Polymers in Tissue Engineering

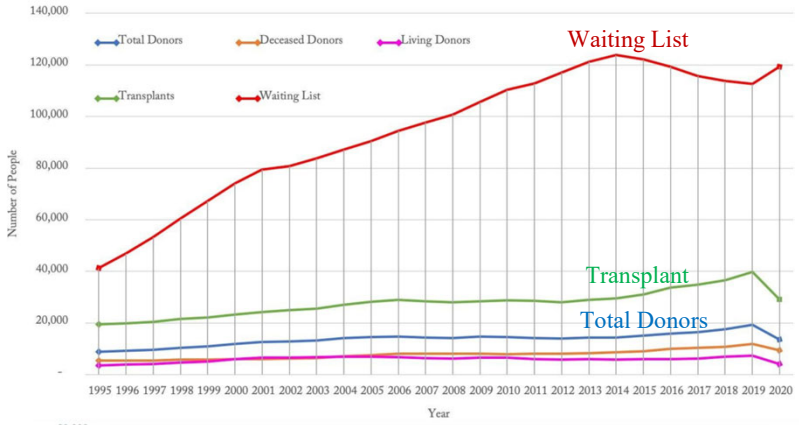
Reading with Goals

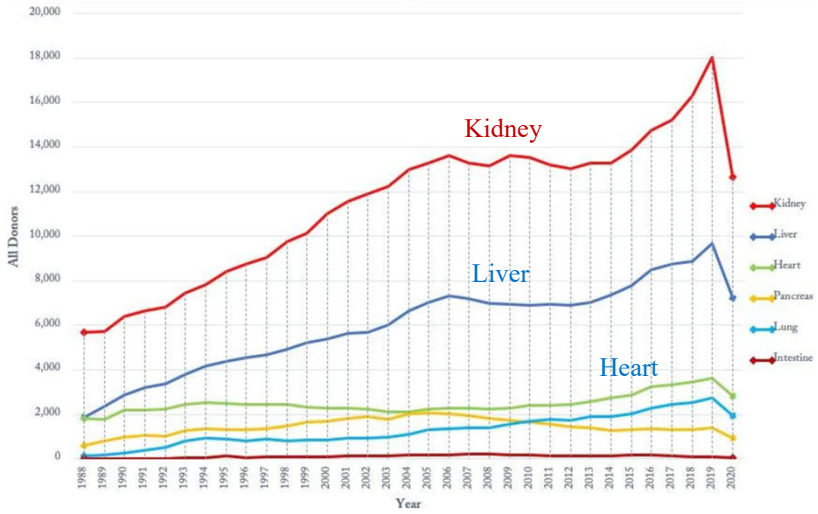
Branding

Organ Donation

Organ donation is essential to save lives, but there are not enough organs available.

Organ Transplant





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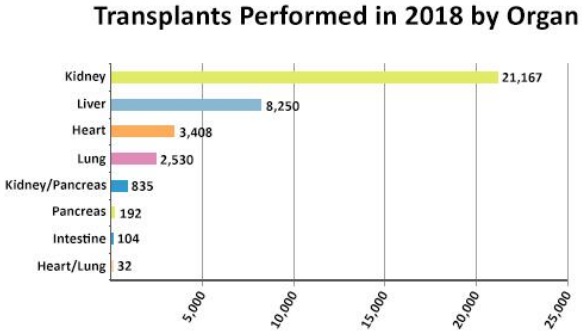
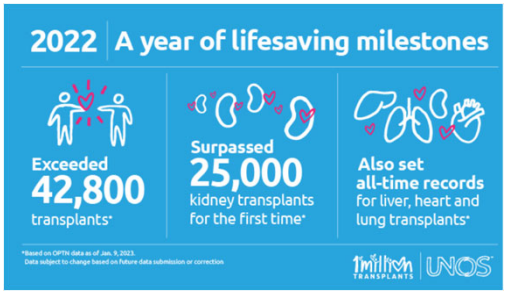
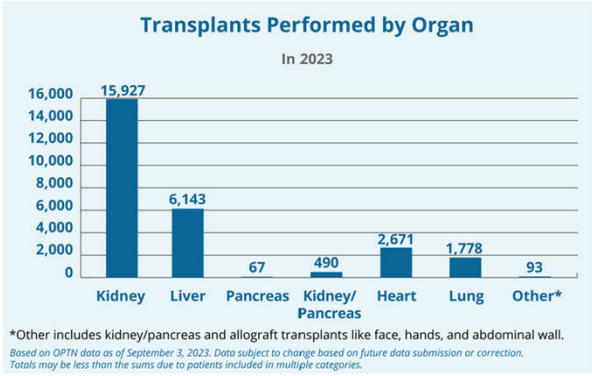
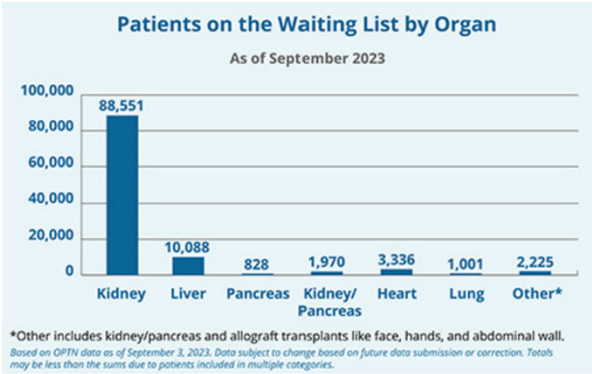




https://optn.transplant.hrsa.gov/news/annual-record-trend-continues-for-deceased-organ-donation-deceased-donor-transplants/
https://unos.org/news/2022-organ-transplants-again-set-annual-records/





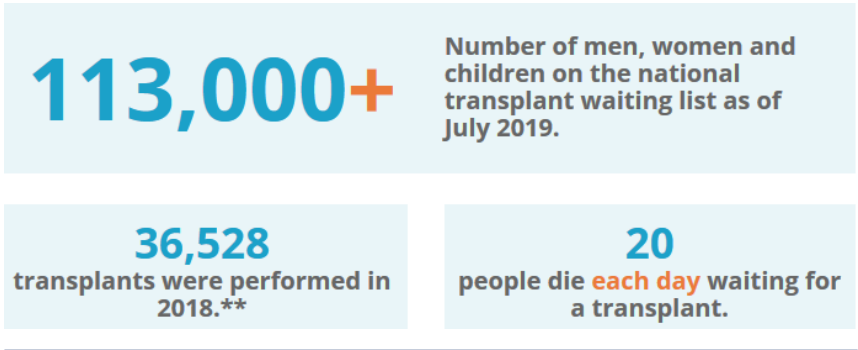
https://www.organdonor.gov/learn/organ-donation-statistics

For current data reports on transplants performed, including by recipient age, ethnicity, gender, or state, visit the OPTN Data Reports (https://optn.transplant.hrsa.gov/).

Organ Donation Statistics

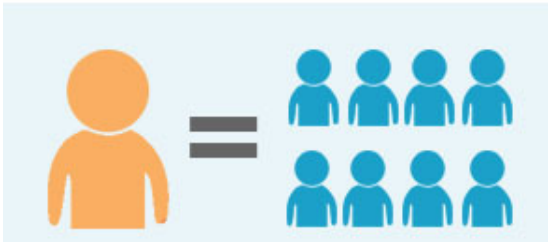
https://www.organdonor.gov/statistics-stories/statistics.html

How many people are waiting for a transplant? Who receives organs and what organs are most needed?



113,000+: Number of men, women and children on the national transplant waiting list as of July 2019.
36,528: Transplants were performed in 2018.**
20: People die each day waiting for a transplant.

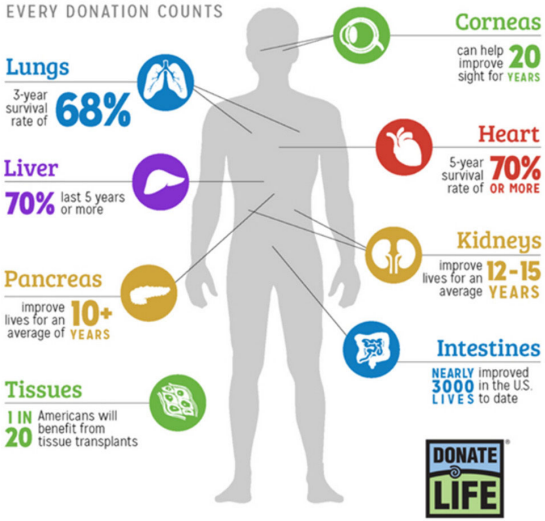
We All Need to Register. Here's Why:
95% of U.S. adults support organ donation but only 58% are actually signed up as donors.
Every 10 minutes another person is added to the waiting list.
Only 3 in 1,000 people die in a way that allows for organ donation.



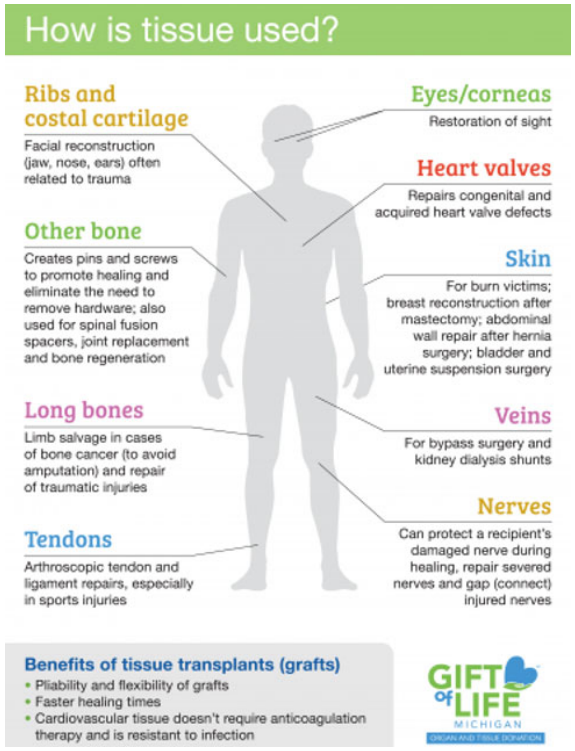
One Donor Can Save Eight Lives. One person can donate up to 8 lifesaving organs.

