment.

Gene therapy

In an effort to get signaling molecules to the cells they trigger, researchers have turned to a field that has had its problems lately: gene therapy. Gene therapists have had a struggle delivering on the field's early promise in part because cells carrying therapeutic genes express them only for a short time. But short-term expression may be enough for remaking bone, Michigan's Goldstein notes. In a flurry of papers last year, researchers from labs in the United States and Germany reported promising early results. In the July 1999 issue of the *Journal of Bone and Joint Surgery*, for example, orthopedic surgeon Jay Lieberman and his colleagues at UCLA reported using an adenovirus carrying a gene that produces BMP-2 to transfect bone marrow cells. They then seeded and grew the transfected cells on a demineralized bone matrix, which they implanted into surgically produced gaps in the leg bones of rats. The treated bones healed normally, while those that received control preparations—either with a non–BMP-producing gene or just the matrix alone—did not heal.

Using a simpler approach, Goldstein and his Michigan colleagues have produced similar results in dogs. Instead of using cells infected with a transgenic virus, Goldstein's team uses circular fragments of DNA called plasmids containing a gene that codes for a protein called human parathyroid hormone, which, like BMPs, helps stimulate the natural bone repair cascade. They trap the plasmids in a polymer matrix, which they implant into a surgically made gap in the leg bones of dogs. In the July 1999 issue of *Nature Medicine*, Goldstein's team reported that surrounding cells picked up the plasmid DNA and expressed it for up to 6 weeks. The treated bones were fully repaired. Again, no effect was seen in control animals. Bone tissue engineering, says Goldstein, "looks to be an area where gene therapy can have one of its earliest, greatest successes."

Based on this and earlier successes with their plasmid gene therapy approach, the Michigan group formed a San Diego–based start-up called Selective Genetics to move the technique into the clinic. The company says that after showing widespread success in animals, they are gearing up to launch a phase I safety trial of the approach in humans.

New cell sources

Some researchers worry that these promising techniques may ultimately hit a roadblock: a shortage of stem cells. Although transplanted signaling molecules attract stem cells to the repair site and cause them to differentiate, the supply may not be sufficient to repair major damage. So several groups are trying to supplement natural stem cells with

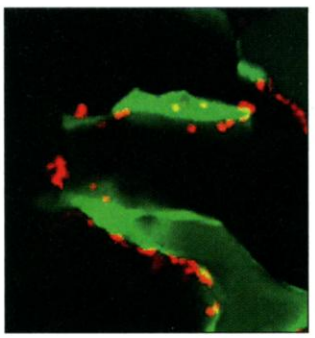
cells grown in culture.

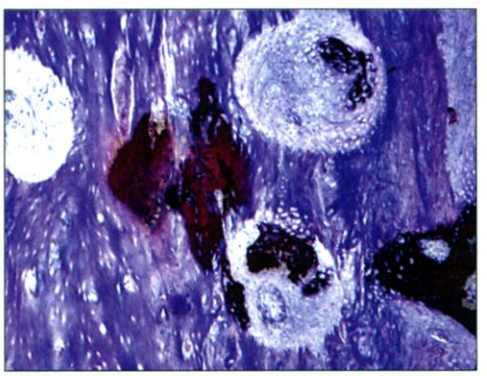
Unlike embryonic stem cells, which can differentiate into any one of the more than 200 cell types in the body, bone marrow stem cells have a more limited repertoire. They are already committed to develop into cells that form a broad class of tissues, including bone, cartilage, and tendons, as well as heart, muscle, and neural tissues. And although they are produced throughout the life of animals, their numbers appear to decline with age, says Arnold Caplan, who directs the Skeletal Research Center at Case Western Reserve University in Cleveland. In newborns, bone marrow stem cells—also called mesenchymal stem cells (MSCs)—account for 1 out of every 10,000 bone marrow cells. That number drops to 1 in 100,000 in teens, 1 in 400,000 in 50-year-olds, and 1 in 1 million to 2 million in 80-year-olds.

