frontiers | Frontiers for Young Minds

HUMAN HEALTH
Published: 23 June 2023
doi: 10.3389/frym.2023.953816

OUT WITH THE OLD AND IN WITH THE NEW: REPAIRING DAMAGED CARTILAGE

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YOUNG REVIEWERS:

AARAV
AGE: 10

BENJAMIN
AGE: 15

BREANNA
AGE: 13

Imagine experiencing aching pain with every step and every bend of your knee. As time passes, the pain only worsens. Sounds terrible, does it not? Such pain could be caused by a disease called osteoarthritis. This disease involves the breakdown of joint tissue, called cartilage, which normally keeps the bones from rubbing together. Even small injuries to cartilage can lead to osteoarthritis because cartilage lacks the ability to heal itself. A surgical procedure called autologous chondrocyte implantation (ACI) can be performed after small cartilage injuries, to help prevent osteoarthritis from developing. In ACI, healthy cartilage cells are grown outside the body and then re-implanted into the damaged area. ACI is not perfect; healing is limited because of the artificial environment that the cells are grown in before implantation. This article discusses the changes these cartilage cells undergo when they are grown in an

OSTEOARTHRITIS

A disease where articular cartilage, the tissue that cushions bone, breaks down and leads to painful bone on bone rubbing. Osteoarthritis can develop from even small joint injuries.

ARTICULAR CARTILAGE

The tissue found in joints that prevents bones from rubbing together and acts as a cushion to absorb forces when the person moves. Cartilage lacks any method to repair itself.

AUTOLOGOUS CHONDROCYTE IMPLANTATION (ACI)

A surgical procedure in which chondrocytes are removed from healthy articular cartilage, grown in a lab, and then put into the damaged area of the joint.

CHONDROCYTES

The cells in cartilage that are responsible for building cartilage by making matrix molecules such as collagen type II and aggrecan.

MATRIX

The structure created by chondrocytes that gives articular cartilage its cushion function.

COLLAGEN TYPE II

An important building block of the cartilage matrix that provides strong structure to withstand pulling and pushing forces.

artificial environment, and how our research is addressing this issue to improve the success of ACI.

OSTEOARTHRITIS: PAINFUL CARTILAGE BREAKDOWN

Osteoarthritis is a disease in which the slippery white tissue between bones in a joint, called **articular cartilage**, breaks down and causes pain. Osteoarthritis can affect any joint in the body but is particularly common in the hip and knee joints [1]. Osteoarthritis can happen with age because of wear and tear, but it can also happen to young people who injure their joints, possibly by tearing their cartilage or dislocating their joint. Even small injuries to articular cartilage can lead to osteoarthritis because cartilage lacks the ability to heal itself.

To prevent osteoarthritis, a procedure called **autologous chondrocyte implantation (ACI)** can be performed following a minor joint injury. Autologous means the cells used in the treatment are from the same patient. Unfortunately, ACI does not always provide lifelong protection against osteoarthritis. Our lab is studying the cause of this limitation and researching ways to make ACI more successful and long lasting—to improve the lives of the people who receive this surgery.

WHAT IS ARTICULAR CARTILAGE?

Articular cartilage is essential for painless movement. It acts as a shock absorber and allows bones to move smoothly over one another. The cells in articular cartilage are called **chondrocytes**, and they are responsible for maintaining healthy cartilage [2]. Chondrocytes keep cartilage healthy by secreting the molecules that form what is called the **matrix**—a kind of living scaffold that gives structural support to the cartilage tissue (Figure 1).

The matrix is what provides cushion to bones. It is made of three main components: **collagen type II**, **aggrecan**, and water [2]. Collagen type II provides the matrix with the ability to endure forces like stretching and compression, and aggrecan gives the matrix the ability to attract water. The aggrecan-water mixture provides the shock-absorbing capabilities of articular cartilage.