The Benefits of Organ Donation

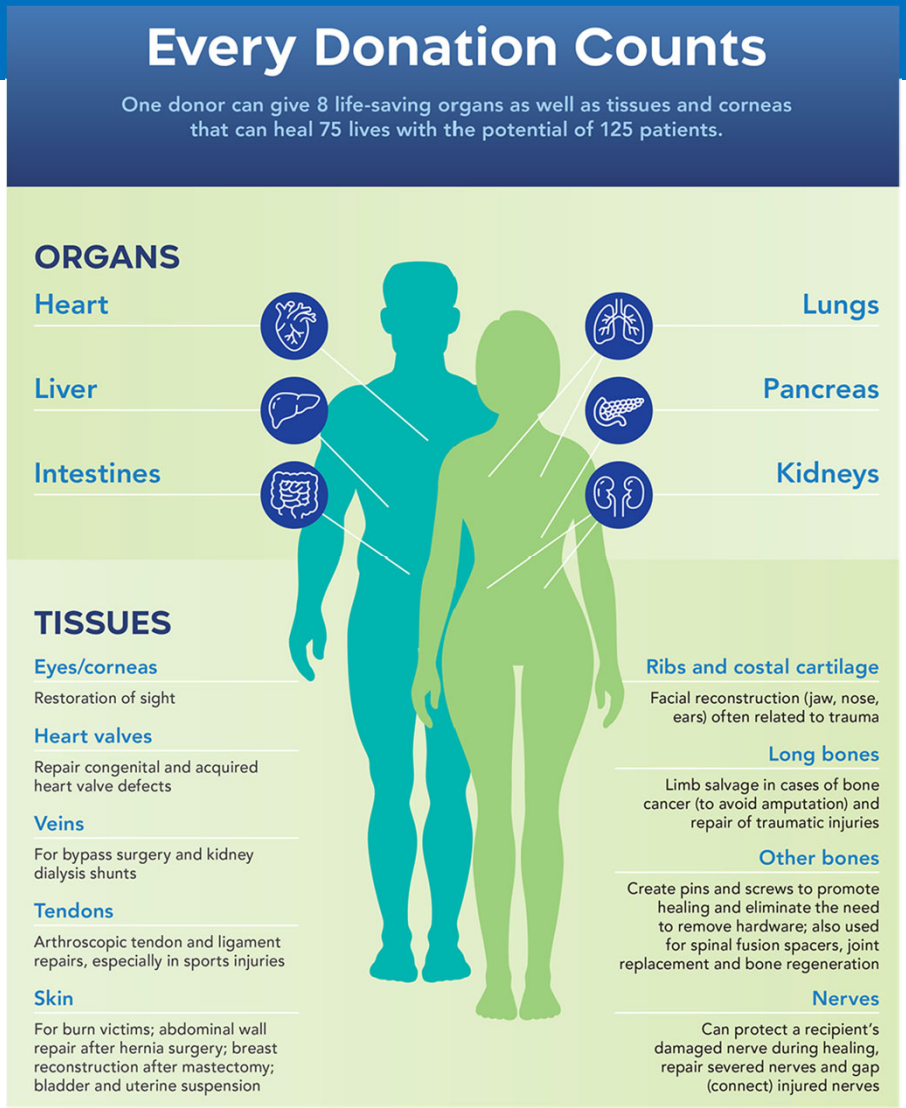
How Organ & Tissue Donation Changes Lives



http://www.wilsontimes.com/stories/donate-life-month-encourages-organ-donor-registration,120471



https://www.giftoflifemichigan.org/about-donation/benefits-organ-donation



https://giftoflifemichigan.org/about-donation/facts-faqs

Antifreeze Polymers

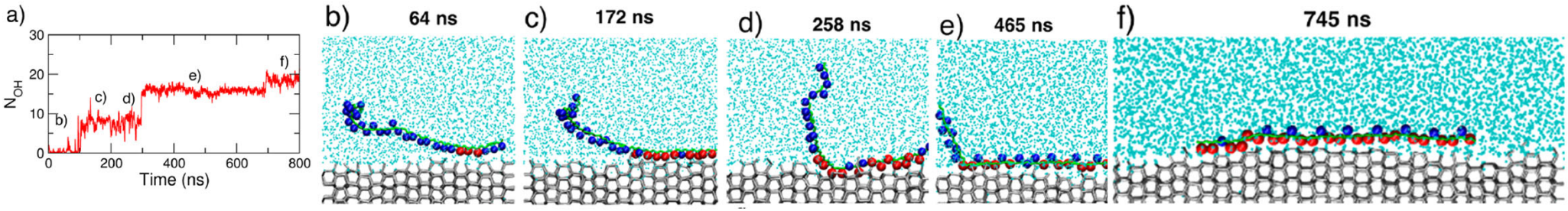
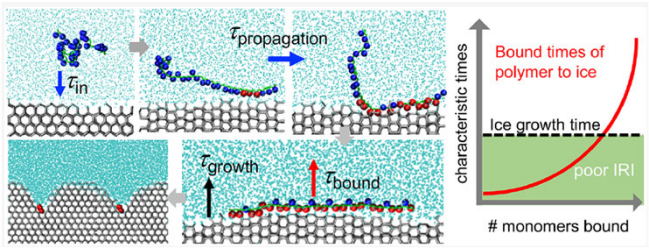


Figure 1. PVA with DP = 30 binds to the prismatic plane of ice in a long process that is reversible at its early stages. (a) Time evolution of the number of OH groups of PVA bound to ice, N_{OH}, where N_{OH} is computed as a running average over 1 ns windows of the trajectory to filter fast fluctuations that do not physically correspond to binding/ unbinding events. (b−f) Snapshots along the binding simulation. The times corresponding to the snapshots are indicated in panel a. Ice is represented with silver sticks, water with cyan points, bound OH groups of PVA with red balls, unbound OH groups with blue balls, and the hydrocarbon backbone with green sticks.



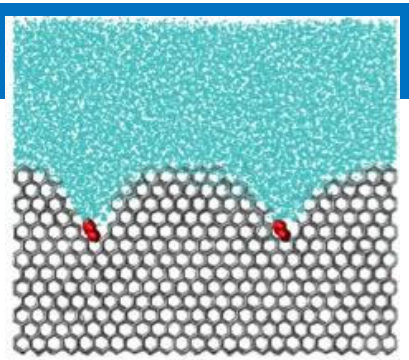
The bound times τ of each oligomer at the ice surface as a function of the average number n_{OH} of OH groups bound for that time interval. We find that τ depends on n_{OH} but not on the degree of polymerization of PVA. The higher the DP of PVA, however, the higher is the number of OH groups that can bind to ice and the longer is the maximum time that the chain can pin the ice surface, preventing its growth.

Ice recrystallization inhibitors (IRI) are of critical importance in biology, cryopreservation of cells and organs, and frozen foods. Antifreeze glycoproteins (AFGPs) are the most potent IRI. Their cost and cytotoxicity drive the design of synthetic flexible polymers that mimic their function. Poly(vinyl alcohol) (PVA) is the most potent biomimetic found to date, although it is orders of magnitude less potent than AFGPs. **A lack of molecular understanding of the factors** that limit the IRI efficiency of PVA and other flexible ice-binding polymers hinders the design of more potent IRI.

The onset of IRI activity of PVA occurs for 15 < DP < 20, in agreement with experiments. We predict that polymers with stronger binding to ice per monomer attain IRI activity at lower DP and identify this as a contributor to the higher IRI potency of AFGPs. The simulations reveal that the limiting step for binding of flexible molecules to ice is not the alignment of the molecule to the surface or the initiation of the binding but the propagation to reach its full binding potential. This distinguishes AFGPs and PVA from rigid antifreeze proteins and is responsible for their different scaling of efficiencies with molecular size.

Naullage 2020, Slow propagation of ice binding limits the ice-recrystallization inhibition efficiency of PVA and other flexible polymers

Antifreeze Polymers



Polymers to the rescue! Saving cells from damaging ice (Feb 13, 2020) Ice can tear apart cells in cryo-storage; Polymers can save the day Cell therapies hold great promise for revolutionizing the treatment of cancers and autoimmune diseases. But this multibillion-dollar industry requires long-term storage of cells at super-cold cryogenic conditions, while ensuring they'll continue to function upon thawing. However, these cold temperatures trigger the formation and growth of ice, which can pierce and tear apart cells. Research published in the *Journal of the American Chemical Society* by University of Utah chemists Pavithra Naullage and Valeria Molinero provides the foundation to design efficient polymers that can prevent the growth of ice that damages cells.

Nature's antifreeze

Current strategies to cryopreserve cells and organs involve bathing them with large amounts of dimethyl sulfoxide, a toxic chemical that messes up ice formation but stresses the cells, decreasing their odds for survival. Nature, however, has found a way to keep organisms alive under extreme cold conditions: antifreeze proteins. Fish, insects and other cold-blooded organisms have evolved potent antifreeze glycoproteins that bind to ice crystallites and halt them from growing and damaging cells. The growing area of cell-based therapeutics demands the development of potent inhibitors of ice recrystallization that can compete in activity with natural antifreeze glycoproteins but do not have the cost and toxicity of dimethyl sulfoxide. This demand has propelled the synthesis of polymers that mimic the action of antifreeze glycoproteins. But the most potent synthetic ice recrystallization inhibitor found to date, polyvinyl alcohol (PVA), is orders of magnitude less potent than natural glycoproteins. "Efforts to identify stronger inhibitors for ice growth seem to have stalled, as there is not yet a molecular understanding of the factors that limits the ice recrystallization inhibition efficiency of polymers," Molinero says.

