

The Bone Builders

Scientists are developing stem cell and gene therapies to repair fractures and treat bone loss

By [Bijal P. Trivedi](#)

For more than 2000 years, since the days of Hippocrates, fractures and broken bones have been treated with nothing more than splints and bandages. In recent times, joints have been replaced with titanium structures and metal, and ceramic pegs hold together broken hips. These approaches, say some scientists, are more in line with fixing an engine rather than a body and they seek to breathe more life into the field of bone repair.

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featured article

A slew of reports describing novel bone building technologies in animals and humans suggests that broken bones and osteoporosis may be among the first disorders to benefit from gene and stem cell therapies.

Many of these new approaches use a local delivery of stem cells—versatile cells that can produce a broad range of tissues—or growth-promoting genes to heal bones. Some initial results in mice and men imply these strategies are promising. In Israel, scientists are repairing bone fractures with stem cells spiked with an additional gene to stimulate bone formation. Scientists in California are using gene therapy to treat osteoporosis in mice. And researchers in Louisiana and Tennessee are using bone marrow transplants to treat infants with a genetic disorder known commonly as 'brittle bone disease.'

"Bone repair is a great candidate for gene therapy because you are enhancing a natural repair process, not curing an entire disease," says Paul Robbins of the University of Pittsburgh, Pennsylvania. Gene therapy has been plagued by problems with keeping a gene active for long periods and only in specific tissues. "But using gene therapy to repair fractured bones only requires that the gene is active for a short period," says Robbins.

Bone repair is also well suited for cell therapies. "Rapid cell turnover makes bone an attractive tissue to work on," says Edwin Horwitz, of St Jude Children's Research Hospital in Memphis, Tennessee. In other tissues, stem cell therapy isn't an option because the tissue is not rapidly replacing itself from the stem cells. But bones turn over rapidly in young children, which means that healthy marrow stem cells are constantly producing healthy, new bone. In children with brittle bone disease, or osteogenesis imperfecta (OI), mutations in the bone marrow stem cells produce weak, fragile bones. Horwitz and his colleague Darwin Prockop, of Tulane University School of Medicine in New Orleans, Louisiana, are treating OI in three infants by replacing their stem cells with healthy ones from a brother or a sister.



Skeleton of Harry Eastlack, a victim of fibrodysplasia ossificans progressiva (FOP). The connective tissue in Harry's body gradually turned to bone. [View larger](#)
Courtesy Mütter Museum, College of Physicians of Philadelphia

Prockop discovered the human gene for collagen in the early 1980s and shortly afterwards identified mutations in it that cause OI. Collagen is the most abundant protein in bone. It is a major structural protein and provides a scaffold on which the bone forms. "If the scaffold is bad then you can't make good bones," says Prockop. The mutations that cause OI prevent collagen from forming the helical protein essential to bone strength.

"Children with OI have bones like eggshells," says Prockop. "A 13-month old girl that took part in our study had to be placed in a crib padded with pillows because simply rolling on her side could cause her ribs to crack." OI children have deformed limbs and a spine that become progressively worse, and this retarded bone growth leads to short stature. The children are frequently confined to a wheelchair. Some cannot sit up or breathe without help. "These kids need 24-hour care," says Prockop.

Prockop's strategy was to treat the OI patients using a bone marrow transplant. Replacing the bone marrow of OI patients, which carries the mutated collagen gene, with healthy bone marrow donated from a sibling would supply the patients with new stem cells that would ideally produce strong new bone.

"The children improved, but the initial results were not dramatic, unlike the second stage of treatment," says Prockop. Six months after the transplant the OI children had grown an average of 7.5 centimeters and their bones had become more dense compared with two severely affected OI infants who did not receive the therapy and had only grown approximately 1.25 centimeters. The growth rate then slowed and eventually reached a plateau. These results appeared in the March issue of *Blood*.

'Children with OI have bones like eggshells.'

To stimulate bone formation, the three children were given a 'booster shot' of special bone-producing mesenchymal stem cells (MSCs) isolated from the original donor's marrow. These MSCs give rise to bone, cartilage and tendon among other tissues and, though rare, are easy to isolate from adults and were grown in large quantities in Horwitz's lab. When, in a second phase, he infused this concentrated dose of stem cells back into the patients, the results were far more striking.

