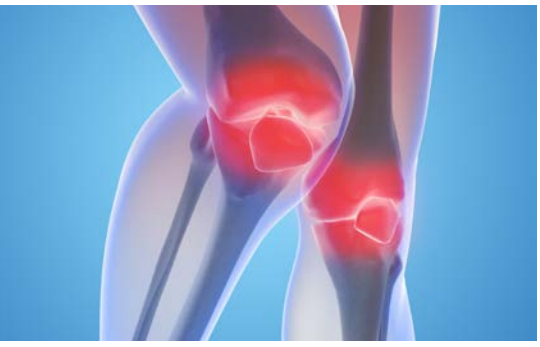
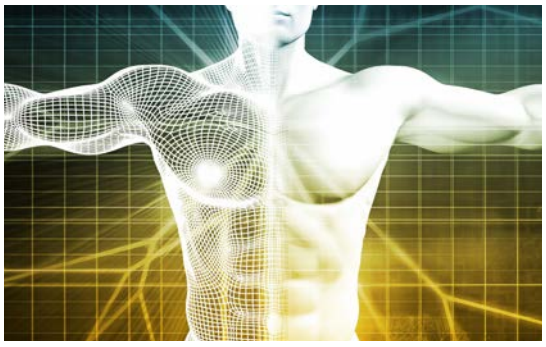
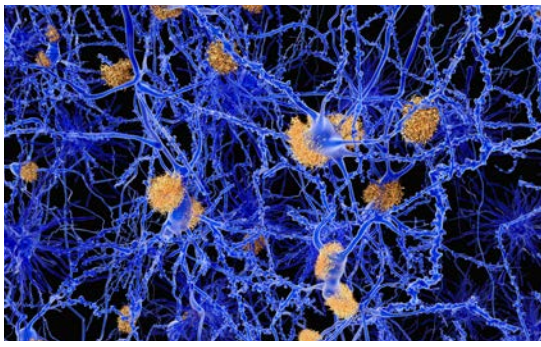
Increasing Healthspan: Tissue Engineering and Medicinal Chemistry for Aging Bodies and Brains

A survey report on the molecular mechanisms of aging and approaches to treating physical disease and injury as well as cognitive decline associated with the aging process.

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Increasing Healthspan: Tissue Engineering and Medicinal Chemistry for Aging Bodies and Brains

About This Report

About The Author

Melissae Fellet has written about chemistry for *New Scientist, Chemical & Engineering News*, and *Chemistry World*. She graduated from the Science Communication program at the University of California, Santa Cruz, and holds a Ph.D. in organic chemistry from Washington University in St. Louis.

I. EXECUTIVE SUMMARY

Lifespans are increasing in societies around the world, in some places at a rate faster than ever before. By 2020, there will be more people older than 60 years old than younger than 5, according to the World Health Organization. This demographic shift happened over more than a century in Europe, but in Asia and Latin America, the population is projected to move from being predominantly young to being predominantly old in just 25 years.[1]

Advances in science, technology, and medicine have increased our life expectancy. Efforts to promote healthy living and prevent disease have also increased the amount of years we have good health. However, those same advances have also increased the number of years that we live with disease: disability as a proportion of life after age 65 is slowly increasing, too. The tissues in our bodies naturally change as we age, with weakened bones becoming vulnerable to fractures and degenerated cartilage contributing to stiff joints. The risk of developing cancer, dementia, or heart disease increases with age, as accumulations of aging cells alter physiological processes. Around the world, researchers are tackling the next challenges to radically increase our healthy life expectancy: preventing cellular aging and repairing aging tissues using tissue engineering.[1]

II. AGING

Although modern medicine has extended our lifespan, we're not always healthy enough to enjoy those extra years traveling, learning new things, visiting family and friends, or pursuing neglected passions. Our "diseasespan"—the portion of our life where diseases of aging take hold—is beginning earlier and lasting longer than that of previous generations.[3]

Aging naturally brings a progressive loss of physical capabilities. Weakened bone and cartilage lead to fragile bones and stiff joints. Our senses dull, particularly hearing and vision. Our bodies become vulnerable to cancer, dementia, and heart disease. Managing these physical changes and disease symptoms is one focus of aging research. Another, less well-studied approach is uncovering the complex molecular mechanisms that cause aging.

Genetic Changes During Aging

Aging is a biochemically complex process that involves several types of genetic changes that alter cellular metabolism.[4] Patterns of gene expression and cellular repair naturally change as cells age. Lifestyle factors such as diet, chronic stress, or carcinogen exposure can damage cells and change epigenetic information that controls gene expression and nucleic acid stability.[5] Unstable chromosomes may rearrange themselves, generating damage that triggers cellular aging.

Much of the difference in lifespan between genetically identical individuals may be due to epigenetics, factors that control gene expression outside of the instructions encoded in DNA.[6] Epigenetic changes involve differences in the structure of the genome at various length scales. In a cell, 147 bases of DNA encircle a complex of histone proteins, like thread wrapping around a spool, to form a nucleosome. The coiled packing of multiple nucleosomes forms chromatin, which further coils into chromosomes.

Some epigenetic signs of aging are apparent at the atomic level. Patterns of methyl groups attached to DNA and acetyl groups dangling from histones change during aging. Aging cells also have different variants of histone proteins than young cells. Other genetic changes during aging impact chromatin packing, which ultimately changes the balance of gene expression. Tightly packed chromatin is inaccessible to transcription and translation machinery, but changes in aging cells relax chromatin packing, making previously inactive genes available for expression. Increased expression of previously inactive genes also happens along with decreased expression of other genes, causing transcriptional drift.

One genetic change that contributes to transcriptional drift is aging by retrotransposition.[7] As the chromatin structure opens in aging cells, mobile elements called transposons, often contained in tightly packed regions of chromatin, become accessible to the protein machinery involved with transcription. Transposons get copied and reinserted into the genome in new locations. This process, observed in yeast, flies, worms, and mice, is linked to cancer and neurodegeneration.

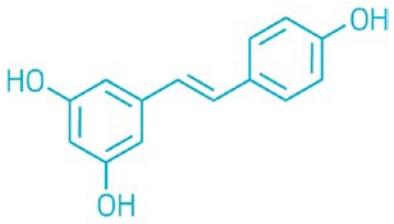
Another age-related cellular change that influences chromatin packing is decreased levels of core histone proteins in aging yeast, worms, and some human cells. With fewer proteins available, DNA cannot form nucleosomes, so portions of the genome that would otherwise be wrapped in a nucleosome are now available for transcription.

When there are fewer nucleosomes, DNA repair and stability are also affected. Old yeast with fewer nucleosomes have more broken DNA and damaged protein complexes involved with chromosome repair. Tweaking the biochemistry of yeast to increase histone production, or prevent destruction of the proteins, increases their lifespan.[8]

Anti-Aging Pills?

The most studied, reproducible, non-genetic intervention known to increase "healthspan" or lifespan in yeast, worms, flies, rats, and primates is reducing total calorie intake 20–40%.[9] Because the relationship between calorie restriction and extended health- or lifespan is observed across so many species, the molecular mechanisms behind this effect are thought to be found in genes common to a variety of species. Although these mechanisms are under debate, calorie restriction is believed to down-regulate highly conserved metabolic pathways that sense high glucose or amino acid levels, and also sense low levels of the energy molecules adenosine monophosphate (AMP) and nicotinamide adenine dinucleotide (NAD+). One theory for this observation is that when a cell recognizes its glucose- and amino acid-sensing pathways are silent, it enters a defensive mode, minimizing cell growth and metabolism to lower rates of cell damage.[9]

Restricting diet can be challenging, compared to taking a daily pill that slows or reverses aging. Researchers are looking for molecules that influence cellular aging processes, including some that might mimic the effects of calorie restriction. However, tinkering too much with biochemical pathways involved in aging can have undesired consequences. Take telomeres, for example. These sacrificial, repetitive bits of DNA dangle at the end of chromosomes, protecting precious genetic information during cell division. When cells copy chromosomes, enzymes cannot copy the full length of a chromosome. With telomeres capping each chromosome, the entire genetic information gets copied with each division, along with as much of the telomere as the proteins can copy. Telomeres naturally shorten during cell divisions, and an enzyme called telomerase lengthens and repairs them. Telomeres that remain shortened lead to premature aging, while active telomerase delays aging. Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak were awarded the 2009 Nobel Prize in medicine for their discovery of telomeres and telomerase. Activating telomerase to repair telomeres was thought to be an attractive route to an anti-aging pill, but these drugs often make cells cancerous.



