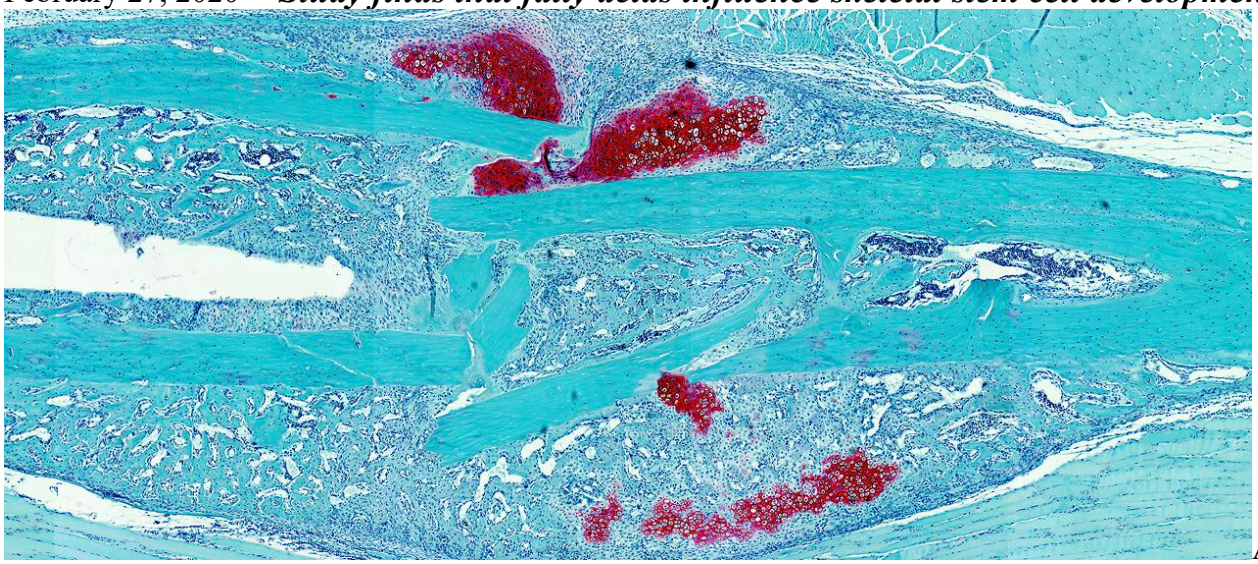
Bone or cartilage: how stem cells repair bone fractures

February 27, 2020 ***Study finds that fatty acids influence skeletal stem cell development***



A histologic section of a mouse bone fracture. Safranin O has been used to colour the cartilage cells red (specifically the proteins produced by cartilage cells); all other tissues are blue. Image courtesy of Nick Van Gastel / KU Leuven

When a bone fracture occurs, the stem cells that repair the injury either form new bone or new cartilage. A new study in the journal Nature has identified how this decision happens: fatty acids in the blood signal to stem cells that they have to develop into bone-forming cells. But if there are no blood vessels nearby, the stem cells end up forming cartilage. The finding that specific nutrients directly influence the development of stem cells opens new avenues for stem cell research.

Bone fractures heal through the action of skeletal progenitor cells: stem cells that are partially specialized, but can still develop into several different types of cells. Bone healing occurs in one of two ways: the progenitor cells evolve into bone-forming cells when the fracture is small, and into cartilage cells when the fracture is bigger. This cartilage is later replaced by bone. Until now, scientists did not know how progenitor cells decide whether to become bone or cartilage cells.

"Our hypothesis was that the presence of blood vessels plays a role," said first author Nick van Gastel, postdoctoral fellow in the lab of HSCI co-director David Scadden. "Despite what many people think, our bones are full of blood vessels, while cartilage does not have any."

When blood vessels surrounding a fracture were blocked, cartilage was formed. When they were not, new bone was created immediately...

This new study in mice confirmed the team's assumption: when blood vessels surrounding a fracture were blocked, cartilage was formed. When they were not, new bone was created immediately.

In a second phase of the study, the researchers tried to find out which signal the blood vessels actually send to the progenitor cells to make them convert into either a bone or a cartilage cell.

"Our previous research had already shown that nutrients play a role in the biology of progenitor cells," said senior author Geert Carmeliet, professor in the Clinical and Experimental Endocrinology Unit at KU Leuven.

In the current study, the team tested how the presence of different nutrients influences progenitor cell fate. The researchers found that the fatty acids present in blood cause progenitor cells to grow into bone-forming cells. If there are no fatty acids nearby, progenitor cells activate the *SOX9* gene, which is the signal for the cell to become a cartilage cell.

"This study is useful for researchers in regenerative medicine, since we still know little about cartilage formation," said Carmeliet. "Research into cartilage disorders such as osteoarthritis may also benefit from these findings. There are indications that cartilage cells receive more fatty acid signals and don't produce enough of the *SOX9* gene in patients with such disorders, which can have adverse effects on the joints. Finally, our study shows for the first time that specific nutrients can inform stem cells which type of cell they should become. That is an important step forward in stem cell research."

