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ORTHOPEDICS today

CURRENT NEWS IN MUSCULOSKELETAL HEALTH & DISEASE

20 Years in Orthopedic Surgery 1981-2001

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Engineering the FUTURE of Orthopedics

Tissue engineering to dramatically change orthopedic practice

Special report examines the National Institutes of Health's groundbreaking research in this emerging field

ORTHOPEDICS TODAY Staff Writer **Patrick McGee** recently spent three weeks on the National Institutes of Health's campus in Bethesda, Md., interviewing investigators working on musculoskeletal tissue engineering projects. McGee begins this special report on page 26 by discussing the inter-agency cooperation and multidisciplinary approach necessary to advance the research that will lead to revolutionary new treatments for musculoskeletal injuries and conditions. "Exploring the use of bone marrow stromal cells for repairing defects," on page 27, shows how these potent cells, which have been used to repair defects in animals, are now ready for clinical trials. "NIH focuses on improving cartilage repair techniques" covers what may be one of the most exciting, but difficult aspects of tissue engineering research. The report concludes on pages 32-33 with a list of NIH-funded tissue engineering research projects and a chronology of tissue engineering achievements.

Exploring the use of bone marrow stromal cells for repairing defects

Researchers at NIH have shown that these potent cells can repair defects in animals. Now they're ready for clinical trials.

by Patrick McGee
ORTHOPEDICS TODAY staff writer

BETHESDA, Md. – The fact that bone marrow stroma contains cells that have the ability to form bone and other types of tissue has been known since the 1960s. Over the intervening years, researchers have slowly come to understand more about how these bone marrow stromal cells (BMSCs) work and their potential applications in orthopedic, plastic and other types of surgery.

One of the centers where research on stromal cells is ongoing is the skeletal biology group at the National Institute for

instead of the total bone marrow cell population has several advantages. First, large numbers of BMSCs can be generated from a small amount of bone marrow aspirate, making additional surgery to harvest autogenous marrow unnecessary. In addition, bone formation by transplanted BMSCs in general occurs more quickly and extensively than with transplanted marrow cells.

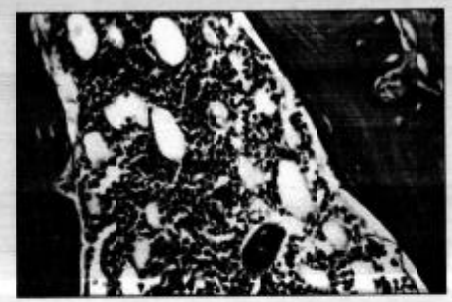
Kuznetsov, who has been conducting research on BMSCs at NIH since 1992, has further refined techniques for culturing stem cells and

Mahesh Mankani, MD, a plastic surgeon specializing in the hand and microvascular reconstruction, is working with Kuznetsov and Robey to investigate ways of making BMSCs applicable in a clinical setting.

Mankani conducted a study with Krebsbach in which they created a mouse calvarial defect model that allowed them to track transplanted cells. The defects were filled with BMSCs derived from transgenic mice with a unique enzyme incorporated into its genome, Mankani said.

New bone formation

In four weeks, new bone had formed in place of the defect in the animals treated with BMSCs, while



COURTESY OF PAMELA GEHRON ROBEY

The bone marrow microenvironment is composed of two different compartments: the hematopoietic stem cells and their progeny, and the physical stroma, which is composed of bone marrow stromal cells upon which hematopoiesis occurs.

BMSCs in the same defect in a canine model. "We've expanded that toward using human cells for the same application and the results are comparable," Mankani said.