Resveratrol

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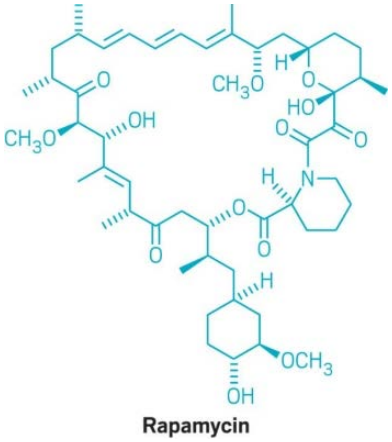
Theoretically, another therapeutic approach for anti-aging drugs is targeting enzymes involved with reversible epigenetic changes in aging. One long-studied target is resveratrol, a molecule in red wine found to reduce inflammation, oxidative stress, and cancer in cell and animal models. Researchers think resveratrol activates sirtuins, a class of enzymes that regulate proteins and genes involved with inflammation and aging. One sirtuin, SIRT1, could be linked to the effects of calorie restriction in animal studies.[10] Another long-studied anti-aging drug is rapamycin, first isolated from a soil bacterium on Easter Island in the 1980s. Rapamycin extends the lifespan of mice, bolsters the immune system of elderly people, and produces biological benefits similar to calorie restriction.[11]

A Tonic for Longer Life?

Rapamycin or related compounds have been shown to increase lifespan or improve other markers of aging in a range of organisms.



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Industrial and academic researchers are developing "rapalogs," analogs of rapamycin, to inhibit a protein called mTOR that is involved with signaling nutrient availability and energy supplies.[12]

Other ways to reverse aging in human cells and mice involve direct genetic manipulation, rather than molecularly altering metabolism. Researchers at the Salk Institute for Biological Studies partially reprogrammed pancreatic and muscle cells in an aging mouse to express the Yamanaka factors, four proteins involved in turning any cell type into a stem cell. Testing the approach in mice that have progeria, a disease that causes premature aging, the researchers found the reprogrammed mice

looked younger, had better organ function, and lived 30% longer than untreated mice without developing cancer.[13]

Because there are several different causes and physiological processes involved in aging, treating aging may be most effective with a personalized approach.[3] In 2014, J. Craig Venter, who competed with the U.S. Government in the race for the first human genome sequence, started a company that aims to be a comprehensive database of genomic, microbiome, and physical characteristic information to solve aging related problems. Other companies are also invested in aging research: In 2013, Google founded Calico, a company of scientists and computer scientists, to develop ways to slow aging and counteract diseases of aging.

Youthful Blood Not Just for Vampires

Some researchers studying aging have revived the 150-year-old technique of parabiosis, surgically connecting the circulatory system of a young mouse to that of an old mouse. Following the surgery, old mice have shiny fur, revitalized organs, and renewed health. The researchers identify substances in young blood linked to these changes, and they have found proteins that seem to reverse age-related memory loss[14] and heart disease.[15]

But parabiosis research can produce conflicting results. A team of researchers at the University of California, Berkeley, argues that these experiments are doing more than just exchanging young and old blood.[16] Through their connected circulatory systems, the old mouse benefits from the young mouse's healthy organs to filter and oxygenate blood. The young mouse serves as a living heart-lung and dialysis machine for the old mouse.

The UC Berkeley researchers performed a different experiment: they used computer-controlled pumps to exchange exactly 50% of the blood between a young mouse and an old mouse. Then they shut off the connection and monitored the physiological changes, which were complex. Old mice recovered better from muscle injury, and neuron and liver cell regeneration was inhibited in young mice. The researchers think identifying and removing substances in old blood that cause decline would be a more beneficial treatment than a dose of young blood.

Some startup companies are taking parabiosis research into controversial human clinical trials. For $8,000, anyone over 35 years old can join a trial, run by the startup company Ambrosia, for a one-time injection of young plasma. The study design does not include a control group receiving a placebo, so some aging researchers question the meaningfulness of the research.[17]

III. CARTILAGE

Cartilage degeneration—as a result of injury, inflammation,[18] or aging[19]—contributes to the development of osteoarthritis, resulting in stiff and painful joints, frequently in the fingers, spine, hips, and knees. Once damaged, cartilage remains compromised because it is essentially unable to repair itself.[20] It contains no blood vessels and only a few cells to maintain its matrix of stretchy collagen fibers.

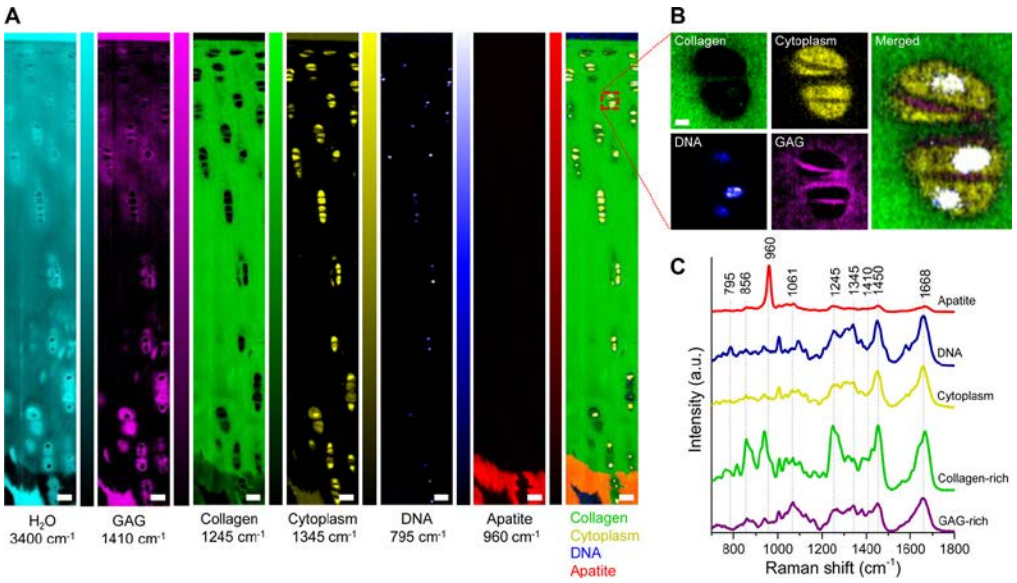
To heal damaged cartilage, orthopedic surgeons scrape the inside surfaces of joints to recruit new cartilage-forming stem cells from the outer layer of bone, but surgical procedures often provide little long-term relief. In the lab, researchers are developing ways to regenerate cartilage by implanting cells or materials that stimulate natural cartilage-forming processes. They are also working to build cartilage in the lab that could be implanted into a person.

Cartilage has few components, so tissue engineers expected it to be easy to recreate in the lab. But they have been unable to create a material with the elastic, stress-resistant, and low-friction properties of cartilage, because they are not yet able to recreate the macro- and microscale structural variations of natural cartilage.

Articular Cartilage

Articular cartilage is the smooth covering on the ends of bones in the hips, knees, and shoulders that helps joints move smoothly. The tissue, a few millimeters thick at maturity, mainly contains a network of collagen protein fibers connected by sugar-covered proteins called proteoglycans and surrounded by water. Only a small proportion of cartilage—approximately 1–5% of its components—are cells known as chondrocytes.[20] During cartilage development, the tissue experiences physical forces that guide the formation of a multilayer structure. Chondrocytes and collagen fibers adopt different orientations and arrangements in each layer. Different fluid flow in each layer also gives the tissue a range of responses to compressive forces. The top layer of cartilage tissue experiences the greatest fluid flow and the highest tensile and compressive forces. Chondrocytes and collagen fibers in this layer arrange themselves in strips that run parallel to the surface of the tissue. The center of the tissue experiences moderate compressive forces and little fluid flow. Chondrocytes dot this layer, and the fibers are randomly oriented. And in the bottom of the tissue, chondrocytes and collagen fibers are aligned perpendicular to the surface of the tissue. Little fluid flows here, and this portion of the tissue experiences strain where it meets the surface of bone.