Interestingly, articular cartilage does not have any blood vessels, so it cannot obtain nutrients and other resources from the blood like most organs and tissues can. So, when articular cartilage is damaged, chondrocytes do not have the resources necessary to repair the damage. When you break a bone, it heals after a while—but articular cartilage does not heal, even after a long time. In fact, even minor cartilage damage can cause further breakdown to cartilage over time,

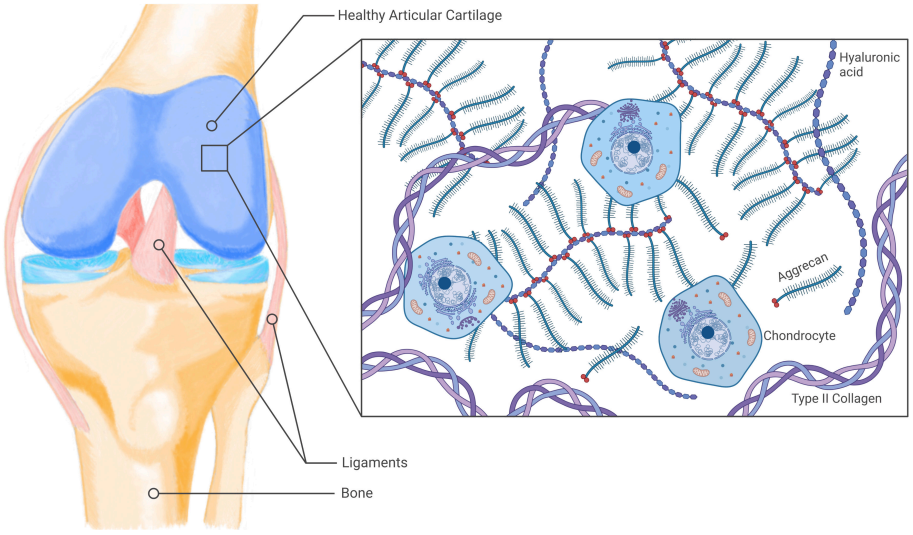

Figure 1

Articular cartilage is the tissue that makes up joints like the knee, and it cushions the bones. Here is a picture of a bent knee joint with the kneecap removed; if we zoom in on articular cartilage **(right)** we can see chondrocytes surrounded by a scaffold-like material called the matrix. The matrix is made up of several types of molecules including type II collagen and aggrecan, which give cartilage its cushioning ability (Image created with Notes, GIMP, and Draw.io).

AGGRECAN

A molecule of the cartilage matrix that draws in water to provide strength for shock absorbance.

which is why even a small injury to articular cartilage can eventually develop into osteoarthritis.

AN OVERVIEW OF ACI

ACI is a common method used to prevent small cartilage injuries from developing into osteoarthritis in the knee, hip, or shoulder joints. ACI is a two-surgery therapy (Figure 2). In the first surgery, a surgeon removes a tiny piece of healthy articular cartilage (approximately the size of a Tic-Tac) from a healthy area in the joint. This tissue is taken to a laboratory where the chondrocytes are separated from the cartilage matrix. The chondrocytes are placed onto plastic dishes filled with a mixture of nutrients that help them to grow. The chondrocytes are grown until there are enough cells to cover the damaged area of the joint. Finally, the surgeon re-implants the lab-grown chondrocytes into the damaged area of the patient's joint.

ACI cannot be used to prevent osteoarthritis that happens due to old age, but it *is* an important tool to prevent osteoarthritis in young people who have enough healthy cartilage to remove and grow in the lab. After injury, ACI allows active people to return to a similar level of physical activity and to require fewer surgeries to repair their articular cartilage. Unfortunately, many patients who receive ACI still end up developing osteoarthritis eventually [3]. This happens because chondrocytes that are grown outside of the body undergo certain changes, so the matrix that they make when they are re-implanted into the joint is different from the normal matrix.