A hidden polymer design variable

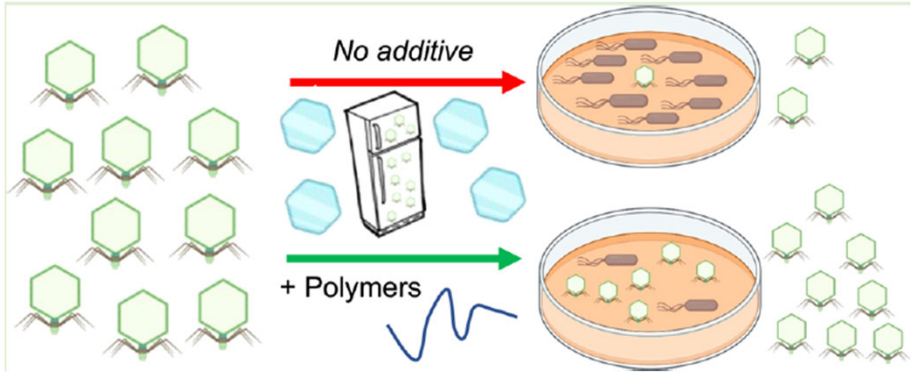
How do molecules prevent ice crystals from getting bigger? Molecules that bind strongly to ice pin its surface–like stones on a pillow–making the ice front develop a curved surface around the molecules. This curvature destabilizes the ice crystal, halting its growth. Molecules that stay bound to ice for times longer than the time it takes to grow ice crystals succeed in preventing further growth and recrystallization. Molinero and Naullage used large-scale molecular simulations to elucidate the molecular underpinnings of how flexibility, length and functionalization of polymers control their binding to ice and their efficiency to prevent ice growth. Their study shows that the bound time of the molecules at the ice surface is controlled by the strength of their ice binding coupled with the length of the polymer and how fast they propagate on the ice surface. "We found that the efficiency of flexible polymers in halting ice growth is limited by the slow propagation of their binding to ice," Molinero says.

The study dissects the various factors that control the binding of flexible polymers to ice and that account for the gap in potency of PVA and natural antifreeze glycoproteins. In a nutshell, each block of antifreeze glycoproteins binds more strongly to ice than PVA does, and are also favored by their secondary molecular structure that segregates the binding and non-binding blocks to allow them to attach faster to ice to stop its growth. "To our knowledge, this work is first to identity the time of propagation of binding as a key variable in the design of efficient ice-binding flexible polymers," Naullage says. "Our study sets the stage for the de novo design of flexible polymers that can meet or even surpass the efficiency of antifreeze glycoproteins and make an impact in biomedical research." Credit: University of Utah

https://scienmag.com/polymers-to-the-rescue-saving-cells-from-damaging-ice/

Polymer-Mediated Cryopreservation of Bacteriophages

Bacteriophages (phages, bacteria-specific viruses) have biotechnological and therapeutic potential. For many biologics, cryopreservation is employed for long-term storage and cryoprotectants are essential to mitigate cold-induced damage. Here, we report that poly(ethylene glycol) can be used to protect phages from cold damage, functioning at just 10 mg/mL (~1 wt %) and outperforms glycerol in many cases, which is a currently used cryoprotectant. Protection is afforded at both -20 and -80 °C, the two most common temperatures for frozen storage in laboratory settings. Crucially, the concentration of the polymer required leads to frozen solutions at -20 °C, unlike 50% glycerol (which results in liquid solutions). Post-thaw recoveries close to 100% plaque-forming units were achieved even after 2 weeks of storage with this method. Initial experiments with other hydrophilic polymers also showed cryoprotection, but at this stage, the exact mechanism of this protection cannot be concluded but does show that water-soluble polymers offer an alternative tool for phage storage. Ice recrystallization inhibiting polymers (poly(vinyl alcohol)) were found to provide no additional protection, in contrast to their ability to protect proteins and microorganisms which are damaged by recrystallization. PEG's low cost, solubility, well-established low toxicity/immunogenicity, and that it is fit for human consumption at the concentrations used make it ideal to help translate new approaches for phage therapy



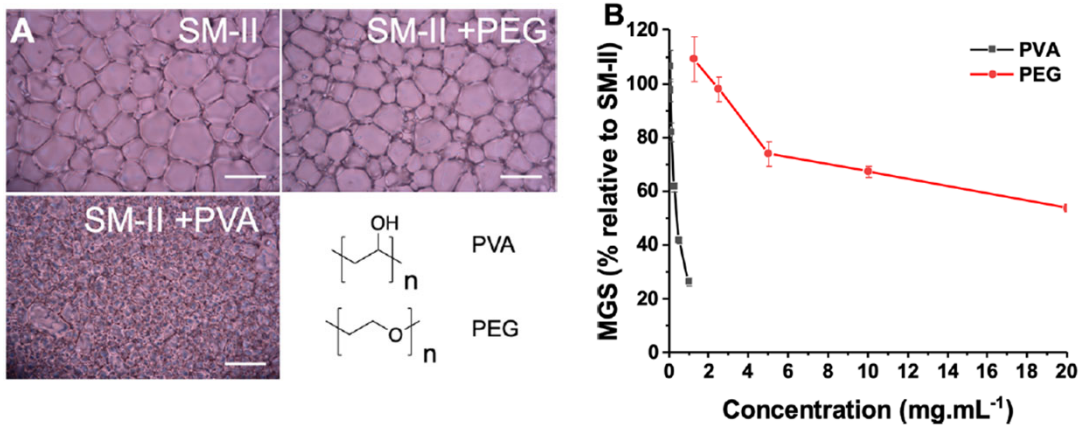


Figure 1. Ice recrystallization inhibition activity of polymers in SM-II buffer. (A) Example micrographs of ice wafers after 30 min annealing at −8 °C. PEG (4000 g·mol−1), 10 mg·mL−1, and PVA (10 000 g/mol), 1 g/mol). Scale bars = 100 μM. (B) IRI activity as a function of polymer concentration. MGS = mean grain size relative to SM-II control.

Marton 2021, Polymer-mediated cryopreservation of bacteriophages

Tumbleweed

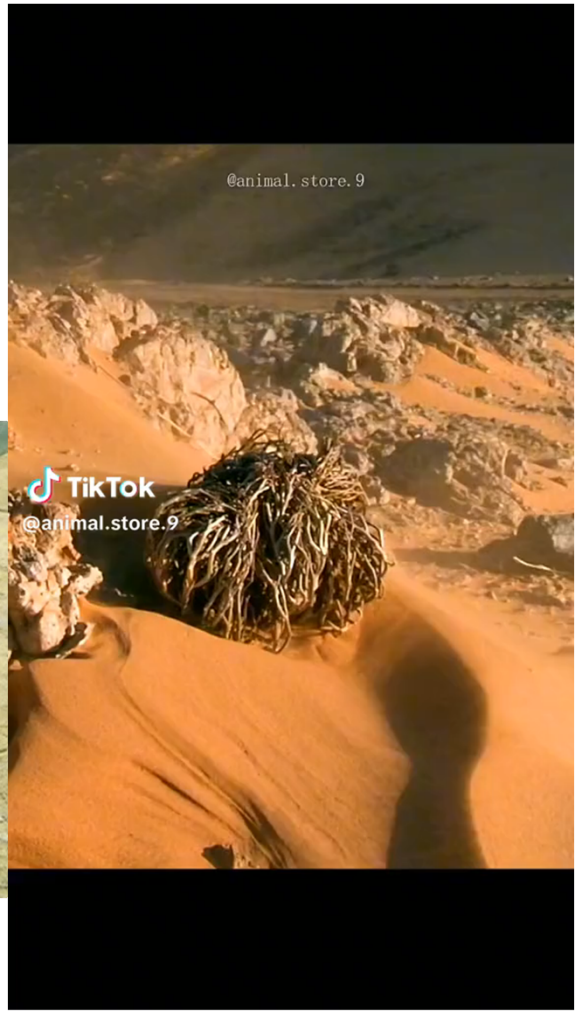
Tumbleweed is a plant that breaks away from its roots and is driven about by the wind as a light rolling mass, scattering seeds as it goes. Examples include pigweed (Amaranth retroflexus, a widespread weed in the western United States) and other amaranths, tumbling mustard, Russian thistle, the steppe plant Colutea arborea, and the grass Spinifex of Indonesian shores and Australian steppes.

https://www.britannica.com/plant/tumbleweed

An Alive Tumbleweed

We have all seen tumbleweeds rolling around on empty stretches of land. However, not many of us have ever seen a tumbleweed that's alive. Here is a treat for your eyes.

https://tigerscroll.com/rare-pictures-that-will-show-you-the-unseen-side-of-things-long/52/



Organs from Animals

Organ Transplants

Organs and Tissues

Loss

- Accidents
- Birth defects
- Hereditary disorders
- Diseases

Current treatments

- Metabolic supplements
- Mechanical devices
- Surgical reconstruction
- Organ Transplant

Organ Transplant Drawbacks

- Shortage
- Tissue mismatch
- Lifelong immunosuppression
- Graft rejection
- Drug therapy cost
- Cancer
- Cost

Transplants

Autograft

Tissue reimplanted into the donor (e.g., use of a patient's own veins or arteries in heart bypass grafting).

Allograft (also known as homograft)

Organs, tissues, and cells from one species transplanted to another animal of the same species (e.g., dog to dog or human to human).

Organ allografts (kidneys, liver, lung, heart, etc.) require immediate surgical revascularization for their metabolic requirements, thereby requiring reattachment by surgical anastomosis of the major arteries and veins.

Tissues and cells do not require revascularization and can be implanted without immediately establishing a direct blood supply (heart valves, blood vessels, orthopedic tissues, skin, cornea, etc.).

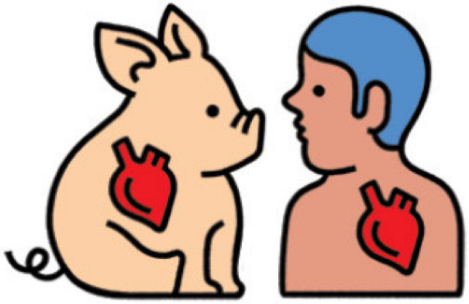
Xenograft

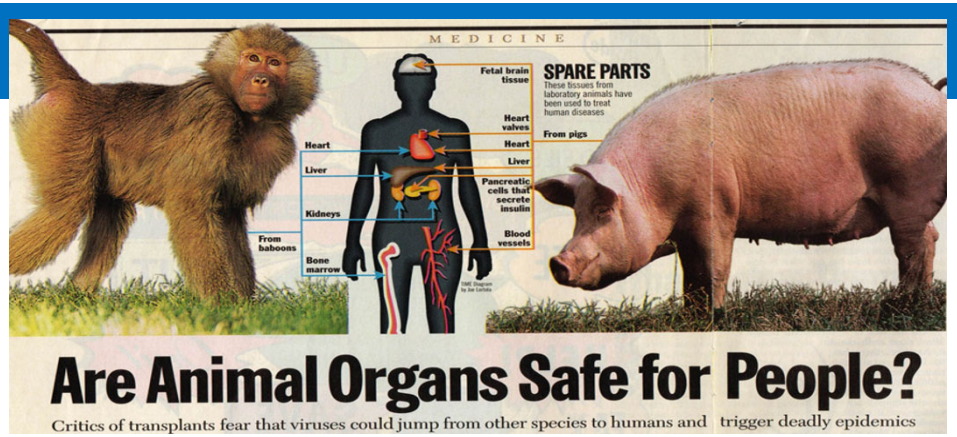
Tissue transplanted from one species to another (e.g., pig to monkey or pig to human).