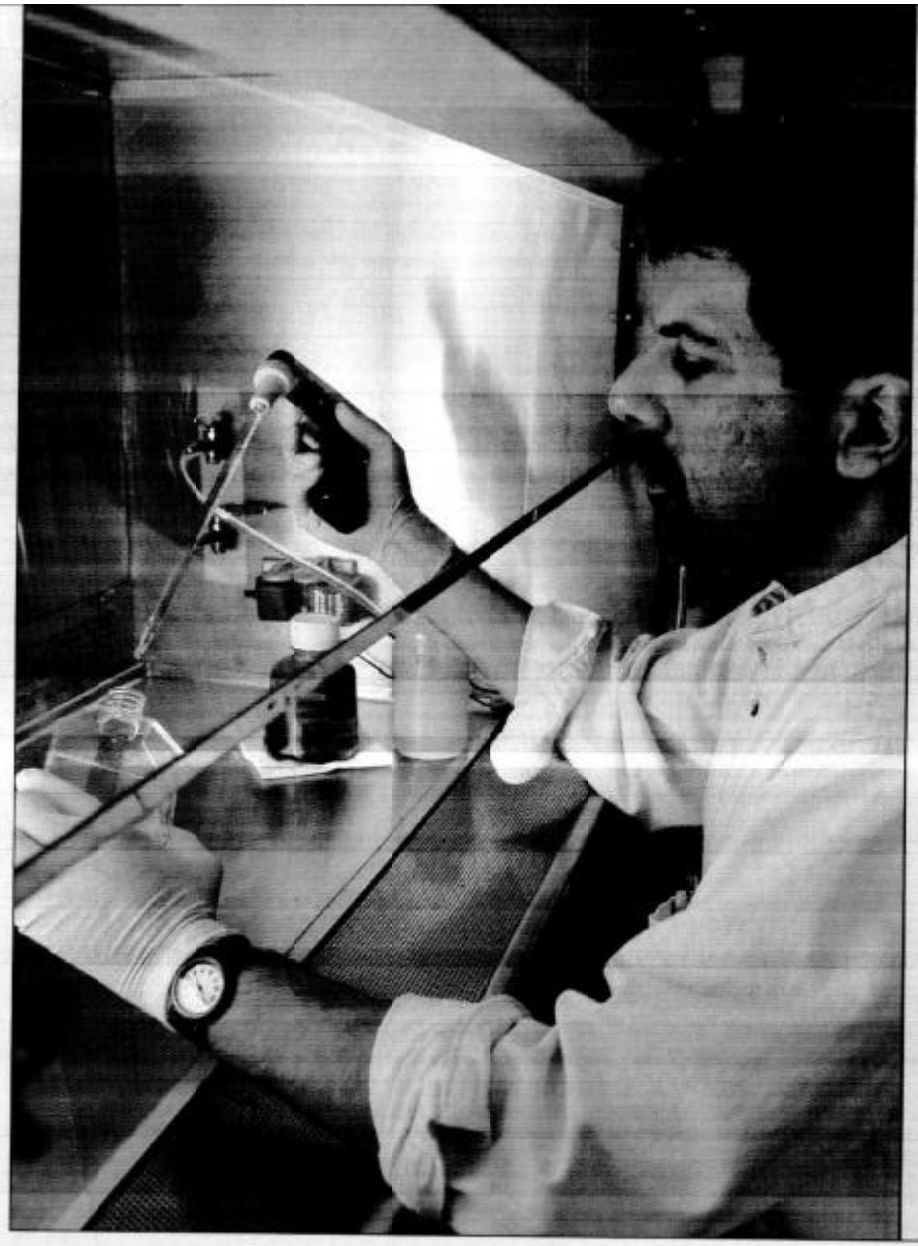
The Food and Drug Administration (FDA) required a proof of principal study in a higher animal before it would approve a clinical trial in humans with similar, non-

Dental and Craniofacial Research (NIDCR) on the National Institutes of Health campus.

"The purpose of this particular group is to try and identify what causes these cells to make bone, as opposed to cartilage or fat, and what makes them support hematopoiesis," said **Pamela Gehron Robey**, PhD, chief of NIDCR's Craniofacial and Skeletal Diseases Branch, where the skeletal biology group is located.

"It turns out that these cells are very plastic. They will shift from one phenotype to another depending on the microenvironment that they are in. We want to characterize what factors cause them to shift," she said.