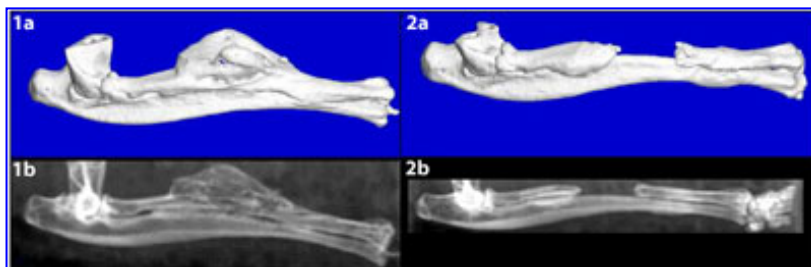
Six months after the booster shot, the children had grown rapidly and experienced a lower rate of bone fractures. Two children showed a growth rate that was between 94 and 67 percent of normal 4-year-olds. The third child showed some growth. Bone samples from the three patients showed that the bone was derived from the healthy stem cells. Prior to the second booster, the three children had almost stopped growing. These results were presented at *Mesenchymal and Nonhematopoietic Stem Cells: Recent Progress and Current Controversies in New Orleans*, Louisiana, in late March.

"The results of this second phase are promising," says Horwitz. It appears that transplanting mesenchymal cells from another sibling is non-toxic to the patient. These cells produce new healthy bone and there is a marked acceleration in growth, he added. "We think there is a real effect here."

Mesenchymal stem cells are also being used to heal fractures in mice. These mouse MSCs are genetically altered to carry a human gene called bone morphogenetic protein-2 (BMP-2, pronounced 'bump'). BMP-2 is a potent bone-inducing gene. It is normally active during embryonic limb development when the human skeleton is being formed, and it is activated again during the repair of broken bones.

Dan Gazit, of Hebrew University-Hadassah Medical and Gene Therapy Center in Jerusalem, Israel, and colleagues are investigating whether BMP-2 carrying stem cells can repair non-

union fractures, which are missing a large chunk of bone and do not heal naturally. Gazit's team coupled BMP-2 to a genetic switch that turns the gene off when the mice are fed the antibiotic doxycycline. The modified stem cells were transplanted next to the fracture and only one of the two groups of mice was fed the antibiotic.



Fracture repair using modified stem cells. **1a and 2a)** 3-dimensional reconstruction of a mouse forelimb 2 months after receiving MSC transplants. **1a)** Repair of a 2.5mm fracture in a mouse whose transplanted MSCs carried an active BMP-2 gene. **2a)** Mice that were fed the antibiotic doxycycline inhibited BMP-2 in the MSCs and could not repair the fracture. **1b and 2b)** Simulated X-ray images showing the healed and unhealed bones. [View larger](#)

Courtesy Dan Gazit

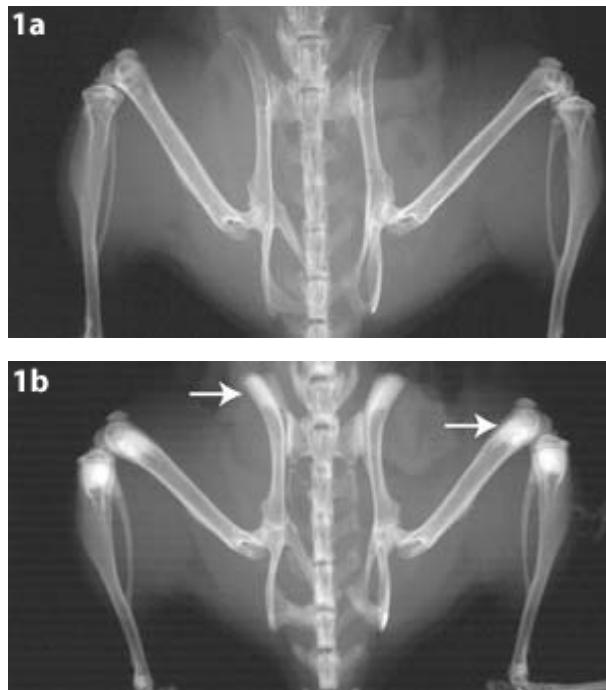
Ten days after the transplant, only the mice that did not consume doxycycline showed new bone growth. The new bone is continuous with the fractured radius, although there was a lump of excess bone formed at the site. Gazit believes the overproduction of bone was caused by the continuous, unregulated activity of BMP-2 throughout the experiment. Gazit's team also discovered that BMP-2 has angiogenic properties and can stimulate blood vessel formation. This study is described in the April issue of *Molecular Therapy*.

"We suspected BMP-2 could induce angiogenesis, which is essential for bone formation and better healing, but it was particularly nice to see this increase in our experiments," says Gazit.