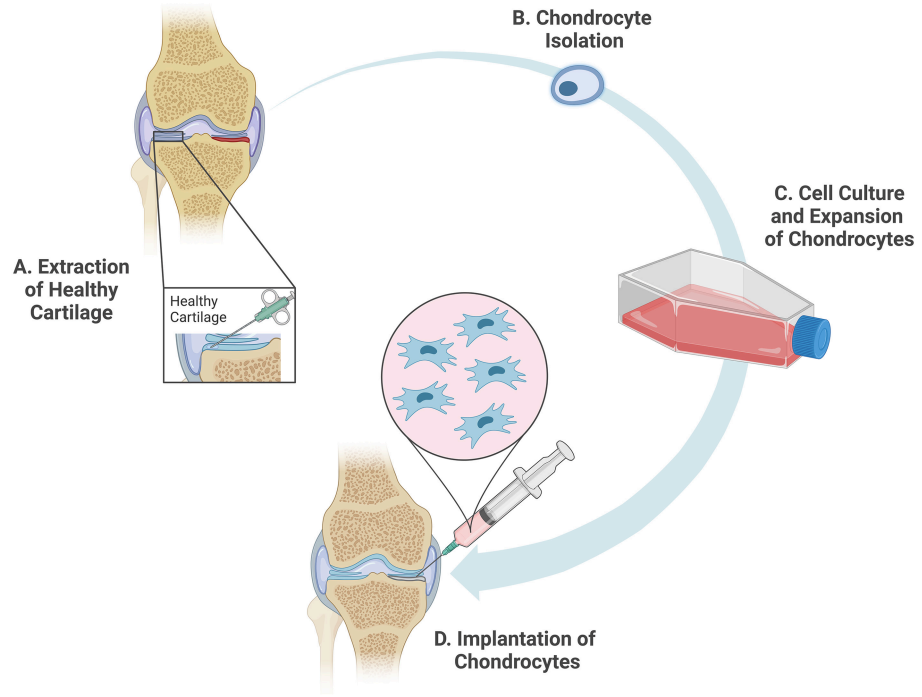

Figure 2

(A) To perform ACI, surgeons first extract a sample of healthy cartilage from the targeted joint. **(B)** Then, scientists isolate the chondrocytes in the lab by breaking down the tissue with enzymes. **(C)** The chondrocytes are grown in the lab, on a stiff plastic dish, until there are enough cells to be re-implanted. **(D)** Finally, the surgeon re-implants the chondrocytes into the damaged region in the joint (Figure created with Biorender).

WHAT HAPPENS TO CHONDROCYTES GROWN IN THE LAB?

The environment in which chondrocytes live has a big impact on their structure and function. When chondrocytes are grown outside of the body, they begin changing their shape until they look different from the way they would look inside the body. The changes are due to differences between the two environments. Within the body, chondrocytes live in a three-dimensional matrix, which is a comfortable environment for them. However, when cells are grown in the lab, they grow on two-dimensional, plastic tissue culture dishes. Although these dishes are ideal for growing more cells, they are stiffer than the chondrocytes' natural environment. This causes the chondrocytes to become wider and flatter, and to produce different molecules than they would make inside the body. This process is called **chondrocyte dedifferentiation**, and we call these cells dedifferentiated chondrocytes (Figure 3).

CHONDROCYTE DEDIFFERENTIATION

A series of significant changes to chondrocytes that happen when they are grown in the lab and affect their ability to produce healthy cartilage.

Normal chondrocytes produce type II collagen for their matrix, while dedifferentiated chondrocytes produce type I collagen, which is more densely packed leading to a less durable matrix [4]. Dedifferentiated chondrocytes also produce less aggrecan. This change in the molecules produced by dedifferentiated chondrocytes leads to the creation of a tissue called **fibrocartilage**. Compared to articular cartilage, fibrocartilage has a shorter lifespan plus it is not as strong and nor absorptive of forces. Therefore, current scientific research

FIBROCARTILAGE

A type of cartilage that is mechanically inferior to articular cartilage. It is produced by cells grown on stiff plastic dishes in the lab. Fibrocartilage limits the lifespan of ACI.