One of the key persons in this research is **Sergei A. Kuznetsov**, PhD, a cell biologist who worked for 14 years with **Alexander J. Friedenstein**, MD, PhD, a Russian researcher who was the father of stromal cell biology. Friedenstein established methods for culturing BMSCs and was the first to transplant them into animals, Kuznetsov said. In addition, great insight into bone marrow stroma has come from a long-term collaboration with **Paolo Bianco**, MD, and his colleagues at the University of L'Aquila in Italy, Robey said.



COURTSY OF NIDCR

Mahesh Mankani transfers bone marrow stromal cells in a lab at NIDCR's skeletal biology group. Mankani and others in the group are working to develop ways to use stromal cells for critical-sized defects in animal and human models.

Several advantages

Transplanting BMSCs alone delivering them where they are needed. In one study, he tested 15 different types of carrier materials for transplanting BMSCs with **Paul Krebsbach**, DDS, before settling on a combination of hydroxyapatite and tricalcium phosphate that the cells seemed to adhere to most readily.

the control animals showed very little new bone formation, he added. By tracking the unique gene, they showed that the transplanted stromal cells were active for at least 14 weeks post-transplantation.

The skeletal biology group is now conducting a study testing the use of

[illegible] bone defects. Robey said she hopes to be able to begin those studies soon: "We've already got the protocols written; we just have to have [FDA] approval before we can do it."

In the long-term, Robey and other researchers working with BMSCs must address several issues. They need to develop quicker and more efficient methods to generate the large quantities of *ex vivo* BMSCs needed to treat larger skeletal segmental defects in humans. The development of more effective carriers to deliver the cells is a necessity as well.

Bone "islands"

Mankani said another area of BMSC research involves the creation of "vascularized bone islands" that could become a promising method of growing bone in the body to repair damage.

"In plastic surgery, we're very interested in transferring bone or even soft tissue that has its own vascular supply to a new location where the environment may be inhospitable to a simple graft, say in a radiation bed or a post-infection bed where there's a lot of scarring," he said.

A 1997 study done by the skeletal biology group demonstrated how BMSCs could be used to generate a vascularized bone graft in a mouse model. Mouse BMSCs were expanded *ex vivo*, placed on a collagen sponge and wrapped around the internal carotid artery and inferior jugular vein.

The construct was then wrapped

in a Goretex membrane to prevent collateral vascularization. Eight weeks later, the transplant contained fully perfused cortical and cancellous bone.

Mankani said this animal model demonstrates the potential of this technique for growing such bone grafts in a patient's body – the thigh, for example – and then transferring them to sites needing repair. In addition, the grafts could be grown in whatever shape is needed by using a form or mold.

Other areas of research

While it is conducting the most roles in the structure and function of bones and teeth.

The Matrix Biochemistry Group, which is headed by **Larry Fisher**, PhD, explores the structure-function relationship of the major non-collagenous proteins of bones and teeth. Finally, there is the Skeletal Clinical Studies Program, which is charged with fostering the exchange of information between researchers and the physicians who see patients in a clinical setting.

