Protein bath helps stimulate old marrow to form bone, study finds

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Bone fractures in the elderly are notoriously slow and difficult to heal. Now, researchers at the Stanford University School of Medicine have identified a simple way to increase the effectiveness of a surgical process called bone grafting that may significantly speed the growth of new, healthy bone in response to trauma.

In studies involving mice and rabbits, the researchers found that a quick dip in a bath of a signaling protein called Wnt3a can rev up sluggish bone-forming cells in older animals that would normally be unable to heal a fracture. If the simple treatment is eventually found to be effective in humans, it may significantly improve the success of bone grafts, which are performed more than 500,000 times every year in the United States.

"We're very focused on designing a treatment that could be easily employed by orthopaedic surgeons in the normal course of bone grafting," said professor of surgery Jill Helms, DDS, PhD. "We've shown that when we temporarily treat bone marrow from aged animals with Wnt before transplanting the cells into a fracture site, we see really robust bone formation."

Helms is the senior author of the study, published July 17 in the *Journal of Bone and Joint Surgery*. Philipp Leucht, MD, a resident in orthopaedic surgery at Stanford, is the lead author.

"Hip fractures in elderly people nearly triple the risk of dying within a

year of the injury, and a rapidly aging population demands more effective treatments for this type of trauma," said Helms.

The temporary nature of the treatment described in the paper, and the fact that it is conducted outside the body, is important because Wnt is a potent stimulator of growth and development for many types of cells. Injecting the protein into the body, where it can affect cells willy-nilly, raises the specter of uncontrolled proliferation and cancer.

Bone grafting involves transplanting whole marrow—which is rich in stem cells that form bone, blood and the cells of the immune system—into a fracture site. Although the use of a patient's own tissue is preferable to avoid rejection, marrow from older people looks and acts nothing like young marrow; as we age, our bone marrow begins to look more like a fatty tissue than the industrious blood- and bone-producing factory of children or younger adults.

As a result, orthopaedic surgeons sometimes use donor bone or marrow, or rely on the activity of drugs that incorporate bone morphogenetic proteins, or BMPs, to stimulate bone growth. But the hunt's been on to find alternatives that allow the use of a patient's own cells without medications.

The new finding is an extension of previous work in Helms' lab aimed at devising a way to produce a biologically active form of the finicky Wnt molecule, which is difficult to purify and dissolve in liquids. In 2010, Helms and Roeland Nusse, PhD, a Stanford professor of developmental biology, showed that they could attach the Wnt protein to tiny, water-friendly molecular bubbles called liposomes that could be injected directly into lab animals with fractures. The previous study found that this treatment promoted the rapid growth of new bone, but safety concerns about its use in humans remained.

In the current study, Helms and Leucht harvested whole bone marrow from laboratory mice genetically engineered to express a fluorescent protein. They then transplanted this marrow into 2-millimeter round holes they'd created in the skulls of anesthetized mice and followed the fate of the fluorescently labeled, transplanted cells.

The researchers found that within seven days, the transplanted marrow cells had remained in the injury site and begun to divide robustly. Over time, the defects in the mice that had received the bone graft healed completely. In contrast, the untreated mice were unable to fill the hole with new bone.

When Leucht and Helms repeated the experiment with marrow from older animals—laboratory mice over 40 weeks old—they saw a very different result. Recipient animals now generated much less bone at the injury site. And a closer look showed that the older marrow expressed lower levels of Wnt protein compared with that of the younger animals.

Finally, the researchers exposed the aged donor marrow to a brief bath of either Wnt3a or a control solution before transplanting them into the recipients. Within seven days, animals that received the Wnt-treated marrow had twice as much new bone at the injury site as control animals.

The researchers repeated their experiments on rabbits, which have longer bones that more closely resemble those of humans. Again, they found that treating old marrow briefly with Wnt3a significantly improved the cells' ability to heal a simulated fracture in the leg of recipient animals.

"Our findings have direct implications for humans," Helms said. "As we age, our healing is much less robust. We now have reason to believe that this might be due in part to a general decline in Wnt signaling. If we can

temporarily activate this signal while the marrow is outside the body, we might be able to provide a transient, much-needed boost to the activity of stem cells in the marrow."

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