Researchers find method to regrow cartilage in the joints

In laboratory studies, Stanford School of Medicine researchers have found a way to regenerate the cartilage that eases movement between bones.

August 17, 2020 - By Christopher Vaughan

Researchers at the Stanford University School of Medicine have discovered a way to regenerate, in mice and human tissue, the cushion of cartilage found in joints.

Loss of this slippery and shock-absorbing tissue layer, called articular cartilage, is responsible for many cases of joint pain and arthritis, which afflicts more than 55 million Americans. Nearly 1 in 4 adult Americans suffer from arthritis, and far more are burdened by joint pain and inflammation generally.

The Stanford researchers figured out how to regrow articular cartilage by first causing slight injury to the joint tissue, then using chemical signals to steer the growth of skeletal stem cells as the injuries heal. The work was published Aug. 17 in the journal *Nature Medicine*.

“Cartilage has practically zero regenerative potential in adulthood, so once it’s injured or gone, what we can do for patients has been very limited,” said assistant professor of surgery Charles K.F. Chan, PhD. “It’s extremely gratifying to find a way to help the body regrow this important tissue.”

The work builds on previous research at Stanford that resulted in isolation of the skeletal stem cell, a self-renewing cell that is also responsible for the production of bone, cartilage and a special type of cell that helps blood cells develop in bone marrow. The new research, like previous discoveries of mouse and human skeletal stem cells, were mostly carried out in the laboratories of Chan and professor of surgery Michael Longaker, MD.

Articular cartilage is a complex and specialized tissue that provides a slick and bouncy cushion between bones at the joints. When this cartilage is damaged by trauma, disease or simply thins with age, bones

can rub directly against each other, causing pain and inflammation, which can eventually result in arthritis.

Damaged cartilage can be treated through a technique called microfracture, in which tiny holes are drilled in the surface of a joint. The microfracture technique prompts the body to create new tissue in the joint, but the new tissue is not much like cartilage.

"Microfracture results in what is called fibrocartilage, which is really more like scar tissue than natural cartilage," said Chan. "It covers the bone and is better than nothing, but it doesn't have the bounce and elasticity of natural cartilage, and it tends to degrade relatively quickly."

The most recent research arose, in part, through the work of surgeon Matthew Murphy, PhD, a visiting researcher at Stanford who is now at the University of Manchester. "I never felt anyone really understood how microfracture really worked," Murphy said. "I realized the only way to understand the process was to look at what stem cells are doing after microfracture." Murphy is the lead author on the paper. Chan and Longaker are co-senior authors.

For a long time, Chan said, people assumed that adult cartilage did not regenerate after injury because the tissue did not have many skeletal stem cells that could be activated. Working in a mouse model, the team documented that microfracture did activate skeletal stem cells. Left to their own devices, however, those activated skeletal stem cells regenerated fibrocartilage in the joint.

But what if the healing process after microfracture could be steered toward development of cartilage and away from fibrocartilage? The researchers knew that as bone develops, cells must first go through a cartilage stage before turning into bone. They had the idea that they might encourage the skeletal stem cells in the joint to start along a path toward becoming bone, but stop the process at the cartilage stage.

The researchers used a powerful molecule called bone morphogenetic protein 2 (BMP2) to initiate bone formation after microfracture, but then stopped the process midway with a molecule that blocked another signaling molecule important in bone formation, called vascular endothelial growth factor (VEGF).

"What we ended up with was cartilage that is made of the same sort of cells as natural cartilage with comparable mechanical properties, unlike the fibrocartilage that we usually get," Chan said. "It also restored mobility to osteoarthritic mice and significantly reduced their pain."

As a proof of principle that this might also work in humans, the researchers transferred human tissue into mice that were bred to not reject the tissue, and were able to show that human skeletal stem cells could be steered toward bone development but stopped at the cartilage stage.

The next stage of research is to conduct similar experiments in larger animals before starting human clinical trials. Murphy points out that because of the difficulty in working with very small mouse joints, there might be some improvements to the system they could make as they move into relatively larger joints.

The first human clinical trials might be for people who have arthritis in their fingers and toes. "We might start with small joints, and if that works we would move up to larger joints like knees," Murphy says. "Right now, one of the most common surgeries for arthritis in the fingers is to have the bone at the base of the thumb taken out. In such cases we might try this to save the joint, and if it doesn't work we just take out the bone as we would have anyway. There's a big potential for improvement, and the downside is that we would be back to where we were before."

Longaker points out that one advantage of their discovery is that the main components of a potential therapy are approved as safe and effective by the FDA. “BMP2 has already been approved for helping bone heal, and VEGF inhibitors are already used as anti-cancer therapies,” Longaker said. “This would help speed the approval of any therapy we develop.”