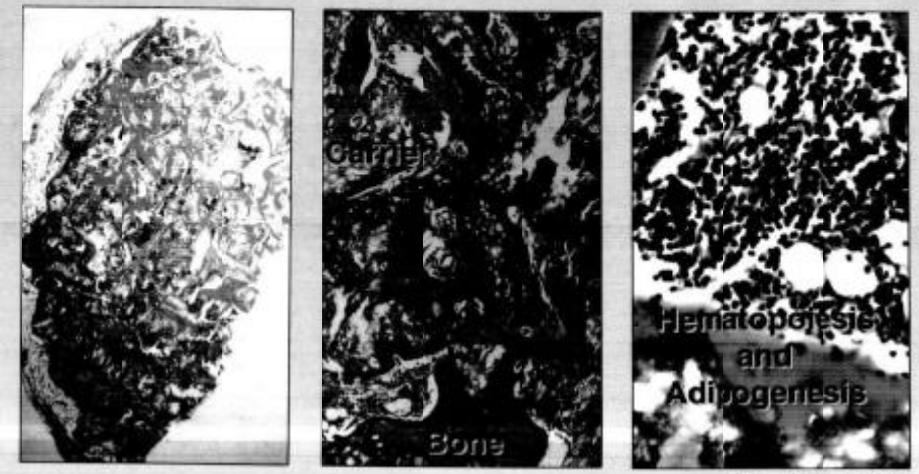
Fibrous dysplasia of bone

Work in Robey's lab is also focusing on what can happen when the normal function of bone marrow stromal cells is disrupted. Branch researchers have collaborated with Bianco on studies of fibrous dysplasia of bone, a disorder characterized by replacement of normal bone and marrow with abnormal bone and marrow.

The disease is caused by a mutation after fertilization of the egg. A mutated protein that regulates a cell signaling pathway causes it to get stuck in the "on" position. This prevents stromal cells from maturing into bone-form-

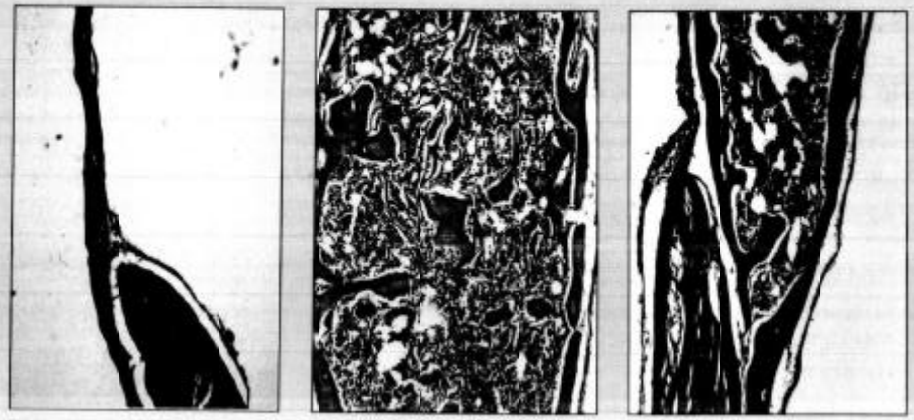
COURTESY OF PAUL KREBSBACH AND MAHESH MANKANI

Expanded bone marrow stromal cells were able to regenerate critical-sized defects in this mouse calvarial model. Researchers are waiting for the FDA to approve a clinical study for similar defects in humans.



COURTESY OF PAOLO BIANCO

After *ex vivo* expansion, human bone marrow stromal cells attached to particles of hydroxyapatite/tricalcium phosphate are able to form an ectopic ossicle when transplanted subcutaneously into immunocompromised mice. These transplanted cells form bone, adipocytes and stroma that support hematopoiesis of recipient origin.



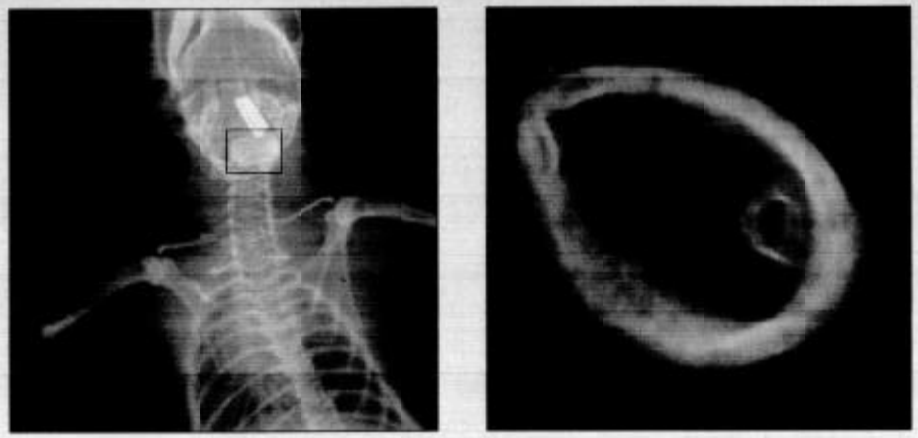
Expanded mouse bone marrow stromal cells were absorbed into collagen sponges and transplanted into critical-sized calvarial defects. After four weeks, the defect was completely filled with bone and marrow derived from the donor cells.