To better understand the structural complexity of cartilage, Molly M. Stevens, at Imperial College London, and her colleagues used Raman spectroscopy to image each component in the full thickness of cow cartilage.[21]



Raman spectroscopic imaging of articular cartilage. (A) Univariate Raman spectroscopy images of articular cartilage showing the band intensity associated with H_2O (3400 cm–1), GAG (1410 cm–1), collagen (1245 cm–1), cytoplasm (1345 cm–1), DNA (795 cm–1), apatite (960 cm–1), and the overlay. Scale bar: 50 μm. (B) High-resolution (~0.3 μm) Raman spectroscopy image of chondrocytes and pericellular matrix obtained by imaging the GAG (1410 cm–1), cytoplasm (1345 cm–1), and DNA (795 cm–1) against collagen (1245 cm–1).

Scale bar: 3 μm. (C) Representative Raman spectra measured from articular cartilage with marked signatures for specific tissue components.

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In this technique, the researchers shone a green laser on the tissue and used unique vibrations from water, collagen fibers, proteoglycans, the DNA and cytoplasm of chondrocytes, and hard apatite of the bone surface to identify their location in the tissue. The researchers statistically correlated each component with its location in the tissue, and found six compositionally different zones. The relative composition of each component in the tissue did not correlate with the elastic modulus, a measure of a material's stiffness, at different depths in the tissue. The researchers suspected that the physical properties of cartilage were due to its microstructure, not variations in its biochemical composition.

To create a material with the multilayered structure of articular cartilage, Sandra Camarero-Espinosa and E. Johan Foster, both at the University of Fribourg, along with their colleagues, created three layers of porous poly(D,L-lactide).[22]

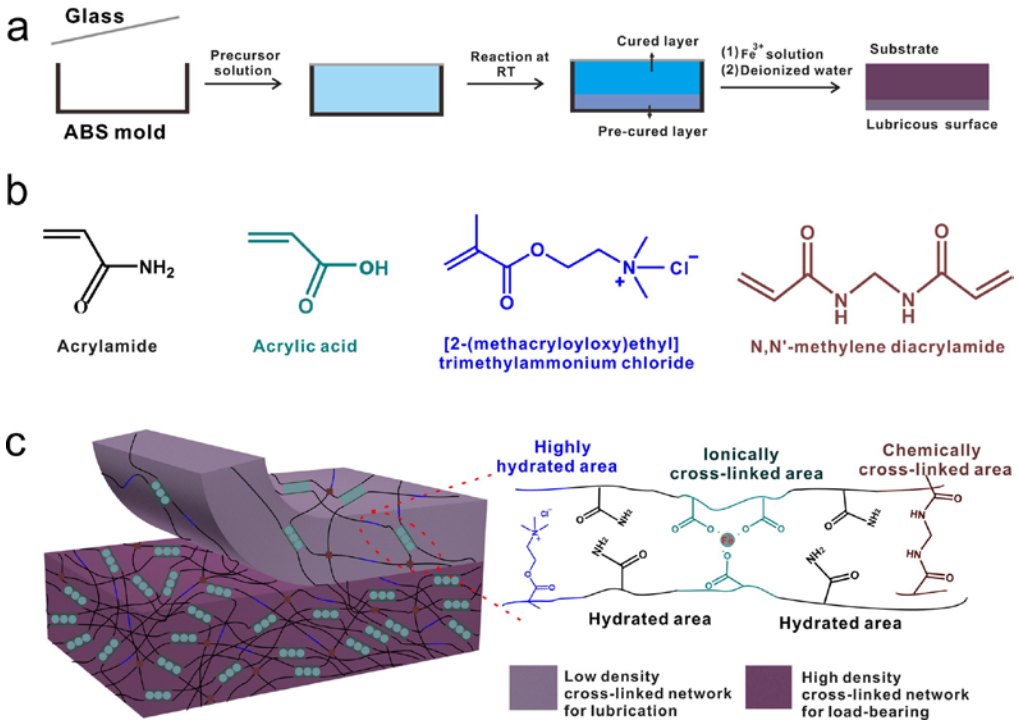
Pores in each layer aligned with the direction of fibers in mature cartilage. In the top polymer layer, which resembles the superficial layer of cartilage, the pores were aligned parallel to the surface. In the middle layer, the pores were not oriented in a specific direction. The researchers also added sulfate cellulose nanocrystals to this layer, mimicking the high percentage of sulfur-containing proteoglycans contained there. Finally, pores in the bottom polymer layer were aligned perpendicular to the surface. The researchers added phosphate cellulose nanocrystals to this layer to promote the formation of hydroxyapatite, the hard material on the surface of bone. They then used solvent welding to bond the three layers together. The result was a material with deep and middle layers both with a thickness of about 2.4 mm, and a superficial layer only 0.2 mm thick. The researchers added human chondrocytes to the polymer scaffold and cultured them for four weeks. The cells formed neocartilage with three distinct tissue structures, and the cell-scaffold construct had a stiffness similar to native tissue.

Advanced materials manufacturing techniques, such as 3D printing, provide researchers more control over the microstructure of engineered articular cartilage by allowing them to vary the pore size or cellular density throughout a material.[23] Liu Yang, at the Third Military Medical University in China, and his colleagues used a 3D printer to create a hydrogel cylinder containing a density gradient of cells, similar to that seen in natural cartilage.[24] The researchers used a collagen-containing ink, containing a known density of chondrocytes, to print a cylinder of gel 6 mm tall. The researchers distributed the cellular ink so that the first 11 layers of the cylinder contained half the total cell density, representing the deep zone of natural cartilage. The middle two layers contained one-third of the total cell density, representing the middle zone of cartilage. The top two layers contained one-sixth of the total cell density, representing the superficial layer of articular cartilage. The researchers set the total cell density of one cylinder to be 1 x 107 cells/mL, which matches the density measured on a human thighbone where it meets the shin bone. After three weeks of culture, cells in this cylinder produced more sugar-covered proteins, called proteoglycans, than did those in printed cylinders containing a higher or lower total cell density.

Along with recreating various aspects of cartilage structure, engineering articular cartilage in the lab is challenging because of the material's mechanical requirements. Any implantable material would need to be able to immediately withstand the compressive forces experienced by an adult body, while also providing lubrication to help joints glide.

To recreate the sliding and compressive properties of articular cartilage, Xiaolong Wang and Feng Zhou, both at the Chinese Academy of Sciences, along with their colleagues, synthesized a hydrogel with one layer that glides on top of another.[25]

The researchers co-polymerized acrylamide, acrylic acid, and [2-(methacryloyloxy) ethyl]trimethylammonium chloride in a 3D-printed plastic box to create a network of interconnected polymers. The polymerization was slower along the bottom and walls of the box than in the center of the mixture, so that when the researchers removed the material from the box, they found a layer of loosely polymerized and well-hydrated material on top of a stiffer material. To further stiffen the material through ionic cross-links, they soaked it in a solution containing iron(III) ions. The hydrogel's maximum tensile strength and compression strength were similar to those of natural bovine cartilage, and the material's coefficient of friction remained less than 0.03 after 72,000 scrubbing cycles with a polydimethylsiloxane ball.



(a) Schematic depiction of the preparation of bilayer hydrogel by a three-step method;

(b) Chemical structure of monomers; (c) Briefly schematic demonstration of bilayer hydrogel containing two layers: low density crosslinked network for lubrication covers on the high density crosslinked network for load-bearing

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Symptoms of aging, such as osteoarthritis, also change the mechanical properties of cartilage. During osteoarthritis, cartilage loses some of the charged sugars that enable it to retain water and resist compression. To restore the water content of damaged cartilage, Mark W. Grinstaff, at Boston University, and his colleagues created a polymer network throughout cow cartilage. The researchers soaked the

cartilage in a mixture of 2-methacryloyloxyethyl phosphorylcholine, which absorbs water and resists compression; ethylene glycol dimethacrylate, a cross-linking agent; and photoinitiating reagents. Then they shone green light on the tissue to trigger polymerization of the components. The combination of synthetic and natural components created a material with compressive strength and wear properties similar to those of natural tissue. Previous methods of using hydrogels to strengthen cartilage only worked at the surface of the material.[26]

Fibrocartilage

Fibrocartilage in the discs that cushion vertebrae and knee joints is the toughest and strongest cartilage in the body. Like articular cartilage, it also has interesting microstructural variations that contribute to its mechanical properties: fibrocartilage contains amorphous regions of proteoglycans interspersed between ordered fibrous domains. Robert L. Mauck, at the University of Pennsylvania; Dawn M. Elliott, at the University of Delaware; and their colleagues wondered if this microstructure influenced how meniscus tissue in the knee joint responds to mechanical strain.[27]

To see if the proteoglycan domains were related to aging or tissue damage, the researchers looked at healthy menisci from fetal, juvenile, and adult cows under a microscope. When they stained the tissue to show the fibrous and proteoglycan-rich domains, they noticed the adult tissue had more proteoglycan-rich domains than the young tissue. The researchers also saw larger proteoglycan-rich domains in knee tissue from donors with arthritis. Next, the researchers wondered how each domain responded to mechanical strain. They built a device to stretch cow meniscus in a confocal microscope, and they imaged changes to the position of fibrous or proteoglycan-rich domains when the tissue was strained. The amount of a domain's movement reflected the amount of strain it transferred to the surrounding matrix. In fibrous regions, about 65% of the applied strain was transferred to the extracellular matrix. But in the proteoglycan-rich microdomains, only 20–25% of applied strain was transferred to the matrix.