Joint replacement surgery has revolutionized how doctors treat arthritis and is very common: By age 80, 1 in 10 people will have a hip replacement and 1 in 20 will have a knee replaced. But such joint replacement is extremely invasive, has a limited lifespan and is performed only after arthritis hits and patients endure lasting pain. The researchers say they can envision a time when people are able to avoid getting arthritis in the first place by rejuvenating their cartilage in their joints before it is badly degraded.

“One idea is to follow a ‘Jiffy Lube’ model of cartilage replenishment,” Longaker said. “You don’t wait for damage to accumulate — you go in periodically and use this technique to boost your articular cartilage before you have a problem.”

Longaker is the Deane P. and Louise Mitchell Professor in the School of Medicine and co-director of the Institute for Stem Cell Biology and Regenerative Medicine. Chan is a member of the Institute for Stem Cell Biology and Regenerative Medicine and Stanford Immunology.

Other Stanford scientist taking part in the research were professor of pathology Irving Weissman, MD, the Virginia and D. K. Ludwig Professor in Clinical Investigation in Cancer Research; professor of surgery Stuart B. Goodman, MD, the Robert L. and Mary Ellenburg Professor in Surgery; associate professor of orthopaedic surgery Fan Yang, PhD; professor of surgery Derrick C. Wan, MD; instructor in orthopaedic surgery Xinming Tong, PhD; postdoctoral research fellow Thomas H. Ambrosi, PhD; visiting postdoctoral scholar Liming Zhao, MD; life science research professionals Lauren S. Koepke and Holly Steininger; MD/PhD student Gunsagar S. Gulati, PhD; graduate student Malachia Y. Hoover; former student Owen Marecic; former medical student Yuting Wang, MD; and scanning probe microscopy laboratory manager Marcin P. Walkiewicz, PhD.

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Ability to repair cartilage with stem cells steps closer

The day that patients with osteoarthritis can ease their painful joints by using stem cell therapy to regenerate damaged cartilage took a step closer recently when researchers reported successfully producing cartilage in rats using embryonic stem cells.

Researchers hope their new stem cell protocol will one day be used to treat the painful joint condition osteoarthritis.

The success is attributed to a new procedure or protocol fur using human embryonic stem cells, developed under strict laboratory conditions, by the researchers at the University of Manchester in the UK.

The researchers report a study about their work – funded by Arthritis Research UK – in the journal *Stem Cells Translational Medicine*. The study shows how they used the new protocol to grow and transform human embryonic stem cells into cartilage cells.

Leading the research is Sue Kimber, a professor in the Faculty of Life Sciences at Manchester, who, with her colleagues, hopes their approach could in future be used to treat the painful joint condition osteoarthritis. She notes:

"This work represents an important step forward in treating cartilage damage by using embryonic stem cells to form new tissue, although it's still in its early experimental stages."

Osteoarthritis mainly affects people over the age of 60, and is a major cause of disability. It is a degenerative disease caused by wearing away of cartilage in joints that have been continually stressed during a person's lifetime, including the knees, hips, fingers and lower spine region.

The World Health Organization estimates that around 9.6% of men and 18.0% of women aged over 60 years have symptomatic osteoarthritis.

Researchers generated precursor cartilage cells from embryonic stem cells

Cartilage cells – also known as chondrocytes – are formed from precursor cells called chondroprogenitors. In their study, the team describes how they used the new protocol to generate chondroprogenitors from human embryonic stem cells.

They implanted the precursor cartilage cells into damaged cartilage in the knee joints of rats.

After 4 weeks the cartilage was partially repaired. After 12 weeks, the cartilage surface was smooth and similar in appearance to normal cartilage.

Later examination of the regenerated cartilage showed that cartilage cells from the embryonic stem cells were still present and active in the tissue.

The study is promising because not only did the new protocol lead to regenerated, healthy-looking cartilage, but there were none of the adverse side-effects that have since dashed the high hopes raised in the early days of stem cell research – the growth of abnormal or disorganized tissue or tumors.

Testing the new protocol is the first step toward trials in human arthritis patients

Testing the new protocol in rats is the first step toward running trials in people with arthritis. But before this can happen a lot more needs to be done to show the protocol works and is safe. The team is already planning their next step to build on their findings.

Another approach to using human embryonic stem cells to generate new cartilage cells is using adult stem cells. Adult stem cells are found in certain “niches” in the body and are not as controversial as embryonic stem cells but their potential is not so great. Also, note the authors, they cannot currently be produced in large amounts and the procedure is expensive.

Dr. Stephen Simpson, director of research at Arthritis Research UK, says he is encouraged by the new study because:

“Embryonic stem cells offer an alternative source of cartilage cells to adult stem cells, and we’re excited about the immense potential of Professor Kimber’s work and the impact it could have for people with osteoarthritis.”

He explains that current treatments for osteoarthritis can only relieve painful symptoms, and there are no effective therapies that delay or reverse cartilage degeneration. Joint replacements are successful in older people, but these options are not effective in younger people or athletes with sports injuries.

Very Technical Article

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8468484/