work with BMSCs at NIDCR, the skeletal biology group is only one of a number of groups at the institute. There were 11 labs and branches in the institute, but after **Harold Slavkin**, DDS, took over as director in 1995, they were consolidated into seven branches. The goal of the reorganization was to ensure that the institute's funding reflected research trends in the field.

Robey's group, the Bone Research Branch, became the Craniofacial and Skeletal Diseases Branch. In addition to the Skeletal Biology Program, the branch includes the Molecular Biology of Bones and Teeth Program, which is headed by **Marian F. Young**, PhD. The program studies the matrix proteins that play key ing cells, thereby producing a matrix that is fragile and easily deformed.

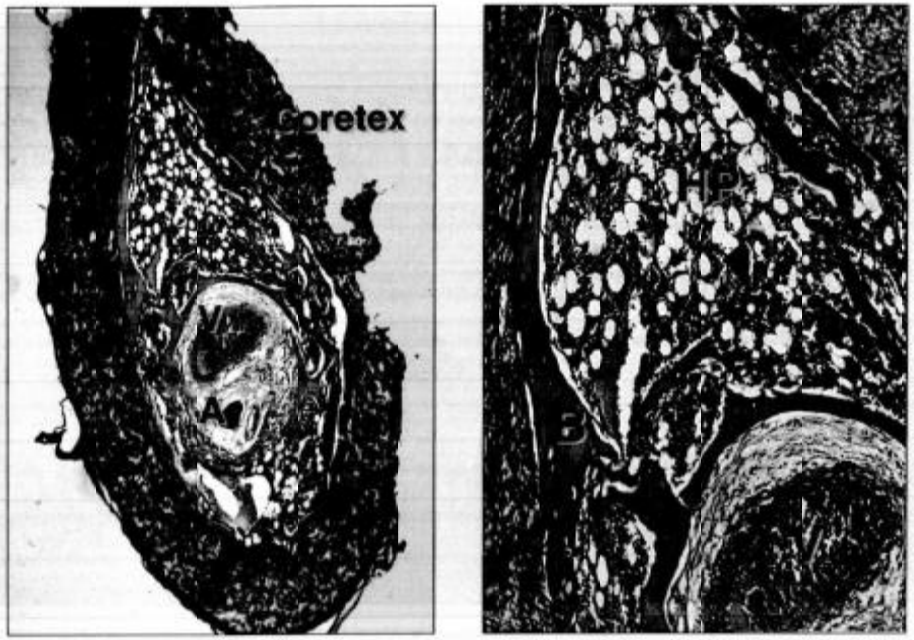
Based on findings from initial research, a series of clinical protocols for studying and treating patients with the disease were initiated by Robey, Bianco and **Shlomo Wientroub**, MD, an Israeli orthopedic surgeon.

Michael Collins, MD, an endocrinologist specializing in metabolic bone diseases, has developed an intensive series of tests that are providing a great deal of physiological information about the disease. In addition to providing more information on cell biology and histopathology, a greater understanding of the pathobiology of this disease will lead to better treatments, Robey believes. ■



COURTESY OF MAHESH MANKANI

Culture-expanded bone marrow stromal cells can also be used to develop "pedicled" bone grafts, or grafts formed around blood vessels. These grafts can then be implanted with the vasculature already established.



To develop a pedicled graft, stromal cells are absorbed into collagen sponges and wrapped around the carotid artery and vein. It is further wrapped with Goretex to prevent collateral

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