Before this stem cell technique can be considered for human clinical trial, however, a reliable method of regulating the BMP-2 gene must be developed. "The doxycycline system of gene regulation is used frequently in bacteria and is very nice for research and development, but it is not a permanent solution," says Gazit.

"Furthermore," says Robbins, "the down side of cell therapy is that there is still concern that stem cells could migrate and form bone where you don't want it, like the liver or the heart."

Balancing bone formation with bone destruction is a tug-of-war between the osteoblasts that build bone, and the osteoclasts that solubilize it. In young adults, these processes are in balance. As people age, bone loss exceeds production, leading to osteoporosis. At Amgen, in Thousand Oaks, California, Jackie Sheng's team is using gene therapy to inhibit the bone-degrading action of osteoclasts to prevent osteoporosis.



Just two weeks after the delivery of the OPG fusion gene mice already show increased bone density (**1b**, arrows) compared with mouse seen in **1a**.

Courtesy Jackie Sheng

Osteoporosis is a chronic disease, particularly common in women over 40. The disease is characterized by accelerated bone loss that leaves the skeleton more susceptible to fractures and slow crushing. The loss of estrogen at menopause accelerates the course of the disease because the hormone normally inhibits osteoclasts. Sheng's approach was to block the formation and function of osteoclasts using osteoprotegerin (OPG).

The Amgen scientists used mice whose ovaries had been removed to mimic estrogen deficiency and osteoporosis seen in post-menopausal women. The researchers then delivered the OPG gene by first wrapping it in a virus, which was used as a tool to transfer the gene into the cells of the mouse.

Sheng and her colleagues made two versions of the OPG gene, a naturally occurring form and a fusion gene in which the active portion of OPG was fused to an antibody gene. The researchers anticipated that a fusion gene might be more stable and remain active longer. They were right.

"The fusion OPG gene remained active for 18 months producing high levels of the OPG protein in all mice that received it," says Sheng. Over 18 months, the level of OPG dropped 1,000-fold from its peak, but even the lowest levels were high enough to produce therapeutic effects. The mice carrying the fusion gene showed a 50-percent increase in bone density, particularly in the tibia and the vertebrae, compared to mice that did not receive OPG. These results are reported in the February issue of *Molecular Therapy*.

"The osteogenesis data from Sheng's study was very exciting. They were able to get high gene expression for over a year, which is particularly impressive," says Robbins.

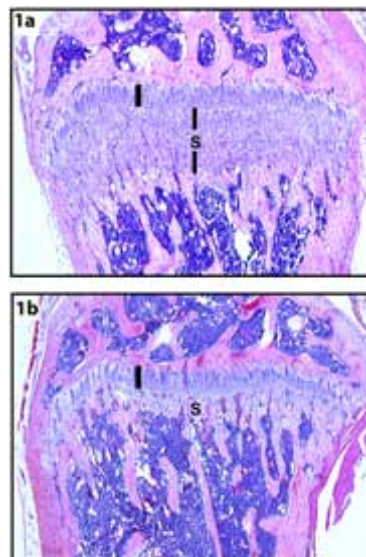
The drawback of Sheng's approach was that the high adenovirus levels required to produce therapeutic levels of the OPG protein caused liver damage in the mice. There is also concern that a protein that is half OPG, half antibody will trigger dangerous immune reactions.

In studies soon to be published, Sheng says that her team has engineered another virus

called adeno-associated virus (AAV) to deliver the naturally occurring OPG gene. According to Sheng, AAV does not trigger a toxic liver response and the protein remains at a constant, therapeutically useful level for an extended period.

"Regardless of whether stem cells or viruses are used to deliver genes, we will probably end up with a cocktail of growth-promoting genes that will enhance bone repair," says Robbins. And if, after further study, the bone marrow and MSC transplant strategies prove successful, these approaches could, in theory, be used to treat any genetic disease of the bone.

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1a) Delivery of the OPG fusion gene resulted in a 2 to 3-fold expansion of the primary spongiosa (**S**)—the primary region of bone growth—compared to the mouse in **1b**).

[View larger](#)

Courtesy Jackie Sheng

[Moutsatsos, I.K. *et al.* Exogenously regulated stem cell-mediated gene therapy for bone regeneration. *Mol Ther* **3**, 449-461 \(April 2001\).](#)

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[Bolon, B. *et al.* Adenoviral delivery of osteoprotegerin ameliorates bone resorption in a mouse ovariectomy model of osteoporosis. *Mol Ther* **3**, 197-205 \(February 2001\).](#)

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