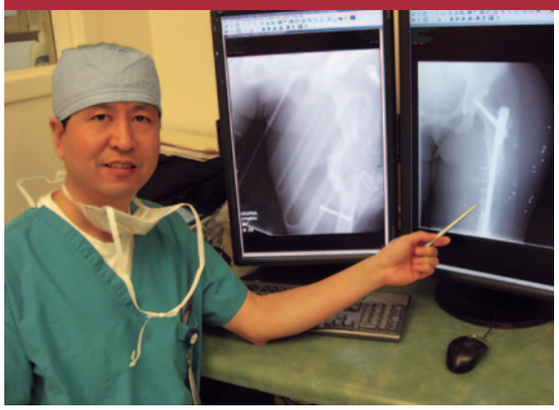
HEALING POWER

Engineering grafts to optimize bone regeneration



▲ Dr. Cui reviews X-rays of a gunshot injury patient whose large bone defect was treated with BMP and an intramedullary nail.

Advances in orthopaedics leave some patients behind simply because their bones refuse to heal. A promising field of investigation to address such problems is the creation of synthetic grafts to treat nonhealing fractures and other bone defects. Researchers are developing grafts that may not only replicate the properties of bone but also deliver cells and proteins that promote bone growth.

Three-time OREF grant recipient **Quanjun Cui, MD**, assistant professor of orthopaedic surgery at the University of Virginia in Charlottesville, is addressing crucial questions in this arena, including: What cells and proteins should be loaded into such grafts?; and How can the potential of these grafts be optimized to regenerate bone and heal defects?

In 2007, Dr. Cui received the OREF/ Zimmer Orthopaedic Career Development Award to study in a mouse model the effect of a graft that delivered bone marrow stem cells to the site of a bone injury. These cells were genetically altered to produce vascular endothelial growth factor (VEGF), a protein that stimulates the growth of new blood vessels, which are necessary precursors to bone repair.

Dr. Cui was awarded a 2010 OREF/ Musculoskeletal Transplant Foundation (MTF) Research Grant for expanding this investigation. His goals are to identify the role of VEGF in promoting bone growth, optimize the effect of VEGF-loaded grafts, and select ideal stem cell candidates.

In 2011, Dr. Cui received the OREF/ Zachary B. Friedenberg, MD Clinician Scientist Grant, which provides a stipend to compensate for the loss of income that would result from devoting less time to clinical practice and more time to research.

This stipend enables Dr. Cui to focus on work supported by his 2010 OREF/ MTF research grant and pursue related investigations funded by other entities. In return, he is required to serve as a role model for orthopaedic residents, interns and medical students; organize and participate in conferences; and work with students, interns and residents in the operating room.

IDENTIFYING IDEAL BONE-PROMOTING PROTEINS

The VEGF protein may have more than one job in the generation of new bone. In addition to its role in blood vessel development, VEGF appears to modulate the activity of bone morphogenic proteins (BMPs). These proteins can induce certain types of stem cells to become bone-generating osteoblasts.

The OREF/MTF Research Grant has enabled Dr. Cui to address critical questions about the additional roles VEGF plays:

- How does VEGF modulate BMP activity?;
- Does VEGF's function vary among different subtypes of BMPs?; and
- Which VEGF-BMP combination yields the greatest degree of bone production?

Dr. Cui explained, "We have cloned VEGF and BMP genes in our lab. We're going to put those genes into selected stem cells. Then with both in vitro and in vivo studies, we'll see if they make more vessels and more bone." The research team is particularly interested in the interplay of VEGF and the BMP-6 subtype, which has been shown in other studies to have robust bone-promoting properties.

The in vitro experiments will enable the researchers to:

- Quantify the degree of VEGF and BMP-6 protein production;

- Identify the molecular chain of events that begins with VEGF activity and leads to blood vessel production and bone formation; and
- Assess various ratios of VEGF-to-BMP-6 concentrations in stem cells to determine which ratio results in the ideal bone-producing activity.