While studying the microstructure of fibrocartilage in knee meniscus, Mauck, Elliott, and coworkers questioned if they could recreate the microstructure of fibrous domains and proteoglycan-rich domains in engineered fibrocartilage. The researchers seeded a fibrous scaffold with cells from a cow meniscus as well as stem cells from cow bone marrow. One cell type produced the collagen matrix, the other produced proteoglycans. After growing for eight weeks, proteoglycan-rich domains in the engineered material were similar in size to those in natural juvenile cow meniscus, and the mechanical properties of the engineered tissue matched those of natural tissue.

One strategy to repair degenerated menisci is to build fibrous scaffolds or squishy hydrogels seeded with cells that doctors can implant to template the growth of new cartilage. However, the challenge for tissue engineers is creating a material that is easy to shape, and that also has the desired compression, fluid flow, and mechanical strength. As with articular cartilage, 3D printing provides precise control over the final shape of the implantable material, but inks that work well for 3D printing do not produce materials with the desired mechanical properties. Benjamin J. Wiley, at Duke University, used a 3D printer to make a custom knee meniscus based on medical imaging of the knee joint from an anatomical model.[28] The researchers printed a meniscus for the model using a double network hydrogel made from interconnected networks of polyacrylamide and poly(2-acrylamido-2-methylpropanesulfonate). By varying the composition of the network, the researchers produced a material with compression strength and elastic modulus greater than those in natural cow cartilage.

IV. BONE

Bone tissue naturally breaks down and regenerates itself. But when degeneration happens faster than rebuilding, bones become porous and vulnerable to fractures. Worldwide, about 30% of women and 20% of men over age 50 will experience osteoporotic fractures, commonly of the hip, spine, and forearm.

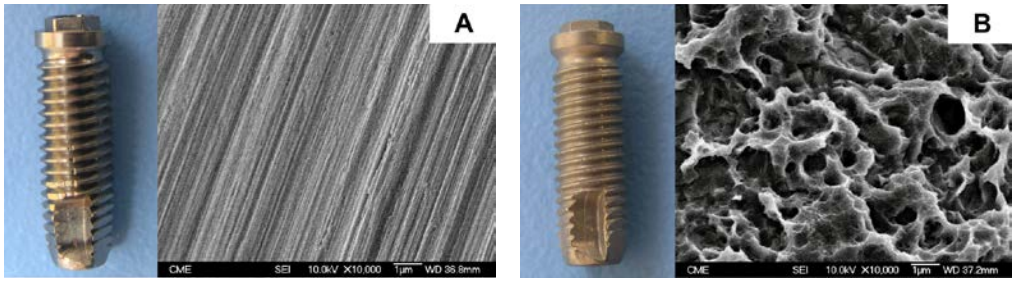
Medicines that maintain bone mass are the most common treatment for osteoporosis, because it's tricky to repair fractures in osteoporotic bones. A broken bone joined with a titanium implant can fail to heal, because forces between the metal pin and bone are too strong for the weakened bone. This can cause microfractures near the interface or prevent the bone from making a smooth interface with the metal implant. Coatings that stimulate bone growth or strong cements surrounding a metal pin can help improve the connection between the bone and an implant. Alternatively, tissue engineers are working to heal fractures with implantable materials that stimulate bone growth.

Coatings

Titanium rods are commonly used to repair broken joints, because titanium has similar stiffness and hardness to the outer layer of bone tissue.[29] Its mechanical properties also make the metal able to support weight. But metal implants do not always form smooth connections with bone tissue. Fibrous tissue between the

implant and the bone can prevent the growth of new bone cells. Over time, bones repaired with metal rods can fracture or get infected.

To help the body incorporate an implant, researchers are developing coatings for titanium rods that support new bone growth or prevent infection. The simplest way to change the rod's surface is etching the metal with acid or sandblasting. In clinical studies, titanium implants with micro-roughened surfaces integrated into new bone better than smooth implants, shortening healing time.



Surface treatment. Gross appearance and surface electron microscopy (SEM) images showing micrometric surface structure of (A) machined, turned implant and (B) sand-blasted and then acid-etched implant. Note the differences in surface roughness at the micrometric level.

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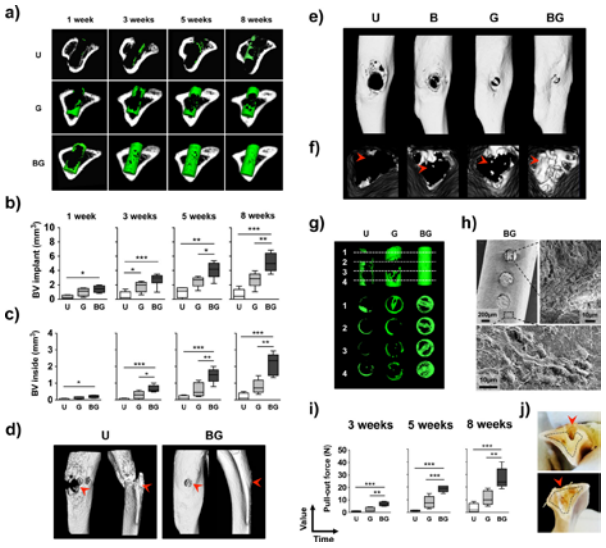
Commercially available coated metal implants contain a layer of calcium phosphate or bioactive glass to encourage bone growth. Calcium phosphate resembles hydroxyapatite, the mineral naturally produced by bone during the healing process. This coating helps new bone cells grow at the bone-implant interface, but it does not stimulate the cells to differentiate into the various types needed for healthy bone.

Bioactive glass, on the other hand, helps new bone grow and differentiate.[30] The most common "bioglass" is made from a mixture of CaO, SiO_2, Na_2O, and P_2O5, and it is used clinically for bone grafts and dental implants. In the body, bioglass bonds to bone, forming a bilayer of hydrated silica and polycrystalline hydroxy carbonate apatite that induces and speeds bone cell growth. The carbonate apatite enhances adsorption of growth factors that help cells flourish. As bioglass degrades, it releases soluble silica and calcium ions, which are cues for cellular growth and differentiation. For this ionic release to impact cell growth, its timing must overlap with the progression of cellular growth.[30] A burst of ions released before cells have developed enough to accept the ionic signals is wasted. To control the degradation rate of bioglass, researchers are adding borosilicate to the recipe. Borosilicate bioglass can also contain additional ions that help bone cells grow.

A team led by João S. Fernandes and Ricardo A. Pires, both at the University of Minho, synthesized borosilicate bioglasses containing calcium, magnesium, or strontium ions.[31] After three days immersed in simulated body fluid, hydroxyapatite formed on the surface of each material, yet each one released ions at different rates. For the strontium-containing material, a burst of ions released after one day corresponded to an increase in solution pH, which the researchers suspected could be associated with the intrinsic antibacterial properties observed in other bioglass coatings. To test the antibacterial activity of their materials, the researchers placed a glass disk containing each material in a petri dish of bacteria. The strontium borosilicate bioglass was particularly effective at killing *Pseudomonas aeruginosa*; cell death was seen using a disc containing 18mg/mL of the bioglass, the lowest known concentration of any bioglass to kill bacteria.

Instead of inorganic ceramics as coatings for metal implants, organic polymers are also an option. Organic polymers can be shaped to mimic fibers of the extracellular matrix or tailored to have physical properties similar to cartilage or the spongy interior of bone. Another advantage of organic polymers is that they can be decorated or filled with molecules that kill bacteria or stimulate bone formation. Paula T. Hammond, at the Massachusetts Institute of Technology, and her colleagues designed a polymer coating containing multiple layers of an antibiotic and a bone growth factor, a protein called BMP-2.[32]



µCT imaging and quantification of bone healing and bone-implant integration.