Human Parts from Animals

Future Facts. Innovations to look out for in 2020 and beyond.
Don Steinberg

TIME, February 3, 2020





Human Parts From Animals
Chinese scientists recently bred piglets born with monkey cells in them. Researchers from California's Salk Institute created embryos containing both human and monkey cells. These and other similar sci-fi-sounding experiments are aimed at creating transplantable human organs, which are often in short supply.

Using animal organs in humans:
'It's just a question of when'.
https://www.theguardian.com/science/2019/apr/03/animal-global-organ-shortage-gene-editing-technology-transplant

The real question is when is 'when'?
Is it 10 years from now or 100 years from now?

Xenotransplantation

Xenotransplantation is any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues or organs. The development of xenotransplantation is, in part, driven by the fact that the demand for human organs for clinical transplantation far exceeds the supply.

Currently ten patients die each day in the United States while on the waiting list to receive lifesaving vital organ transplants. Moreover, recent evidence has suggested that transplantation of cells and tissues may be therapeutic for certain diseases such as neurodegenerative disorders and diabetes, where, again human materials are not usually available.

Although the potential benefits are considerable, the use of xenotransplantation raises concerns regarding the potential infection of recipients with both recognized and unrecognized infectious agents and the possible subsequent transmission to their close contacts and into the general human population. Of public health concern is the potential for cross-species infection by retroviruses, which may be latent and lead to disease years after infection. Moreover, new infectious agents may not be readily identifiable with current techniques.

https://www.fda.gov/vaccines-blood-biologics/xenotransplantation

From Pig with Love to Human

In a First, Man Receives a Heart From a Genetically Altered Pig

The breakthrough may lead one day to new supplies of animal organs for transplant into human patients. (He lived two months)

A 57-year-old man with life-threatening heart disease has received a heart from a genetically modified pig, a groundbreaking procedure that offers hope to hundreds of thousands of patients with failing organs. It is the first successful transplant of a pig's heart into a human being. The eight-hour operation took place in Baltimore on Friday, and the patient, David Bennett Sr. of Maryland, was doing well on Monday, according to surgeons at the University of Maryland Medical Center. "It creates the pulse, it creates the pressure, it is his heart," said Dr. Bartley Griffith, the director of the cardiac transplant program at the medical center, who performed the operation.

"It's working and it looks normal. We are thrilled, but we don't know what tomorrow will bring us. This has never been done before."

"This is a watershed event," said Dr. David Klassen, the chief medical officer of the United Network for Organ Sharing and a transplant physician. "Doors are starting to open that will lead, I believe, to major changes in how we treat organ failure."

But he added that there were many hurdles to overcome before such a procedure could be broadly applied, noting that rejection of organs occurs even when a well-matched human donor kidney is transplanted. "Events like these can be dramatized in the press, and it's important to maintain perspective," Dr. Klassen said. "It takes a long time to mature a therapy like this."

. Bennett decided to gamble on the experimental treatment because he would have died without a new heart, had exhausted other treatments and was too sick to qualify for a human donor heart, family members and doctors said.

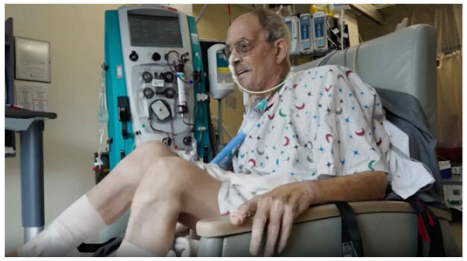
The New York Times. Jan. 10, 2022. Roni Caryn Rabin

Dr. Bartley Griffith, left, performed the operation on David Bennett Sr. to receive a new heart from a genetically modified pig.

Surgeons performed an eight-hour transplant of a genetically modified pig's heart at the University of Maryland Medical Center.

One month after experimental pig heart transplant, doctors say they see no signs of rejection or infection

Nadia Kounang, CNN (October 20, 2023)
https://www.cnn.com/2023/10/20/health/pig-heart-transplant-maryland-update



Lawrence Faucette does physical therapy after being the second person in the world to receive experimental pig heart transplant. (University of Maryland Medical Center)

Second person to receive experimental pig heart transplant dies nearly six weeks after procedure

Nadia Kounang, CNN (November 1, 2023)
https://www.cnn.com/2023/10/31/health/lawrence-faucette-second-pig-heart-transplant-dies/index.html

In this photo provided by the University of Maryland School of Medicine, Lawrence Faucette sits with wife, Ann, in the school's hospital in Baltimore, Md., in September 2023, before receiving a pig heart transplant. Lawrence Faucette, the second person to receive a transplanted heart from a pig has died, nearly six weeks after the highly experimental surgery, his doctors announced Tuesday, Oct. 31, 2023. (Mark Teske/University of Maryland School of Medicine via AP, File)

Pig Kidneys Transplanted Into Brain-Dead Man as Patients Face Organ Shortages

First-of-its-kind surgery in Alabama is seen potentially leading to clinical trials of animal-to-human transplants

Jamy Marcus on January 20, 2022.
https://www.wsj.com/articles/pig-kidneys-transplanted-into-brain-dead-man-as-patients-face-organ-shortages-11642680005

Should We Be Breeding Pigs Just for Their Hearts?

Jan Dutkiewicz/January 20, 2022
https://newrepublic.com/article/165074/pig-heart-transplant-ethics

Pig Kidneys to Human

Pig Kidneys Transplanted to Human in Milestone Experiment

Experts predict that such nonhuman-to-human “xenotransplants” may become a viable option within the next decade

It’s an exciting time to be an organ transplant physician. Just two weeks ago, doctors in Baltimore reported completing the first successful transfer of a pig heart into a living human patient. Now pig kidneys might be just around the corner.

In late September 2021 a team of researchers transplanted a gene-edited pig’s two kidneys into the body of a person who had undergone brain death (the irreversible loss of all brain function) in a procedure designed to fully simulate clinical transplantation. Once inserted, the new kidneys sustained blood flow and even produced urine until the study ended 77 hours later. The results were published on Thursday in the American Journal of Transplantation.

“It really demonstrated that we have the infrastructure to be able to do this,” says the new study’s lead surgeon Jayme Locke, a transplant surgeon at the University of Alabama at Birmingham (UAB). The investigation’s standardized process “is going to be just as important as demonstrating that the pig kidneys are viable in humans.”

An organ transplant is full of risks. The human immune system is remarkably good at distinguishing between “self” and “nonself,” and when it detects a foreign entity—whether a virus, a strange bacterium or someone else’s internal organ—it mounts an attack. This is great for fighting disease. But in the context of transplantation, a strong immune response can eventually cause the body to reject the new organ. To avoid this, doctors prescribe immunosuppressing drugs to the recipient. Unfortunately these medications also leave the patient susceptible to viruses and bacteria. “The biggest risk is [miscalculating] this balance between rejection and infection,” says Dorry Segev, a kidney transplantation specialist at Johns Hopkins University, who was not involved in the research.

For patients receiving a nonhuman organ, a procedure called a xenotransplantation, that risk is multiplied. Xenotransplants (and, in rare cases, poorly matched human organ transplants) can trigger a phenomenon called hyperacute rejection, in which the body begins aggressively attacking the new organ within hours or even minutes of surgery. “It’s a different type of rejection. And it’s a fundamental barrier,” says Paige Porrett, director of vascularized composite allotransplantation and of Clinical and Translational Research at UAB’s Comprehensive Transplant Institute and lead author of the study.

Porrett’s team overcame this obstacle by using kidneys from a designer swine with 10 key genetic tweaks to make its organs a better match for humans. For instance, the donor pig was equipped with genes to help prevent blood clots and regulate blood vessel strength. Another gene, involved in responding to growth hormones, was knocked out to ensure that the transplanted kidneys stayed human-sized inside its recipient. “I certainly wouldn’t want a pig-sized kidney,” Locke says.

The team’s procedure was not the first pig-to-human kidney transplantation: that operation took place on September 25 at NYU Langone Health, and the recipient was also a person without brain activity. “It was pretty exhilarating,” says Robert Montgomery, director of the NYU Langone Transplant Institute, who performed the surgery with his team. His and his colleagues’ research was designed primarily to test the viability of the single kidney. While the organ functioned successfully, removing waste from the blood and disposing of it in the form of urine, it was attached to a blood vessel in the recipient’s upper leg rather than implanted in the abdomen, where kidneys normally go.

In contrast, the UAB team executed a full clinical transplant procedure, from assessing organ compatibility to removing the recipient’s kidneys and replacing them with the xenotransplants. The researchers also took pains to ensure that the donor pig was raised in a pathogen-free facility, and they had the entire process reviewed by an ethics board. “At times that felt harder than the actual science that we were doing,” Porrett says.

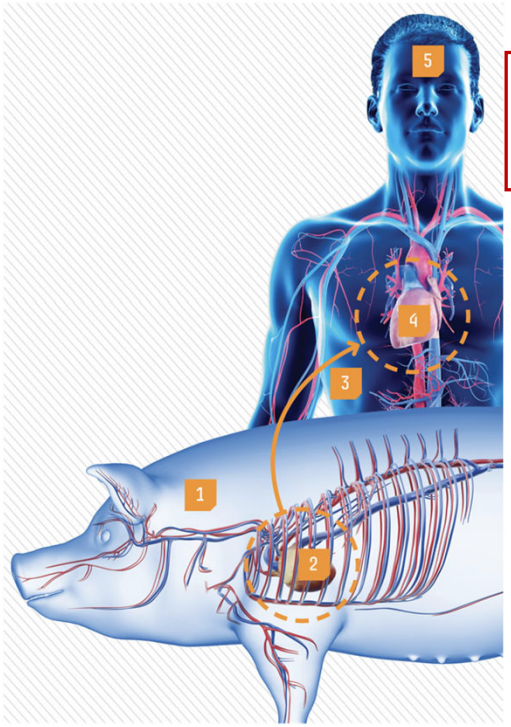
Joanna Thompson on January 20, 2022.
https://www.scientificamerican.com/article/pig-kidneys-transplanted-to-human-in-milestone-experiment/?utm_source=newsletter&utm_medium=email&utm_campaign=today-in-science&utm_content=link&utm_term=2022-01-20_featured-this-week&spMailingID=71141861&spUserID=NTY3NzEwMjIyMQS2&spJobID=2231333715&spReportId=MjIzMTMzMzcxNQS2

Jamy Marcus on January 20, 2022.
https://www.wsj.com/articles/pig-kidneys-transplanted-into-brain-dead-man-as-patients-face-organ-shortages-11642680005

From Porcine Pump to Future Breakthroughs

PORCINE PUMP

How scientists grow a pig's heart and transplant it into a patient



5 CAUSE OF DEATH
Bennett's autopsy revealed no sign of organ rejection but a thickening and stiffening of heart muscle, which led to heart failure.