In subsequent in vivo experiments with a mouse model of bone defect, the researchers will test the ability of the ideal VEGF-BMP concentration to promote healing.

IDENTIFYING IDEAL STEM CELLS

Stem cells loaded into bone grafts have two purposes: to serve as vehicles for delivering genes for VEGFs and BMPs; and to exercise their capacity to become bone-generating osteoblasts.

Cells known as multipotent stem cells have the potential—with a certain set of molecular prompts—to become a number of different cells in the body. Multipotent stem cells that can be induced to become osteoblasts can be isolated from a variety of sources. For example, although bone marrow would be an obvious source, multipotent stem cells from fat tissues can also be induced to become osteoblasts.

Populations of multipotent stem cells with slightly different characteristics may demonstrate significant differences in their potential to contribute to bone growth and healing. To understand the functional differences, Dr. Cui and his colleagues are investigating the relationship between certain proteins on the surface of stem cells (surface markers) and the ability of those cells to repair bone defects in mice.

Photos courtesy of Dr. Cui

Dr. Cui explained, "Cell populations can be identified by variations in their surface markers, and each population can behave quite differently. Our goal is to identify the most potent stem cell population for graft implantation."

Dr. Cui and his research team have selected five stem cell populations, each with a characteristic surface marker profile—a combination of either the presence or absence of three markers. Each cell group is also engineered to express genes for VEGF and BMP-6. With a mouse model of bone defect, Dr. Cui is assessing the potential of each cell population to induce bone regeneration.

The outcome of both sets of experiments may suggest an ideal protein–stem cell payload for a synthetic bone graft at the site of fractures and other bone defects.

SHAPING THE FUTURE OF ORTHOPAEDICS

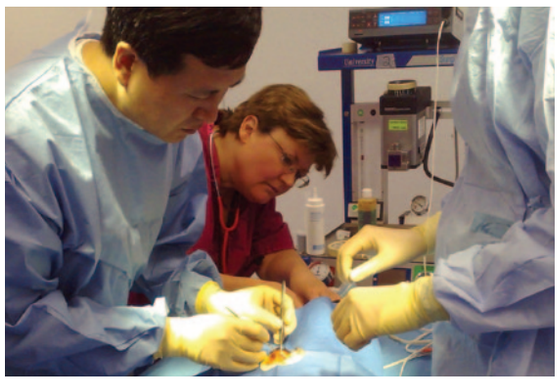
With the stipend from his OREF/Friedenberg Clinician Scientist Grant, Dr. Cui will have the opportunity to take his current work in additional directions.

While a number of his in vivo studies have focused on bone repair for traumatic injury or fractures, he has also turned his attention to an animal model of osteonecrosis, the death of bone tissue due to the lack of an adequate blood supply. Using essentially the same treatment strategies, he will test the ability of synthetic grafts loaded with VEGF/BMP-producing stem cells to regenerate bone at sites where bone tissue has died.

The grant also enables him to dig deeper into the basic science underlying VEGF and BMP activity in bone growth. Although there is ample evidence of the synergistic effects of VEGF and BMP on bone formation in animal models, the complex mechanisms leading to these outcomes have not been ascertained.

Dr. Cui explained, "If we don't get the pathway sorted out, the clinical translation of this work is going to be weak. When we can explain the molecular steps that lead from the expression of these proteins to the generation of new bone, then the orthopaedic community can be more confident in the potential of the synthetic grafts to repair bone defects."

Dr. Cui emphasized the importance of the OREF/Friedenberg grant not only to fuel research and support his career as a clinician scientist but also to demonstrate to students and residents the value of integrating research and clinical practice. He noted, "With the support of this grant, we can set up an environment—a real model—that shows medical students and residents how to pursue an academic career, research and clinical practice at the same time. We are taking care of our patients and training the next generation of clinician scientists."



▲ Dr. Cui (left) works on an osteonecrosis model. Photos courtesy of Dr. Cui.