(a) Radiographs and 3D reconstruction of new bone around implants at 1, 3, 5, and 8 weeks after revision. (b–c) Quantitative healing indices derived from µCT measurements: bone volume around implants and inside the channels (using cylindrical ROIs in A). BMP-2 was critical for good healing. n = 4 per group. (d) In severe case of the untreated, substantial bone destruction led to bone fracture. (e) 3D images of front views, (f) top-down views in marrow space, and (g) implants show complete bone deposition on the BG. (h) SEM images of the BG coated implants pulled out at 8 weeks post-revision demonstrate tissue infiltration. (i) Box-and-whisker plot shows mechanical pullout force at predetermined time points, n = 4. (j) Bright-field images of excised tibiae treated with BG at 5 weeks post-revision. Red arrows denote implant sites. *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA with Tukey post hoc test.

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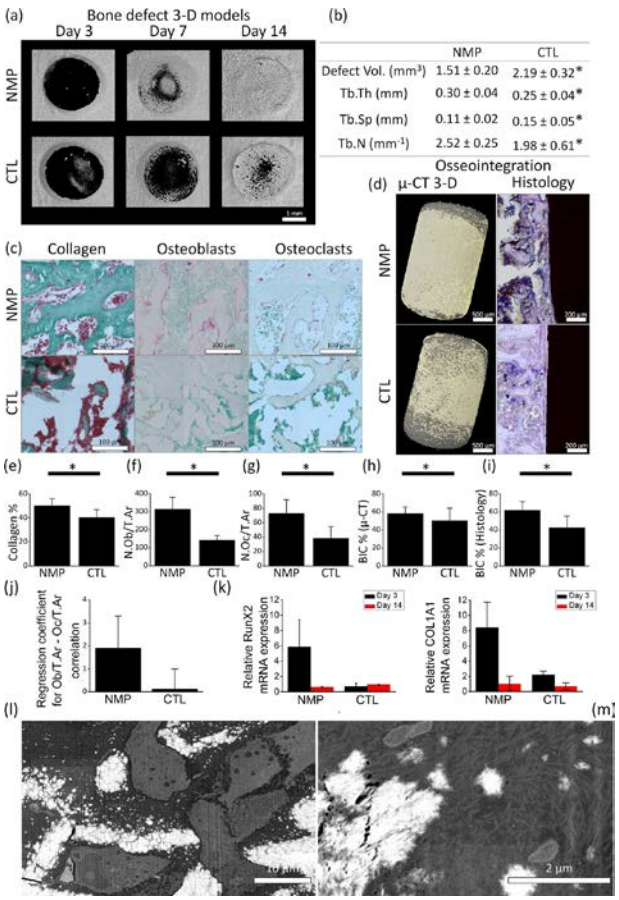
They measured the release profile of each molecule in solution, noticing a 60 µg burst of antibiotic after one day, followed by sustained release of 1 µg/day for the next 40 days. BMP-2 release followed a slower trajectory: 110 ng released each day for six days, followed by 13 ng for the remaining 34 days. Next, the researchers induced bacterial infection in the shinbone of rats and surgically placed coated and uncoated polymer implants in the bone. After eight weeks, animals who received untreated implants had severe infection and bone damage. Over the same time period, animals with coated implants had healthy bone tissue growing on and around them, and their shinbones were 15 times stronger than those in untreated animals.

Cement

To seal metal pins in bone, or just fill gaps in damaged bone, surgeons implant bone cement. The most common bone cement is a paste of calcium phosphate microparticles that mimics the natural calcium mineral in bone. After implantation, crystals precipitate out of the paste and form a solid material. There are two common formulas for calcium phosphate cements, but they both have limitations. One hardens quickly, speeding healing time, but has 25% the strength of outer covering of bone. The other formula requires several hours to set into a material about 40% as strong as bone. However, magnesium phosphate cements offer better strength, setting time, and resorption rate than calcium phosphate-based materials.[33] Similar to calcium ions, magnesium ions are important to increasing bone density, cell adhesion, and stability. Magnesium phosphate cements can be up to 65% as strong as bone, and some formulas have inherent antibacterial activity. One magnesium phosphate cement, made by the Texas company Bone Solutions, Inc., is approved for use by U.S. regulators.

By tuning the ingredients in magnesium phosphate cement, researchers have more control over the time it takes the material to harden, compared to hardening rates for calcium phosphate cements. This control enables them to explore producing injectable materials, a feature that would simplify bone repair and that few calcium-based cements have.[33] A team led by Jake E. Barralet and Faleh Tamimi, both at McGill University, made an injectable hydrogel containing 2D sheets of nanocrystalline magnesium phosphate.[34] When they are suspended in water, positive and negative charges on the outside of the sheets helps them form a thixotropic gel that stiffens under pressure and returns to a gel when the pressure is released. The researchers tailored the composition of the ingredients—sodium hydroxide, magnesium hydroxide, and phosphoric acid—to produce a material that flowed through a needle as easily as water. The material stiffened after injection into the



NMP accelerates bone healing and implant osseointegration. (a) μ-CT 3D models of the bone defects at days 3, 7, and 14 show enhanced bone healing among NMP-treated defects. (b) μ-CT analyses show smaller defect volume, less trabecular separation (Tb.Sp), more trabecular thickness (Tb.Th) and trabecular number (Tb.N) in NMP-treated defects. (c) Maison trichrome stain shows more collagen (e), ALP stain shows more osteoblasts (f), and TRAP stain shows more osteoclasts (g) in NMP-treated defects. (d,h,i) μ-CT 3D models and coronal histological sections of Ti-implants show more bone (yellow in μ-CT and pink in histology) in contact with the implant in the NMP-coated implants. (j) The regression coefficient of the correlation between N.Ob/T.Ar and N.Oc/T.Ar shows that the high number of osteoclast in NMP samples was promotionally correlated to an even higher number of osteoblast (r = 0.56); this correlation was not observed in the control sites (r = 0.08). (k) qRT-PCR shows that the expression of COL1A1 and RunX2 were up-regulated in NMP treated defects at day 3 compared to the control, however, no significant difference was observed at day 14. (l–m) FIB images showing bone matrix (l) and collagen fiber (m) undergoing mineralization by osteoblasts in NMP-treated defect at day 7. Statistical analyses were assessed by two sample student t test and accepted as statistically significant at $p < 0.05$*.

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shinbone of a rat, helping to almost completely fill the defect with new bone within 14 days after implantation.

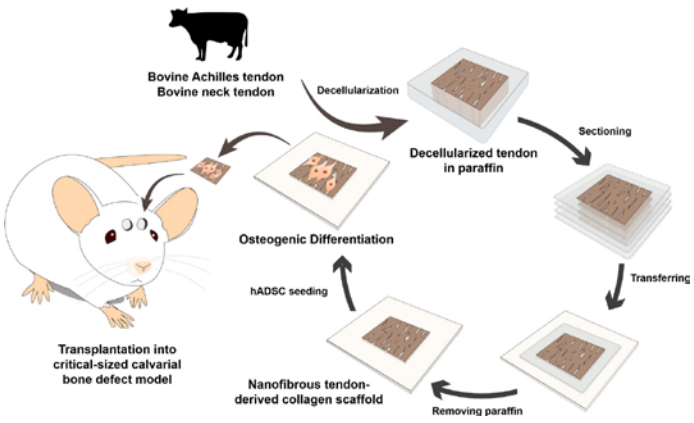
A team led by Sarah L. Kieweg and Cory J. Berkland, at the University of Kansas, wanted to make an injectable bone cement using only natural materials. The researchers sought to use a slurry of nanoparticles, rather than microparticles, because they thought it would be easier to tune the material's flow properties with different additives. The increased surface energy of nanoparticles gives them a higher affinity for additives than that of microparticles. The researchers made a gel containing nanoparticles of hydroxyapatite, a hard mineral in bone, and hyaluronic acid, a sugar in cartilage.[35] They then added microparticles of human demineralized bone matrix and decellularized cow cartilage, to include components of extracellular matrix that might encourage bone growth. Including the extracellular matrix components improved the flow properties of the colloidal gel, compared to

a gel containing only the nanoparticles. Preliminary biocompatibility tests showed that the material appeared to promote cell viability, and the researchers planned to study the material's impact on bone regeneration processes in cells.

Grafts

One challenge with using implants to join fractures is that the pins physically block the growth of new blood vessels, thus hindering healing. To help bone heal naturally, doctors also seal a break by implanting tissue harvested from a patient, a cadaver, or a different animal. Using a patient's own cells, or autografting, usually results in fewer complications from immune system rejection. But this process requires a second operation to harvest tissue for the graft.