4 PROMISING RESULTS
The implanted pig heart functioned well for several weeks and showed no signs of rejection.

3 THE SWITCH
Surgeons disconnected Bennett's unhealthy heart from his circulatory system and installed the pig alternative in an eight-hour-long surgery.

2 EDITED HEART
The pig's heart underwent ten genetic modifications, including the removal of three immune rejection-related genes, the insertion of six human genes and a growth gene to control the heart's size.

1 GROWING PIGS
Gene editing occurs by transferring genetic information between the nuclei of pig egg cells. When the pigs grow, their bodies contain the edited genes.

5 Breakthroughs in Medical Treatment in 2023

1 ALZHEIMER'S
A new drug called lecanemab targets a plaque that builds up in the brain of people with Alzheimer's disease, called amyloid, to slow the rate of decline.

2 AI AND SEPSIS
Using artificial intelligence (AI), researchers created an algorithm to detect several risk factors for sepsis, the leading cause of hospitalisation and death worldwide. The AI was able to detect sepsis nearly six hours earlier than traditional methods.

3 FIGHTING MALARIA
Vaccines using mRNA to tackle COVID-19 have helped research teams develop experimental vaccines which may be effective in reducing malaria infections and decreasing its transmissibility.

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4 NEW ASL DRUG
The US Food and Drug Administration approved the use of a new drug called Relyvrio to treat a nervous system disease called amyotrophic lateral sclerosis (ASL). The results of a preliminary study showed that Relyvrio may slow the rate of decline for ASL sufferers.

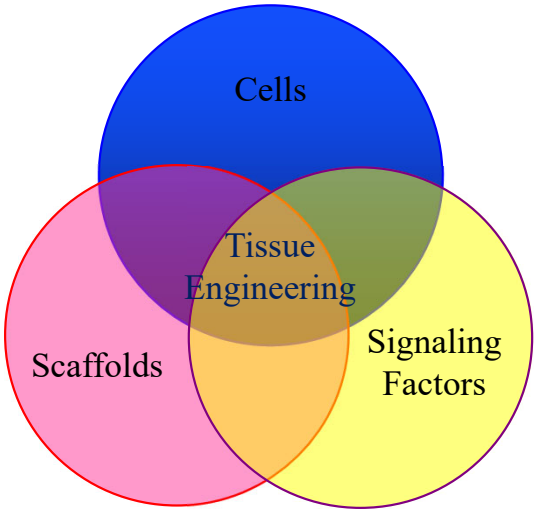
5 BATTLING CANCER
A 13-year-old girl who relapsed with leukaemia became the first patient to receive a genetically engineered immune cells treatment, which sent her into remission.

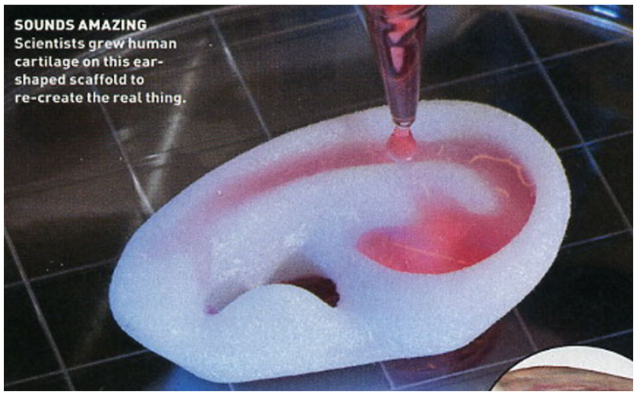
Tissue Engineering

Since there are not enough donated organs to meet the demands, human organs need to be obtained by other means. One way is to obtain organs from animals, but this has numerous problems now. Alternatively, artificial organs can be made in laboratories, and such synthetic approaches resulted in a new research field known as tissue engineering. The tissue engineering approach is still far from perfect and practical. Still, it provides a tool to develop artificial organs in the future with a series of innovations by young scientists.

Tissue Engineering

Application of the principles and methods of engineering and the life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and development of biological substitutes to restore, maintain, or improve function. (Skalak 1988)





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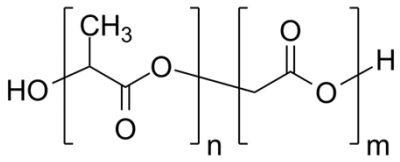
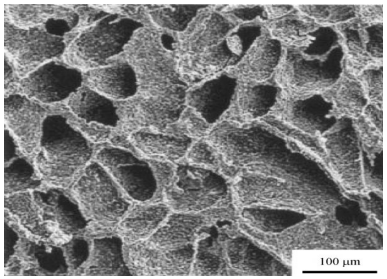
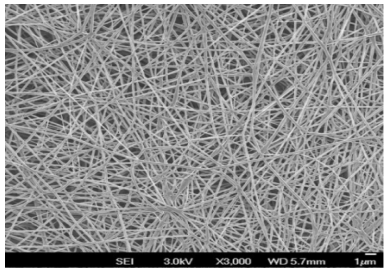


Lactide Glycolide





Science (1993) 920-926

Tissue Engineering

A

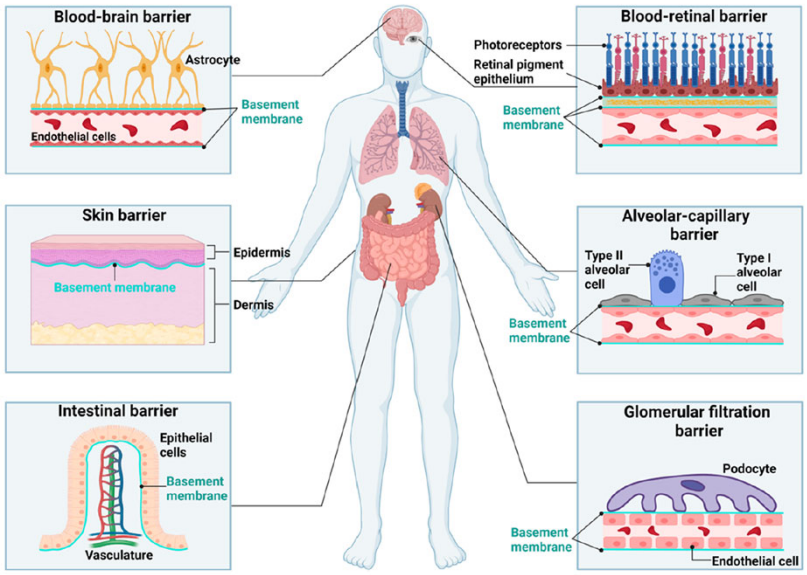


Figure 1. Basement membrane location: Basement membrane (BM) is ubiquitous in the human body and is located adjacent to the epithelium, endothelium, and parenchymal cells including muscle, adipose as well as nerve cells. It is involved in many vital physiological processes and is found in many organ barriers including the brain, retina, kidney, intestine, and lung. The schematic displays examples of some of the vital organs where the BM can be found. This includes the underlying areas of the epithelium and endothelium, where it supports and physically separates the different cellular layers.

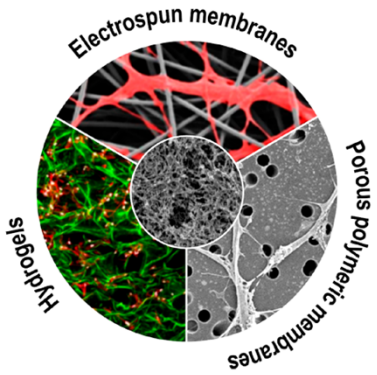


Figure 3. Structural resemblance of native basement membrane with synthetic mimics: Schematic represents the structural resemblances

Jain 2022, Mimicking the natural basement membrane for advanced tissue engineering