Figure 3

Growing chondrocytes in the lab leads to dedifferentiation. Chondrocytes from articular cartilage are round when they are removed from the joint. After growing on hard plastic lab dishes, they turn into dedifferentiated chondrocytes, which are spread out and do not resemble circular chondrocytes. When dedifferentiated chondrocytes are re-implanted into the joint, they lead to the production of fibrocartilage, which is not as functional as healthy articular cartilage.

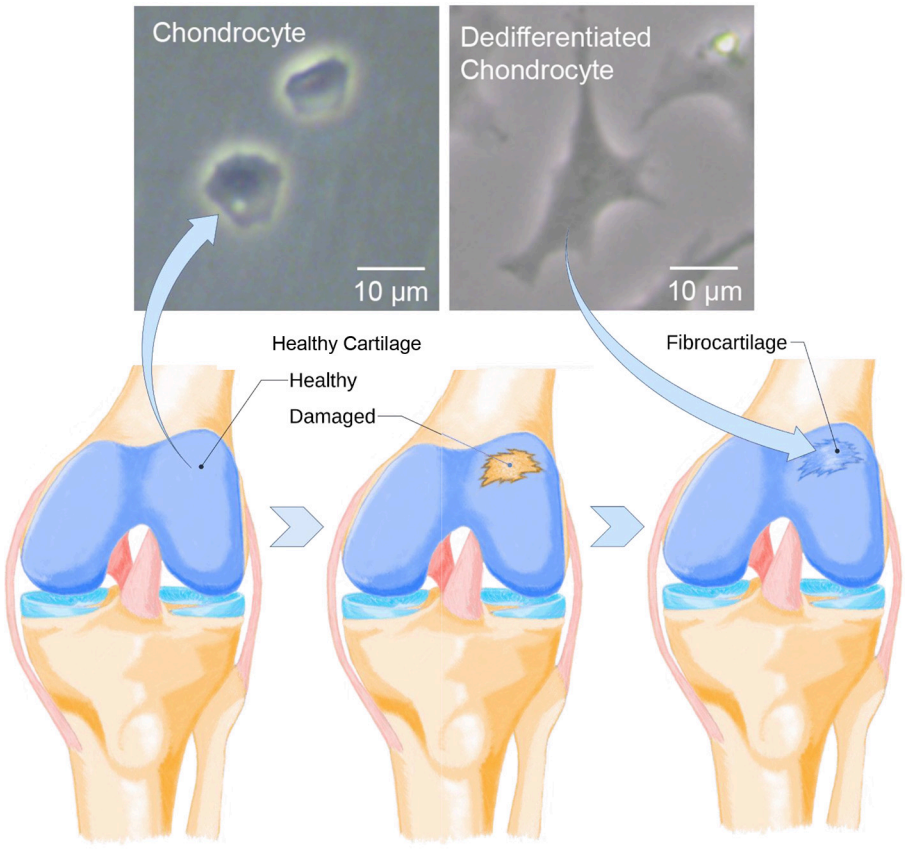

Figure 3

is trying to to reverse chondrocyte dedifferentiation, to improve the matrix produced by these cells.

HOW CAN WE IMPROVE ACI?

Currently, there are no ways to heal cartilage permanently, to prevent osteoarthritis. Our research aims to improve ACI by increasing the number of healthy chondrocytes that can be re-implanted into a joint. We have found that when chondrocytes are grown on a softer dish that is more like their natural environment, they produce matrix that is more similar to that produced by chondrocytes in the body. We try to reverse the initial shape changes chondrocytes undergo in the lab, and turn them back into their round, healthy form. We are discovering the molecules that cause this shape change and, by studying those molecules, we are trying to reverse the dedifferentiation process.