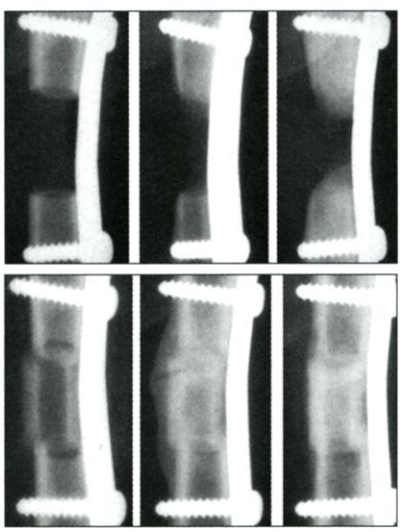
That's bad news for anyone who has lost large sections of bone in an accident or through cancer. Animal studies show that BMP therapies and other cell-signaling approaches have trouble mending gaps larger than about 25 centimeters because they can't recruit enough stem cells to the area, says Annemarie Moseley, president and CEO of Osiris Therapeutics, a Baltimore, Maryland–based tissue engineering start-up. In these cases BMPs begin by recruiting stem cells to the ends of the healthy bone and regenerating new tissue toward the center of the gap, but "if you look at the center of the matrix you don't see any evidence of bone growth," says Moseley. The same problem hampers a related approach of simply harvesting healthy bone marrow from a patient and transplanting it in the repair site. "You can put as much marrow in there as you want, but it won't help" if there aren't enough stem cells, says Caplan.

For that reason, Caplan, Osiris, and others have been working to implant stem cells directly into bone repair sites. Caplan's lab helped launch the field about 12 years ago when they first isolated MSCs and came up with a means to expand cell numbers in culture. Since then, Caplan, DePuy's Bruder, Moseley, and others have experimented with a variety of MSC-based implants. In 1989 and 1990, for example, Caplan's group published papers showing that MSCs seeded on a porous, calcium-based ceramic substrate could heal 8-millimeter gaps in the leg bones of rats. They have since reproduced these results for larger bone defects in larger animals. These and other successes prompted Caplan in 1992 to launch Osiris Therapeutics, which aims to carry the approach to humans.

Since the early 1990s, Osiris has shown that the MSC-based therapy works in rats, rabbits, and dogs. And today the company is preparing to launch a phase I safety trial with MSCs in humans. Pamela Robey, a cell biologist with the National Institute of Dental and Craniofacial Research (NIDCR) in Bethesda, Maryland, has made similar progress. Robey says her group has shown that stem cells seeded on a matrix—hydroxyapatite in this case—work to seal large bone gaps in mice, rats, rabbits, and dogs. She is also awaiting FDA approval to launch human clinical trials.







Stem cells. Mesenchymal stem cells (stained red, above image) loaded onto a matrix lead to new bone growth (stained burgundy, right image) when implanted at repair site. Gap in dog femur fills during 16 weeks after treatment (lower panels), while untreated bone remains unrepaired (upper panels).

Still, MSCs have their own drawbacks. The biggest concern is time. The current procedure involves extracting stem cells from a patient, growing them in culture, and transplanting them back into that same person, a process that takes weeks. Not only does this rule out emergency repairs, but it also makes the procedure expensive, says Bruder. To get around this problem, Osiris has been experimenting with implanting MSCs from one animal into another, hoping to come up with cell-based implants that surgeons can simply remove from the freezer and implant in a patient's body. The approach has potential, says Moseley, because MSCs don't express the cell surface markers that T cells recognize in rejecting implanted tissue. Thus far, studies on about 40 dogs and "untold numbers" of rats have showed that the transferred cells not only do not spark an immune reaction, but go on to form normal bone, she says.

Putting the pieces together

As researchers push different approaches to tissue engineering and companies stake out their claims on technologies, commercial competition is heating up. And that worries some researchers, who fear that it may make it hard to determine which strategies work best. "I don't think it's clear to me or the field in general which of these techniques is useful for different applications," says Michigan's Mooney. Adds Bruder: "Companies are worried that combination therapies will be superior to their single bullet" and are therefore reluctant to test their products along with those of their competitors.

So strong is this concern, says Robey, that it has kept her from working with BMPs. "One of the reasons I turned to stem cells was because I couldn't get BMPs to do my work," she says. And the result is that progress on determining the most effective combinations is slow. Last year, for example, researchers at Osiris and Novartis collaborated to transfect MSCs with the gene for BMP-7, seed them on matrices, and implant them in rats. The results were excellent, says Moseley, but she says the research has since been dropped because Stryker Biotech owns key rights to BMP-7.

Stryker's Kemler says his company is not trying to quash competition but is pursuing its own "proprietary" combination therapies, which he declines to specify. Nevertheless, Robey and others say the balkanized landscape of intellectual property in tissue engineering prevents them from testing novel therapies. "I do consider that to be a real logjam, and I am not sure how that will be broken," says Robey. Moseley says she believes the logjam will eventually give way as the field matures over the next few years. Says Caplan: "Tissue engineering is just getting off the ground."

–ROBERT F. SERVICE

CREDITS: (STAINS) OSIRIS THERAPEUTICS; (PANELS) S. BRUDER *ET AL., J. BONE JT. SURG.* **80-A** (JULY 1998)