To simplify autografting, tissue engineers are building 3D materials that help stem cells isolated from fat or bone marrow develop into healthy bone tissue. They generally combine two types of stem cells: endothelial cells that can become blood vessels, and mesenchymal stem cells that can become bone cells. Each of the two types of cells helps the other develop properly, and new blood vessels help the implant integrate into existing tissue. In the body, stem cells naturally attach to and grow along a porous network of collagen fibers called the extracellular matrix. To recreate this physical structure, tissue engineers use various types of 3D scaffolds as templates for stem cell growth. One scaffold involves using natural extracellular matrix from animal tissue, modified to remove the cells and leave the fiber network behind. Then researchers seed human stem cells onto the decellularized matrix. Qiaobing Xu, at Tufts University; Seung-Woo Cho, at Yonsei University; and their colleagues used thin sheets of decellularized cow tendon as a material for bone regeneration.[36]



Schematic illustration to describe the fabrication of nanofibrous tendon-derived scaffolds and hADSC transplantation using fabricated tendon scaffolds in a critical-sized calvarial bone defect.

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They seeded the scaffold with human stem cells derived from fat and waited seven days for the cells to become

bone cells. They then implanted the material into a hole in mouse skull and secured it in place using glue that contained factors to stimulate bone and blood vessel growth. Eight weeks after transplanting the cell-seeded tendon material, 86% of the bone had regenerated in the hole. When the scientists placed cells seeded on a commercially available collagen-hydroxyapatite scaffold, only 52% of bone regenerated. To grow bone and blood vessels at the same time, the researchers stacked alternating tendon sheets seeded with stem cells for either bone or blood cells. Eight weeks after implantation, they removed the material, soaked it with antibodies that label bone and blood vessels, and found layers of bone and vascularized tissue using fluorescence microscopy.

In another study, instead of using natural scaffolds to provide physical cues for cell growth, Hae-Won Kim, at Dankook University, recreated scaffolds using polymer nanofibers infused with bioglass nanoparticles.[37] Silicon and calcium ions released during bioglass degradation help improve bone and blood vessel growth. The researchers seeded the material with blood vessel cells and implanted it under the skin of rats. After two weeks, they found new blood vessels growing in the material.

A final way to help stem cells develop into bone is to use nanoparticles to recreate the mesoscale of bone tissue building blocks, instead of the microscale of the extracellular matrix. The basic unit of bone is an osteon, which has thin layers of bone tissue encircling a central channel filled with blood vessels. Rings of osteons form bone tissue. To create an osteon-like subunit, Hongsong Fan, at Sichuan University, and his colleagues added human blood vessel stem cells to collagen microspheres patterned with human bone cells similar to those that make new bone.[38] Next, they added gelatin microspheres to link the cell-seeded collagen spheres into a mesoscale tissue structure. The researchers envisioned that the gelatin spheres, when implanted in the body, would quickly degrade and leave space for new blood vessels to grow. Meanwhile, an enzyme preloaded into the collagen microsphere would degrade the collagen, creating space for bone and blood cells to grow. The researchers implanted the mixture under the skin of mice. After eight weeks, they used fluorescence microscopy to identify blood vessels and bone tissue. When the implant contained collagen spheres pre-seeded with cells, new tissue formed in what used to be the center of the sphere. But when the researchers implanted collagen spheres and cells, without pre-seeding the cells in the spheres, they only saw new tissue growing on the outside of the spheres.

V. HEART DISEASE

Cardiovascular disease accounts for 40% of deaths in people aged 65 and older,[39] and is the leading cause of death worldwide. Risk factors for heart disease include stress, smoking, family history, high blood pressure, and cholesterol plaques collecting along artery walls. The symptoms of heart disease can be sudden and dramatic. Plaque buildup narrows the space for blood to flow through arteries. Completely clogged vessels stop blood flow to a portion of the heart, causing cells in that region to die and triggering a heart attack. Human heart tissue has limited ability to regenerate itself, so the damaged tissue alters heart function and contributes to heart failure. Most treatments for heart disease work to reduce risk factors with lifestyle changes like diet and exercise, and lower cholesterol or blood pressure with medicine. In the lab, researchers are working to repair the impacts of heart disease by regenerating damaged heart tissue.

Cardiac Regeneration

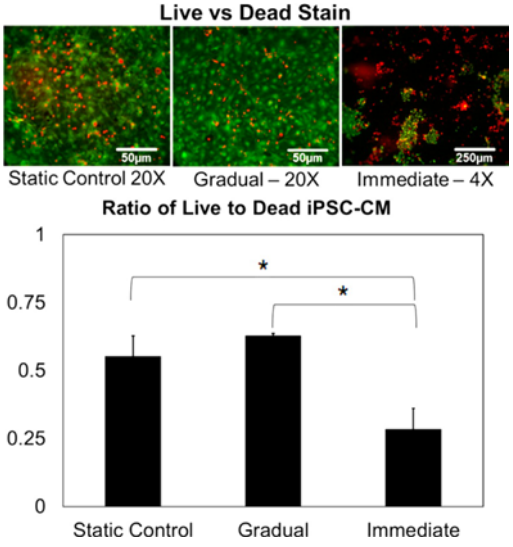
While heart muscle cells can slowly regenerate themselves into adulthood, their regeneration potential following a heart attack or other damage is not well understood. Heart transplants are a procedure of last resort for patients with severe heart failure, but the availability of donor organs limits how many patients can receive this treatment. One route to universal treatments would be to heal damaged heart tissue using a patient's own cells.

Over the past ten years, scientists have discovered a variety of small molecules that encourage the growth of undamaged heart muscle cells, or that stimulate stem cells or cells from the membrane surrounding the heart to form heart muscle cells.[40] Small molecules can also be used to reprogram scar tissue formed after a heart attack to produce heart muscle cells instead. However, only a few of these molecules have advanced to in vivo experiments. Often, these drugs target metabolic pathways that improve cellular regeneration in vitro, but can trigger cancer development in vivo. However, researchers expect more molecules to advance to in vivo tests as they discover new pathways to target, and modify known molecules to have desirable pharmacological properties.

One challenge with testing cardiac stem cells in animal experiments is getting the cells to survive and integrate into existing tissue. In addition to chemical cues, heart muscle stem cells need electrical and mechanical cues to form the proper shape and automatic contraction that generates a heartbeat. To understand how electrical cues help diseased heart cells regenerate, a team led by Mehdi Shamsara, at the

National Institute of Genetic Engineering and Biotechnology in Iran, and Mohammad Massumi, at the University of Toronto, prepared two 3D scaffolds of polymer fibers made from poly(ether sulfone) and polyaniline, an electrically conductive polymer.[41] One scaffold had fibers oriented in the same direction, while the other had randomly oriented fibers. The researchers collected skin stem cells from a patient with heart disease, induced the cells to become pluripotent stem cells, and introduced the cells to the scaffolds. They placed each scaffold inside a bioreactor containing chambers with electrodes. The researchers then delivered electrical pulses through the chamber for one hour a day for 15 days, finding that cells grown on the scaffold with aligned fibers differentiated into heart muscle cells more efficiently than those growing on the scaffold with random fibers. The researchers suspected the aligned fibers provided unidirectional electrical stimulation, similar to that experienced during natural heart development.

Simultaneous electrical signals generate muscle contractions that contribute to a regular heartbeat. Each heartbeat pumps blood into and out of the chambers of the heart, and this fluid flow generates mechanical forces on the muscle cells. Palaniappan Sethu, at the University of Alabama at Birmingham, and colleagues wondered how these mechanical forces affected the growth and development of cardiac stem cells under simulated in vivo conditions. The researchers built a chamber that held a 3D culture of heart muscle stem cells. By pumping fluid through the culture chamber in a circulatory loop, they mimicked the fluid flow in the left ventricle.



(Top) Live (green)/dead (red) staining performed on iPSC-CMs following gradual and immediate application of hemodynamic load. The lower magnification image of the immediate sample was required to reliably quantify the increased cell loss experienced within the sample.

(Bottom) Ratio of live to dead cells shows statistical significance between immediate and other samples ($p < 0.05$, $N = 3$). No statistical significance was found between the static and gradual samples ($p > 0.05$, $N = 3$).