If we can reverse chondrocyte dedifferentiation, we will be able to grow chondrocytes in the lab that are healthy and function similarly to those that grow in the body. If more healthy chondrocytes are available for implantation, ACI can increase the quality and lifespan of regenerated cartilage. We anticipate that our research will result in

fewer surgeries for people who damage their articular cartilage—and more time running, jumping, and living the lives they love!

ACKNOWLEDGMENTS

We would like to thank Marissa Heino for her help with the early stages of our project and Hiba Oumial and Cameron Schweiger for help with the editing process. This work was supported by the Delaware Center for Musculoskeletal Research from the National Institutes of Health's National Institute of General Medical Sciences under grant number P20GM139760.

REFERENCES

1. Katz, J. N., Arant, K. R., and Loeser, R. F. 2021. Diagnosis and treatment of hip and knee osteoarthritis: a review. *JAMA* 325:568–78. doi: 10.1001/jama.2020.22171
2. Sophia Fox, A. J., Bedi, A., and Rodeo, S. A. 2009. The basic science of articular cartilage: structure, composition, and function. *Sports Health* 1:461–8. doi: 10.1177/1941738109350438
3. Macmull, S., Parratt, M. T., Bentley, G., Skinner, J. A., Carrington, R. W., Morris, T., et al. 2011. Autologous chondrocyte implantation in the adolescent knee. *Am. J. Sports Med*. 39:1723–30. doi: 10.1177/0363546511404202
4. Ma, B., Leijten, J. C., Wu, L., Kip, M., van Blitterswijk, C. A., Post, J. N., et al. 2013. Gene expression profiling of dedifferentiated human articular chondrocytes in monolayer culture. *Osteoarthritis Cartilage* 21:599–603. doi: 10.1016/j.joca.2013.01.014

SUBMITTED: 26 May 2022; **ACCEPTED:** 31 May 2023;
PUBLISHED ONLINE: 23 June 2023.

EDITOR: Bahtiyar Yilmaz, University Hospital of Bern, Switzerland

SCIENCE MENTORS: Nishma Gupta and Bingyun Li

CITATION: Gonzalez-Nolde S, Benito CG, Inguito KL, Schofield MM, Manzoni TJ, Richardson-Solorzano S, Chowdhury VP and Parreno J (2023) Out With the Old and in With the New: Repairing Damaged Cartilage. Front. Young Minds 11:953816. doi: 10.3389/frym.2023.953816

CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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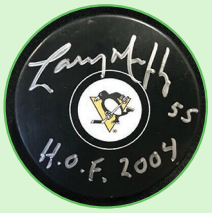
YOUNG REVIEWERS

AARAV, AGE: 10

I am a ten-year-old in Grade 6, who is very interested about anything related to buildings, maths and science. When I grow up, I want to be an architect. My favorite pastime is solving Rubik's cube. I love riding my bike, playing cricket, writing poems, and sketching. I am curious to understand the world around me.



BENJAMIN, AGE: 15

When I was introduced into this world, I first lived in an apartment with my dad and mom. Life was great, I had no responsibilities to take care of. Three years later, my sister joined us. After five years, I left that apartment to live in a house. This was also when I was introduced to hockey. When I started my middle school, we moved into a giant house, after that our life has been going smoothly.

BREANNA, AGE: 13

Hello! I am a seventh grader! I love drawing, figure skating, dancing, and tennis! I like watching movies and reading books when I am bored. I have a brother, a helpful mom and dad, and my best friend dog. My house has a surrounding of many trees and beautiful birds. My favorite foods are ice-cream, banana bread, and pasta. I like being myself!

AUTHORS

SOFIA GONZALEZ-NOLDE

Sofia Gonzalez-Nolde was born and raised in New Jersey, where she grew up with her parents and two siblings. She studied biology at the University of Delaware from 2017–2021 and completed her bachelor's degree. Currently, she is working on her master's degree in biology at the University of Delaware, where she works under the supervision of Dr. Justin Parreno. In his lab, she is studying the role that the structure of the top layer of chondrocytes regulates the molecules they produce. In her free time, you can find Sofia at the skatepark, rock gym, or playing her guitar.