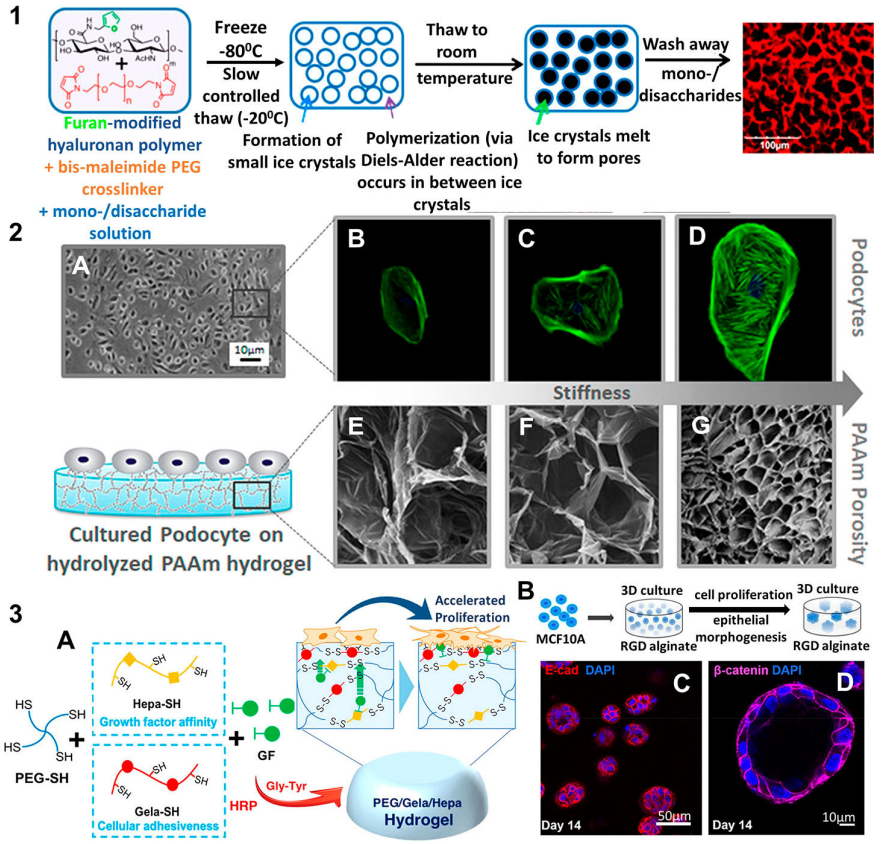


Figure 5. Optimization of hydrogel properties to mimic basement membrane structure and function: Modification of physical and chemical properties of hydrogels have been addressed in varying methods that allow their use as scaffolds to mimic BM: (1) Limitations of pore formation have been overcome using Diels−Alder click chemistry and cryo-gelation of the agarose and hyaluronic acid hydrogels. The polymer mixture is frozen, where the ice crystals slowly melt and are replaced by pores. (2) Tunable physical properties of the hydrolyzed polyacrylamide (PAAm) such as stiffness (0.3-300 kPa) and pore size have been exploited to mimic the glomerular filtration barrier with the podocyte cells and also to study the influence of scaffold mechanical properties on such a filtration barrier in vitro. (3A) Cell adhesion and proliferation on hydrogel BM mimics have been enhanced including the development of biofunctional PEG hydrogels using HRP mediated crosslinking of thiolated polymers, where the 4-arm PEG-SH was conjugated with thiolated gelatin (Gela-SH) and heparin (Hepa-SH). (3B) hydrogels are commonly used to realize a 3D environment specifically for proliferation of breast epithelial cells as well as to mimic a tumor environment, where MCF10A cells are embedded in RGD functionalized alginate gels to form (3C) spheroids and (3D) acini like structures similar to in vivo.

Development of Tissue Engineered Artificial Organs

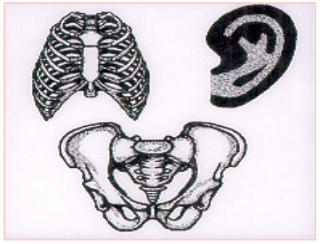
Templates/Scaffolds
Biocompatible and biodegradable materials
Natural: Collagen, Hyaluronic acid, Gelatin.
Synthetic: PGA, PLA, PLGA.

Scaffold structures
Non-woven
Fiber bonding: thermal treatment,
membrane lamination

Scaffold preparation
Solution casting
Spray casting
Melt molding: compressing molding
Electrospinning

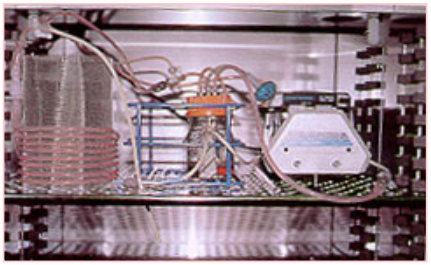
Porous structure
Emulsion freeze-drying: lyophilization
Particulate leaching
Gas saturation (high pressure)
Phase separation

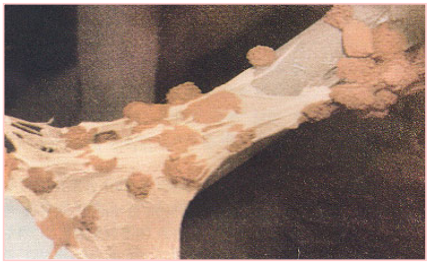
Natural Organs



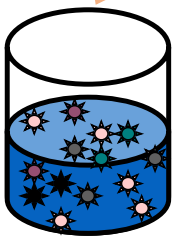
Scaffolds in Organ Shape and Size

Cell Seeding

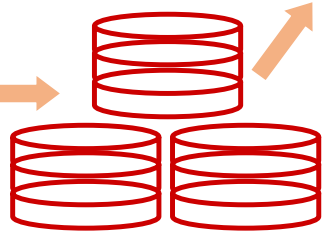




Preparation of Artificial Organs



Isolation of Cell *in vitro*



Large Scale Cell Culture *in vitro*

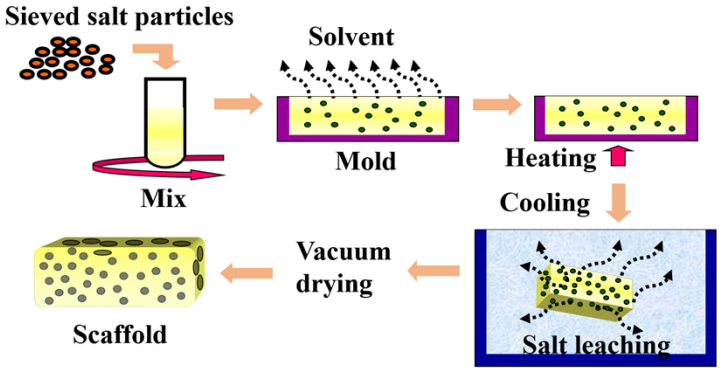
New Tissue Engineered Organs

Professor Gilson Khang, Chonbuk National University, Korea

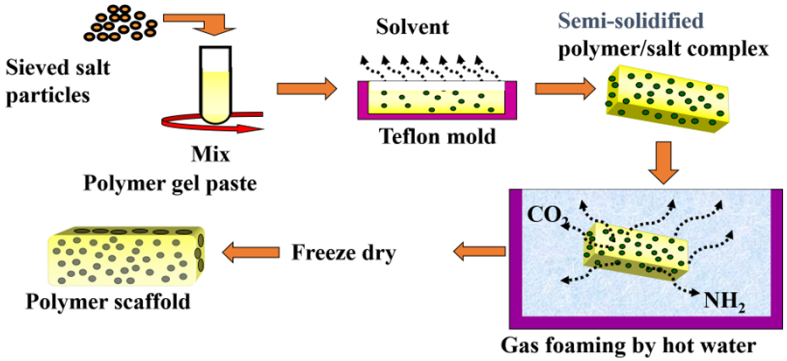
Porous Scaffold Preparation

The key here is that the pores have to be interconnected, i.e., making continuous channels where cells can migrate and grow.

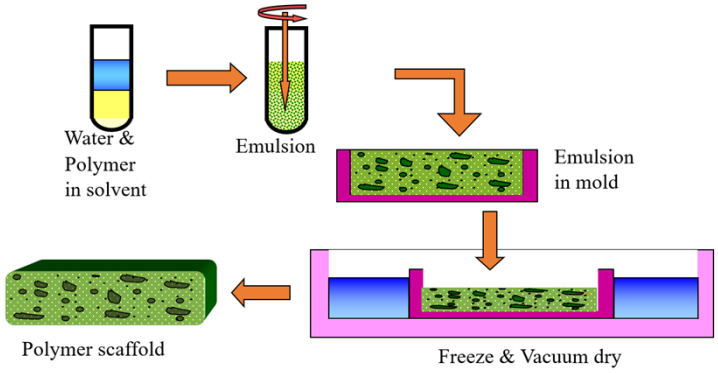
Particulate Leaching



Gas Generation

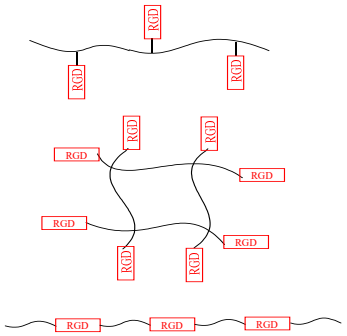


Emulsion Freeze-drying



Signaling Factors

RGD	Fibronectin, Collagen, Fibrinogen, Laminin, Vitronectin
YIGSR	Laminin
IKVAG	Laminin
LRE	Laminin
REDV	Fibronectin
DGEA	Collagen
VTXG	Thrombospondin
VGVAPG	Elastin



Professor Gilson Khang, Chonbuk National University, Korea

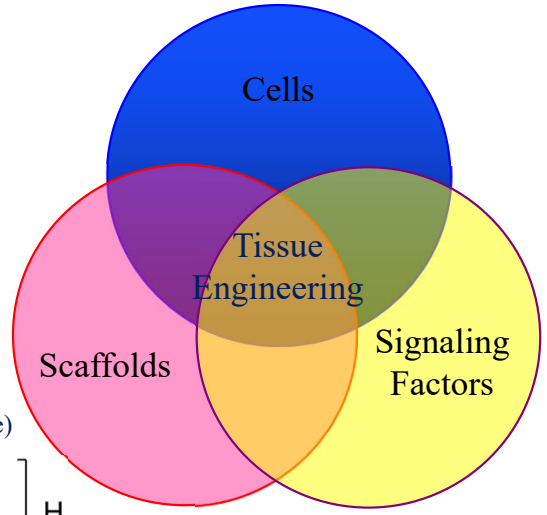
The Extracellular Matrix (ECM)

Before we discuss tissue engineering, we need to recall our understanding of the extracellular matrix (ECM).

Biological tissues and organs consist of cells surrounded by a complex molecular framework that is known as ECM. ECM is a complex mixture of different macromolecules, such as collagens, proteoglycans, glycoproteins, and elastin and one of its important roles is to provide tissues with the appropriate structural integrity. ECM can be simply considered to be a house for cells.

Examples of ECM Functions

1. Immobilization of the cells to prevent fusion, aggregation, and necrosis.
2. Improved oxygen transport, such as is provided with perfluorocarbons. (Movie Abyss)
3. Presentation of an acceptable physical environment for attachment-dependent cells.
4. Provision of an acceptable chemical environment (e.g., collagen gels have been shown to induce epithelial cells to grow into duct-like structures and to promote neonatal endocrine pancreatic cells to organize into islet-like structures.)
5. Physical limitation of the cell volume within the chamber.
6. Physical support for the outer membrane.