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To simulate the conditions of damaged heart tissue, the researchers flushed the culture chamber with serum containing few nutrients, and noticed that many cells died. But when they flushed the culture chamber with fluid that had high nutrients, the

stem cells responded similarly to those growing in a static culture, indicating that the extra nutrients helped the stem cells survive the mechanical stress from circulation.[42] The highest number of cells survived when the researchers gradually increased the mechanical stress by gradually increasing fluid flow over two days, compared to immediately delivering a fluid force that cells would experience inside the heart.

VI. CANCER

The risk of receiving a cancer diagnosis increases with age, and one of the main biological processes involved with aging could also be linked with cancer. During senescence, cells stop dividing when their genetic information experiences damage that could make the cells cancerous. Senescent cells do not die, although they announce their status change by producing chemical signals that travel to neighboring cells, perhaps to trigger repair and immune responses. In young cells, the process is thought to keep cancer from spreading. But as senescent cells accumulate with age, the chronic presence of their secretory signals can also stimulate tumor growth.

Aging, senescent, and cancerous cells have two things in common: damaged DNA and unstable genomes.[43] The explosion of genetic sequencing technologies over the past decade now provides insight into this genetic variation. With that knowledge, scientists look to use a tumor's genetic information to deliver personalized cancer treatment. They also look for new ways to grow tumors in the lab so that they can study how genetic variation influences the effectiveness of chemotherapy. Another form of personalized cancer treatment, engineering a patient's immune system to attack cancer cells, has repeatedly eliminated some forms of leukemia and lymphoma during clinical trials. However, concern over side effects, and even deaths, during the trials are driving researchers to reduce the treatment's toxicity while maintaining its effectiveness.

Personalized Cancer Treatment

Over the past decade, improvements in sequencing technologies have increased the amount of genetic information available. Sequencing the 3 billion bases of the human genome, which first took a decade, can now be done in a few days at a 50,000-fold drop in cost.[44] With increased access to genetic information come new ideas about how to use it to improve health. Government initiatives in the United States, European Union, United Kingdom, China, and Australia aim to develop ways to use genetic information to help doctors provide personalized treatment

for various diseases. With some cancers, for example, genetic sequencing can indicate that particular treatments will or will not be effective.[45]

Genetic changes are a hallmark of cancer cells, and these changes can be highly varied within one tumor as well as different tumors of the same type. Typical solid tumors have 30 to 70 mutations that turn the cells cancerous, in addition to many others that occur during natural cell aging.[46] Tumor-causing mutations include extra copies of genes, repetitive DNA sequences, or large sections of chromosomes. In a particular gene, one of the bases may be changed or modified with a methyl group. In one tumor, some mutations may confer resistance to treatment, while others make cells more susceptible to a drug.[45] To study this variation, researchers sequence the genome from many individual cells, looking for unique changes as well as shared changes that allow them to categorize cells into various groups by their mutations.[47] However, getting meaningful data is a challenge for single cell sequencing. Errors can be introduced during cell isolation, genome amplification of each cell, and data interpretation.

A team led by X. Sunny Xie, at Harvard University, developed a method to improve the accuracy of genome amplification.[48] Typical approaches to this step can preferentially amplify certain regions of the genome, resulting in an incomplete genome after sequencing. In this new method, the researchers randomly inserted pieces of DNA called transposons. These transposons enabled linear, rather than exponential, amplification. With this method, the researchers could identify micro copy-number variations, additions or deletions of portions of the genome smaller than 100,000 bases, with a resolution of 10,000 bases.

Despite the variety of ways to obtain genetic information about tumors, for this information to be most useful in the clinic, one oncologist believes that it also needs to be linked to a patient's clinical history, including information such as tumor grade, type of relapse, and tumor stage at diagnosis.[49]

Organoid Models

To catalogue genomic mutations linked to cancer, a research project sponsored by the National Institutes of Health started to sequence lung, brain, and ovarian cancers in 2006. When active sequencing for the Cancer Genome Atlas ended eight years later, the project had sequenced more than 10,000 tumors from 33 types of cancer, and identified millions of cancer-causing mutations.[50] The Atlas remains the largest collection of tumor data analyzed for genomic information. To confirm that a genomic mutation identified in the Atlas causes cancer, researchers either intentionally engineer the mutation into healthy cells to see if they turn cancerous,

or they look to reverse cancerous processes in cells by treating tumor tissue with genes that target the intended mutations. This means researchers test cells in a dish, or use animal models either implanted with tumors or engineered to be living models for particular cancers. However, both of these methods have experimental limitations.

Traditional cell culture is riddled with variability.[51] To improve their research tools, international research organizations are collaborating to create 1,000 new cancer cell models that better resemble the variety in tumor type and architecture than current cell models do.[52] However, growing cells in a dish does not recreate the 3D structure of natural tissue. Studying cancer in model organisms such as mice places the tumor in context of all the other physiological systems, but animal studies are expensive and time consuming. A new, different way of growing cells in the lab could combine the benefits of both cell culture and animal models. Mini-tissues called organoids, grown from mouse or human stem cells, have 3D structure, differentiation patterns, and cellular architecture resembling organs such as the brain, liver, kidney, prostate, taste bud, and retina.[53] The intestine organoid was the first to be developed in 2009.[54] To make this organoid, researchers isolated mouse intestinal stem cells and cultured them in Matrigel, a protein matrix produced by mouse sarcoma cells, which was supplemented with chemical cues to guide cell differentiation. The stem cells formed a hollow cyst of tissue that developed folds and ridges resembling the structure of natural intestinal lining. The organoid also contained all four types of epithelial cells found in natural intestinal tissue.

In 2015, Toshiro Sato, at Keio University School of Medicine, and his colleagues engineered organoids to be models for colorectal cancer.[55] The researchers started with colorectal organoids derived from healthy cells, and used CRISPR/Cas-9 gene editing to change five genes commonly mutated in colon cancer. Organoids that expressed all five mutations grew like cancer cells in vitro, and produced tumors when implanted in the kidney capsule of mice.

Organoids can also be a living repository of cancer genomes. A team led by Hans Clevers, at the Royal Netherlands Academy of Arts and Sciences, and Mathew J. Garnett, at Wellcome Trust Sanger Institute, collected colon cancer cells, as well as healthy intestinal cells, from 20 patients and synthesized organoids from each group of cells.[56] The researchers sequenced the genomes from cells in each tumor and healthy organoid. The most common mutations they found were also those identified in the Cancer Genome Atlas for colorectal cancer. To test the tumor organoids' drug sensitivity, the researchers screened them against 83 drugs in clinical use, undergoing clinical trials, or in experimental development. They identified compounds that inhibited cell growth and statistically correlated the protein known to be targeted by the drug to mutations in each affected organoid. The researchers were also able to use the organoids to confirm the effects of drugs

in clinical use and under development. They concluded that organoids could be used to guide patient-specific treatment within weeks after harvesting the cells.

Cancer Immunotherapy

Personalized cancer treatments are attractive as a way to target tumors that can be difficult to treat. Along with using genetic information to guide treatment decisions, another way to personalize some cancer treatment is to harness the power of a patient's own immune system. For patients with some forms of leukemia and lymphoma, cancer immunotherapy with engineered T-cells caused their cancer to completely disappear. However, toxic side effects, and several deaths, during clinical trials are leading researchers to reduce the treatment's toxicity without sacrificing its efficacy.

T-cells naturally cruise through the body looking for foreign substances. If the cells find something suspicious, they can kill cells directly or they can recruit other immune cells to help. Cancer, however, evades T-cell recognition by modulating checkpoint proteins that naturally deactivate T-cells—a mechanism that normally prevents them from damaging healthy tissue.

To recruit T-cells as cancer killers, researchers engineer them to express chimeric antigen receptors (CARs).[57] A CAR has three protein components: a target-binding domain, typically derived from an antibody, presented outside the cell; a hinge that anchors the receptor in membrane; and several domains dangling inside the cell involved with intracellular signalling and activation. When a CAR T-cell encounters cancer, its antigen recognition fragment binds to surface markers on the cancer cell. That binding stimulates T-cells to multiply and secrete chemicals that kill and lyse cancer cells. To make CAR T-cells, doctors first draw blood from a patient, remove the T-cells, and send them to a manufacturing lab. In the lab, scientists introduce a viral vector containing genetic instructions to make a CAR and allow the engineered cells to proliferate for several days. Before receiving a transfusion of engineered cells, patients get chemotherapy to reduce white blood cells and increase chances their body will accept engineered cells.