CARLOS G. BENITO

Carlos G. Benito was born and raised in New Jersey, where he grew up with his three sisters. He is majoring in biology with minors in Spanish for healthcare and entrepreneurship. He is currently working on pursuing an undergraduate thesis in Dr. Parreno's lab. His current research focuses on structures within the eye, with a focus on age-related loss of vision. Outside of the lab, he enjoys gardening, repairing antique houses, and working as chief business officer of Sia Precision Education.

KAMERON L. INGUITO

Kameron L. Inguito was born and raised in Newark, Delaware with two parents and two siblings. He received his bachelor's degree in economics from the University of Delaware, and he started medical school in July of 2022. In the past, he served as a research assistant in a biology lab, under the supervision of Dr. Justin Parreno. Much of his research involved analyzing how the cellular structure of tendons, the tissue that connects muscle to bones, plays in disease. Currently, he is a medical student at Sidney Kimmel Medical College. During his free time, he loves to hike, spend time with friends, and listen to podcasts.

MANDY M. SCHOFIELD

Mandy M. Schofield is a master's student at the University of Delaware. She was born in Harrogate, England, and moved to Baltimore, Maryland when she was a kid. She got her undergraduate degree in biology from Towson University in May of 2020. She always knew that she loved science and is excited to be doing research that can help people someday. With Dr. Parreno, she is researching how the actin cytoskeleton can be targeted to improve ACI. When she is not in the lab, she loves doing Zumba, baking, or playing with her dog.

THOMAS J. MANZONI

Thomas J. Manzoni is from Dallas, Pennsylvania. He graduated from the University of Delaware in 2021, where he studied mechanical engineering. At that time, he focused on biomechanics and medical device design, and how to use various technologies such as 3D printing to develop mechanical loading prototypes. Currently, he studies biology at the University of Delaware, focusing on using bioprinting and biotechnology to develop new methods for cartilage repair. Outside of research, he likes to spend time outdoors and go fishing.

STEPHANIE RICHARDSON-SOLORZANO

Stephanie Richardson-Solorzano is a Ph.D. student from Orange County, California, concentrating on molecular biology, biochemistry, and genetics. She completed her undergraduate degree at the University of San Diego in biochemistry while focusing on organometallic research. Once she entered the workforce, she fell in love with the idea of combining chemistry and biology, and she enrolled at the University of Delaware. Her current research in focuses on the development of lab-based models of human cartilage to use in drug screening for personalized medicine to treat osteoarthritis. When outside of the lab, she enjoys traveling to natural parks, hiking, and reading.

VISNU PRITOM CHOWDHURY

Visnu Pritom Chowdhury is from Bangladesh. He studied medicine and afterward earned his master's degree in biotechnology. His interest lies in the domain of molecular and cell biology, especially in the field of molecular and personalized medicine. He is currently pursuing his Ph.D. in molecular biology and genetics at the University of Delaware. He wants to learn the art of drug discovery so that, in the future, he can work on finding solutions to diseases that do not have any solutions yet. Although he loves music, drawing, and calisthenics, his world revolves around his family.

JUSTIN PARRENO

Justin has always been intrigued by the structure-function relationship in cells; specifically, how the organization of actin within cells can determine the way cells behave. The actin cytoskeleton was once thought to be difficult to target to prevent or treat diseases. But with the increasing understanding of the diversity of actin networks, targeting the actin cytoskeleton is now possible. His lab is focused on targeting the actin cytoskeleton to prevent and treat diseases such as osteoarthritis and cataracts. Outside of the lab, Justin enjoys discovering new coffee shops, playing and watching basketball, and most importantly spending time with his family and dog. *jparreno@udel.edu

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