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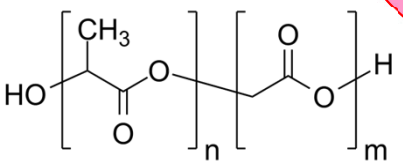
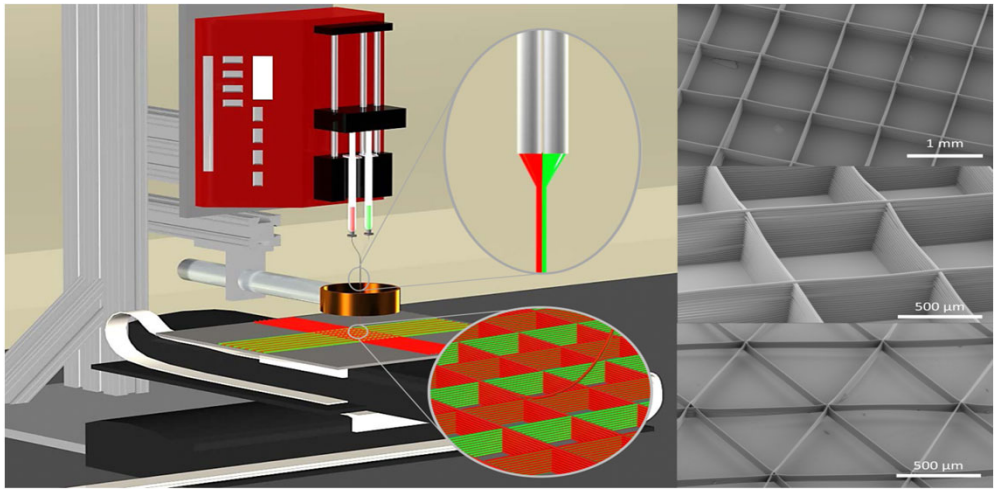
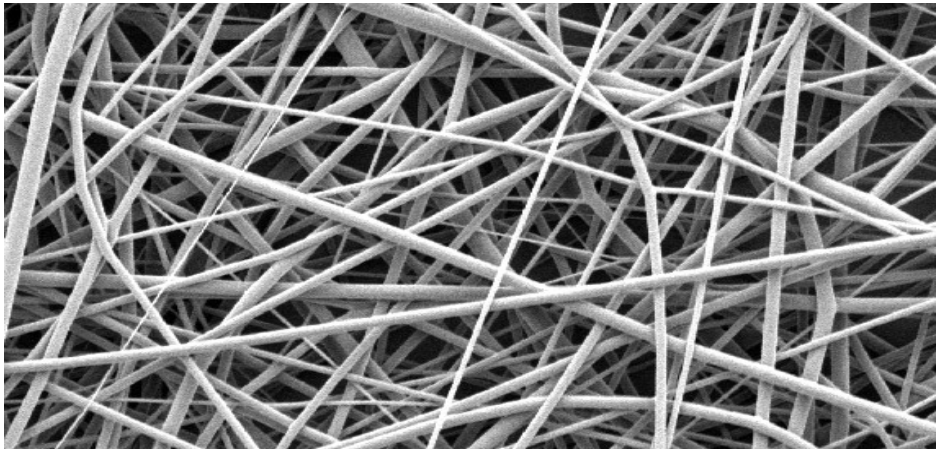
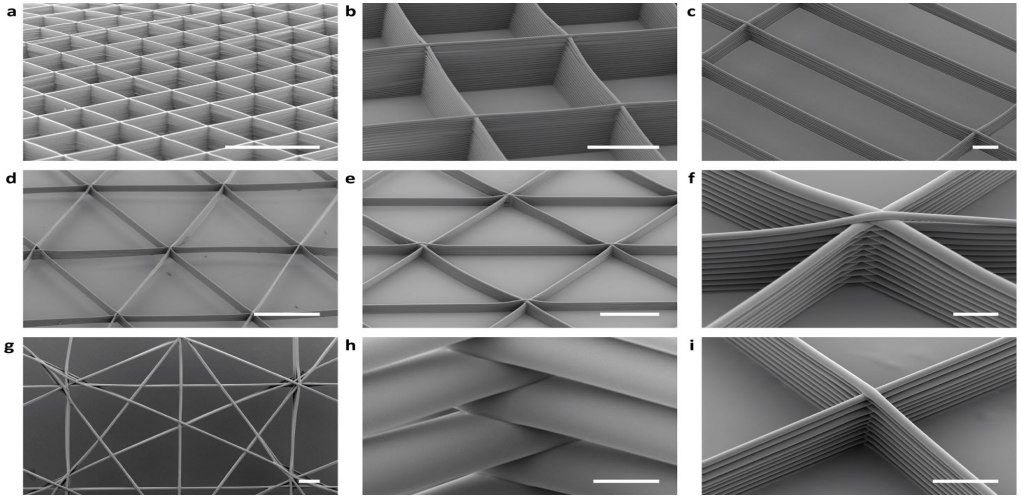


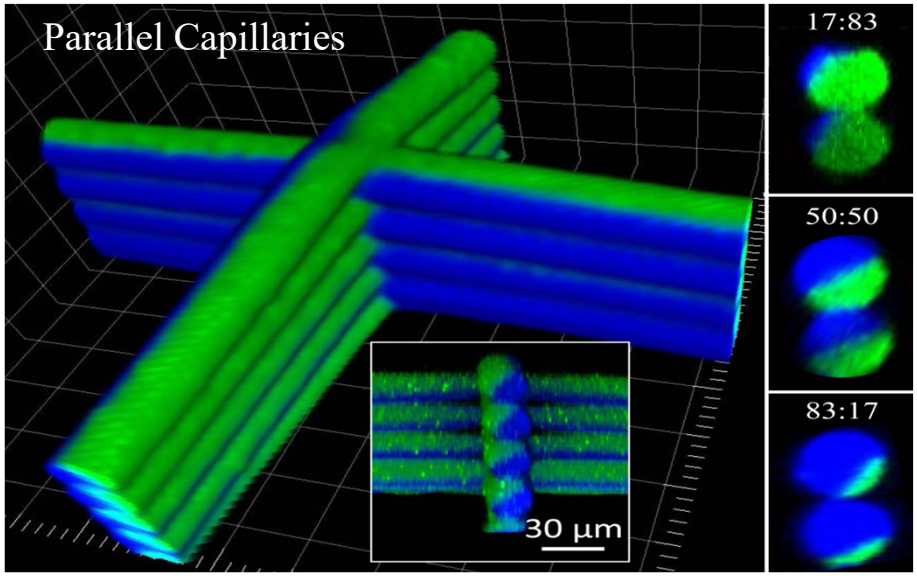
Lactide Glycolide

New Scaffolds



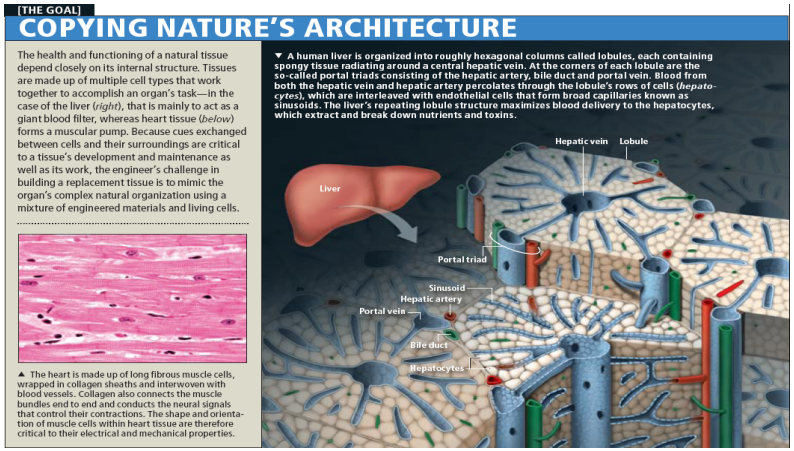




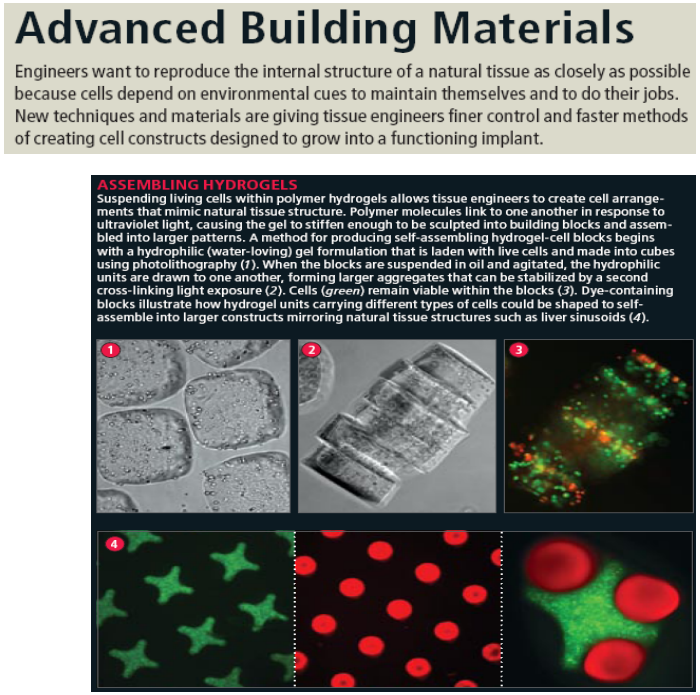


ECM

Natural Tissues



Synthetic Materials



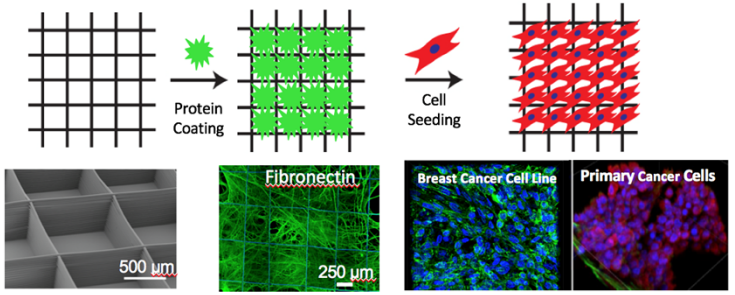
Professor Luis Solorio

Tissue Engineering, Osteoarthritis, Drug Delivery, Medical Imaging.

Development of tissue engineered tumor models to study the environmental drivers of metastasis

The tumor microenvironment is a complex milieu of cells, extracellular matrix (ECM) proteins, and soluble signaling factors that drive phenotypic changes in cancer cells during processes such as the metastatic cascade. It is a challenge to determine how these microenvironmental factors act both individually and in concert to drive phenotypic changes in cancer cells as part of cancer progression.

Our research is focused on applying principles of tissue engineering, medical imaging, and drug delivery for the development of modular, 3D tissue-engineered constructs that can used to evaluate the cancer cell response to microevniornmental cues. Taken in aggregate our tumor models can be used to develop patient avatars for precision medicine strategies, as part of high-throughput drug screening applications, and for the rational design of controlled drug release systems.