In 2011, a team led by Carl H. June, at the University of Pennsylvania's Abramson Cancer Center, gave CAR T-cells to three patients with advanced chronic lymphoid leukemia who had few treatment options left. After CAR T treatment, their cancer essentially disappeared. Since then, several CAR T clinical trials treating acute lymphoblastic leukemia in children, young adults, or adults has resulted in 70-90% of patients going into remission.[58] However, the clinical trials also revealed dramatic side

effects, including neurological toxicity and inflammation and fever associated with cytokine release syndrome. In some CAR T trials, patients died from brain swelling.

To improve CAR T therapy, researchers are adding a second recognition receptor to help the engineered T-cells better recognize cancer cells over noncancerous tissue. Biotech companies developing CAR T therapies are adding "suicide switches" into CAR T-cells that trigger the engineered cells to self-destruct in the presence of a small molecule. If side effects become too severe, doctors can administer the small molecule to kill the engineered CAR T-cells.[59]

VII. DEMENTIA

Dementia describes a variety of chronic or progressive brain diseases that impact memory, behavior, and a person's ability to do daily tasks. Although dementia is not a normal part of aging, the risk of developing it increases with age, and the social impact of dementia is growing worldwide. By 2018, the cost of dementia is expected to reach $1 trillion,[60] and the number of cases worldwide is expected to triple by 2050.

There is no treatment for any form of dementia, which is linked to two different physical processes. First, small strokes can injure blood vessels feeding the brain. Also, certain proteins forming clumps and tangles in the brain are thought to cause diseases such as Alzheimer's or Parkinson's. More than 40 million people worldwide live with Alzheimer's,[60] the most common form of dementia. Although a troubled history of failed clinical trials haunts Alzheimer's drug development, academic and industrial researchers continue to search for medicines that can stop or reverse neurodegeneration.

Amyloid Hypothesis

The 25-year-old hypothesis that clumps of amyloid-β protein (Aβ) coating neurons initiate Alzheimer's disease is the prominent theory driving Alzheimer's drug research.[61] Following this hypothesis, researchers work to stop amyloid proteins from forming plaques. But many clinical trials for anti-amyloid drugs have failed, some in late stages, due to a lack of noticeable effect. Since the amyloid hypothesis is still subject to debate,[61] some see these failures as evidence that discredits the theory.

One big blow for Alzheimer's drug research came in 2012, when several clinical trials for high-profile drugs failed, often due to problems with study design.[62]

Some patients in the trial were misdiagnosed with Alzheimer's, while others had disease too advanced for the drug to have an effect. At that time, a lack of clear imaging tools and biomarkers for the disease also prevented researchers from tracking the effect clearly. Now, current clinical trials for a drug developed by Biogen aim to tackle those challenges. A company executive said that they wanted to ensure that all participants in the Phase I trial had Alzheimer's disease, and that the trial is the first to require positron emission tomography (PET) scans to confirm reductions in plaque accumulation.[62] Biogen's drug is an antibody called aducanumab that binds Aβ and prevents it from accumulating in the brain. In a Phase Ib trial of 166 people, the drug significantly reduced the amount of plaque in brains of people with early-stage Alzheimer's. The results were impressive enough to allow the drug to progress directly to two Phase III trials.

Another approach to drug design aims to prevent plaque formation by inhibiting an enzyme called β-secretase (BACE), which trims a larger protein into Aβ. Early BACE inhibitors also inhibited other proteins, although these toxicity and safety issues were improved with later versions of the drugs. However, this anti-Alzheimer's tactic has not fared well in clinical trials either: BACE inhibitors produced by Merck[63] and Lilly[64] have failed Phase II clinical trials in the past few years.

A growing focus for Alzheimer's treatment is on molecules that target tau, a protein that forms tangled fibers inside neurons. The accumulation of amyloid plaques on the outside of neurons triggers the formation of tau tangles inside the cells. As the disease progresses, at some point, tau tangles spread quickly between neurons. Targeting tau tangles is difficult because they accumulate inside cells, not outside like amyloid plaques. Timothy M. Miller, at Washington University in St. Louis, tested an anti-tau therapy produced by Ionis Pharmaceuticals. The drug is 20 nucleotides of antisense RNA that bind to mRNA that codes for the tau protein, preventing protein synthesis. In mice, injections of the antisense RNA significantly lowered levels of tau in the brains and cleared pre-existing tau tangles.[65]

Despite the variety of approaches to developing anti-Alzheimer's drugs, the difficulties with clinical trials has led investors to be wary of supporting the research. One reason is that to get to market, Alzheimer's drugs have to improve both cognition, such as learning and memory, and function, such as activities of daily living. However, changes to cognition and function happen at different times as Alzheimer's progresses. In the early stages of the disease, cognition declines before function, and by the time impaired function is apparent, the disease may be too advanced for drugs to work. To speed the process of developing effective anti-Alzheimer's drugs, a group of researchers and patient advocates argue that regulators need separate categories for drugs that improve cognition, and those that improve function.[66]

Non-Amyloid Treatments

Other experimental approaches to Alzheimer's treatment sidestep Aβ accumulation and look to treat other symptoms of the disease, such as neuronal death and inflammation. Along with accumulating outside neurons, Aβ also sneaks into cells and infiltrates mitochondria, the organelles that produce energy for the cells. For unknown reasons, Aβ intrusion causes the mitochondria to produce fewer energy molecules and more reactive oxidants, leading to neuronal death.

To interrupt this process, a team led by Taeghwan Hyeon, at the Institute for Basic Science, and Inhee Mook-Jung, at Seoul National University, wanted to fill mitochondria with antioxidant molecules. The researchers chose ceria nanoparticles because they can degrade both superoxide and hydrogen peroxide, the two most common reactive oxygen species overproduced in Aβ-impacted mitochondria. Previous antioxidant molecules that targeted mitochondria only neutralized hydrogen peroxide. The researchers injected ceria nanoparticles, coated with positively charged triphenylphosphonium to help them enter mitochondrial membranes, into four-month old mice with Alzheimer's gene mutations. Two months later, when the mice would normally experience advanced Alzheimer's disease, they examined slices of mouse brains under a microscope. Treated mice had 40% more neurons than untreated mice, though they still had fewer neurons than healthy mice did.[67] More studies are needed, the researchers said, to identify why the nanoparticles prevent neuronal loss.

Altering Brain Wave Patterns to Treat Alzheimer's

Neurological disorders such as Alzheimer's often disrupt brain waves called gamma waves that oscillate from 60 to 90 Hz. To learn how this change related to the disease, a team of researchers led by Li-Huei Tsai, at the Massachusetts Institute of Technology, restored the waves in the brains of mice engineered to produce excess Aβ.[68] To control brain wave patterns in these mice, the researchers engineered particular brain cells, called interneurons, in the mice to express light-activated ion channel proteins. A fiber optic cable implanted in the mouse brains sent flashes of light that triggered the ion channel proteins, causing the engineered nerve cells to fire and communicate with other neurons to generate oscillations of brain activity. The researchers found that inducing gamma waves at 40 Hz significantly reduced Aβ levels in the mice, and that the Aβ accumulated in brain immune cells called microglia. To test a less-invasive method of inducing oscillations, the researchers had the mice watch a light flickering at 40 Hz for one hour a day for a week. Compared to mice that sat in the dark, these mice had fewer plaques in their brains. The researchers think re-establishing the disrupted gamma waves stimulated immune cells to clear plaque.

VIII. CONCLUSION

Aging, and the associated decline in health is inevitable. But we may have more control over the process than we may think. Lifestyle choices, particularly in midlife, can help us live healthier longer. Regular exercise helps patients with arthritis, heart disease, balance problems, and high blood pressure. A diet rich in whole foods and avoiding smoking and alcohol also reduces the risk of cancer and dementia.

Elizabeth Blackburn, at the Salk Institute for Biological Studies, and Elissa Epel, at the University of California, San Francisco, say specific food, sleep, exercise, and stress reduction habits linked to longer telomeres indicate the best choices to forestall cellular aging.[69] Stressful situations linked to shortened telomeres indicate an increased potential for senescent cells. Accumulations of senescent cells increase inflammation, a risk factor for any age-related disease.

Along with diet and exercise, changing our mindset to reduce stress and fostering relationships with others are actions that induce our cells to lengthen telomeres. Long, stable telomeres at old age seem to reduce the number of years a person experiences ill health due to diseases of aging. Although the molecular basis for aging is more complex than telomere length, the concept points to a new paradigm for aging: increasing healthspan to enjoy an increased lifespan.

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