History of Tissue Engineering

History of Tissue Engineering

The term "tissue engineering" as recognized today was first introduced at a panel meeting of the National Science Foundation in 1987, which led to the first tissue engineering meeting in early 1988. However, tissue engineering strategies date back to the seventies and eighties for developing skin substitutes. Despite these early approaches for replacement, repair, and regeneration of failing organs, the true emergence of tissue engineering as a medical field started in the early nineties when tissue engineering was defined as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function.

Goals of Tissue Engineering

Tissue engineering aims to restore tissue and organ function by employing biological and engineering strategies to clinical problems. The functional failure of tissues and organs is a severe and costly healthcare problem, as their replacement is limited by the availability of compatible donors.

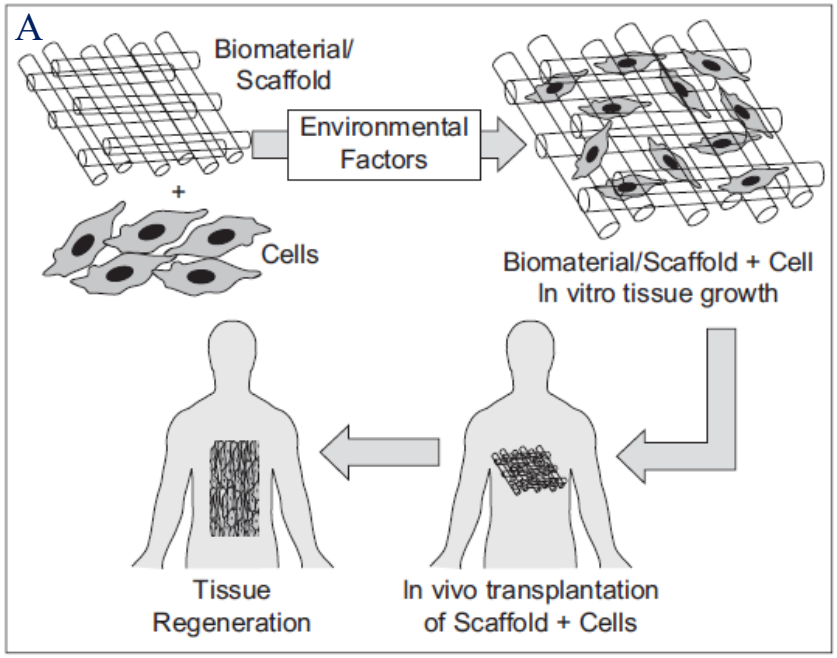
Artificial prostheses and mechanical devices save and improve the lives of millions of patients, but are not ideal since they are subject to mechanical failure upon long-term implantation. Furthermore, mechanical devices rarely integrate with host tissues, and can trigger a host immune response damaging healthy tissue around the implant. In addition, surgical reconstruction of organs and tissues are attempted where the organs or tissues are moved from their original location to replace a damaged tissue, e.g., saphenous vein as bypass graft, patella tendon for anterior cruciate ligament (ACL) repair. However, often this strategy fails to replace all the functions of the original tissue. Additionally, development of malignant tumors, surgical complications, and morbidity at the donor sites are major problems in surgical reconstruction of tissues. Thus, tissue engineering has emerged as another alternative for tissue or organ transplantation. The primary goal of tissue engineering is to provide a biological substitute to treat tissue/organ loss or failure by integrating multiple aspects of engineering, biology, and medicine. By recapitulating the normal tissue development process, tissue engineering represents a strategy to restore, maintain, and improve tissue function, which ultimately aims toward complete organ replacement.

Sarkar 2013, Overview of tissue engineering concepts and applications, in Biomaterials Science (Third Edition), 2013. pp. 1122-1137.

Traditional Tissue Engineering Approaches: Two Main Strategies

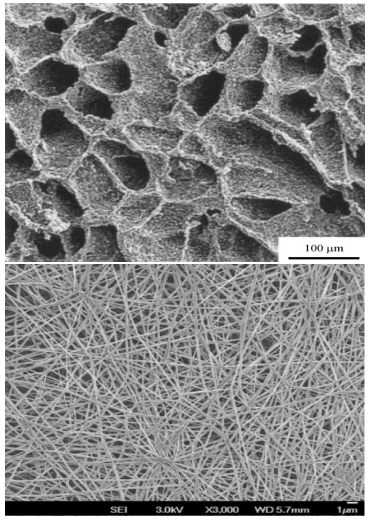
(1) Transplantation of a tissue grown *in vitro* consisting of an artificial matrix with cells and growth factors

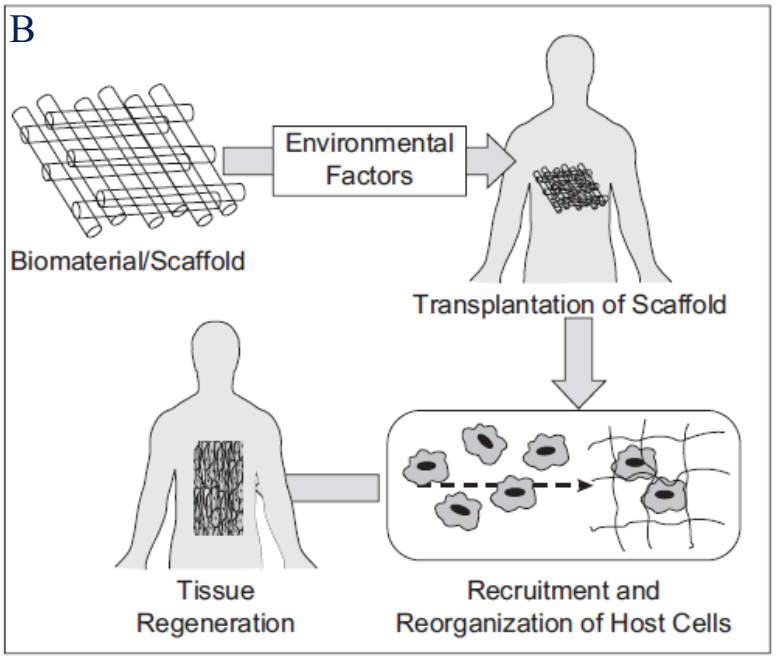
(2) *in situ* regeneration of tissue utilizing a combination of an artificial matrix and growth factors as a guiding template to induce host cell regeneration of the tissue *in vivo*.



In vitro tissue engineering followed by transplantation.

Note that scaffolds need to be porous for cells to grow and build their own extracellular matrix. Example structures are shown below.





In vivo tissue engineering by transplantation of scaffold and recruitment and reorganization of host cells

FIGURE II.6.2.1 Tissue engineering approaches may be classified into two categories: (A) transplantation of in vitro grown tissues; and (B) promotion of tissue regeneration in situ. In both approaches the scaffolds or artificial matrices, often biodegradable polymers, are integrated with microenvironmental factors (such as cytokines, growth factors, mechanical forces, physico-chemical factors, spatial and temporal signals, and extracellular matrix molecules).

Sarkar 2013, Overview of tissue engineering concepts and applications, in Biomaterials Science (Third Edition), 2013. pp. 1122-1137.

Traditional Tissue Engineering Approaches

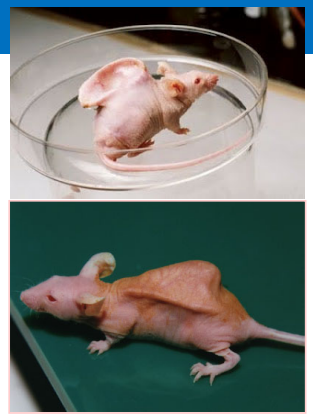
In addition to traditional tissue engineering approaches, other methods used for tissue regeneration include local and systemic cell injection without a scaffold, and closed looped systems used as implantable or extracorporeal devices. While classically these methods are not regarded as tissue engineering, they have contributed significantly to tissue regeneration.

Cell Therapy. Cell therapy involves delivery of cells through systemic injection into the bloodstream or through direct transplantation into a local tissue. The major requirement for this strategy is to harvest the cells and grow them in large numbers for *in vivo* transplantation.
Direct injection of cells to a local site is a common strategy attempted to promote tissue regeneration. However, the survival of the delivered cells is typically low, often due to a lack of a rich nutrient and oxygen supply. Alternatively, cells can be administrated via systemic injection, which relies on cells traveling through circulation to engraft in the target site. Cell transplants from bone marrow, peripheral blood or umbilical cord have been used to treat several blood-related diseases including leukemia, multiple myeloma, and immune deficiencies. The main goal of these strategies is to deliver hematopoietic (blood) stem cells to treat blood-related diseases. Recently, mesenchymal stem cells, connective tissue progenitor cells, which repair or regenerate non-hematopoetic tissues, have been systemically injected to treat diseases including myocardial infarction, bone diseases, and brain injury in clinical trials. The main challenges for cell transplantation are: growing large number of cells without bacterial contamination; preservation of cell phenotype; and preventing accumulation of genetic mutations during culture expansion. Although cells have been successfully delivered to the heart to treat ischemic tissue following myocardial infarction, and into the joint to treat arthritis, irrespective of the delivery route, cell therapies face challenges due to widespread death of the transplanted cells, poor engraftment, and loss of control over the fate of the transplanted cells.

Closed Loop Methods. A variety of closed loop systems are used as extracorporeal or implantable devices which house the transplanted cells in a semipermeable membrane. The membrane permits diffusion of nutrients and excreted products, but prevents the movement of antibodies, pathogens or immunocompetent cells. There are several types of designs for such devices. Vascular type design uses a conduit structure around which the cells are transplanted in a chamber.
As blood flows through the conduit it provides nutrients to the transplanted cells, while cell-secreted substances diffuse into the bloodstream. Additionally, micro/macrocapsule-based systems have been used as closed loop systems where cells are encapsulated within hydrogel droplets. The encapsulated cells can then be cultured in vitro or transplanted in vivo, either to repopulate a defect site or to produce growth factors or other molecules that will have an effect on the targeted cell population. Closed loop extracoporeal devices have been used for the treatment of liver, pancreas, and kidney pathologies. Major problems associated with these types of devices include fouling, fibrous tissue overgrowth, restricted and hindered diffusion, and immunogenic response.

Sarkar 2013, Overview of tissue engineering concepts and applications, in Biomaterials Science (Third Edition), 2013. pp. 1122-1137.

Tissue Engineered Organs



When tissue engineering first began in the mid 1980's, scientists made many tissue-looking objects. An ear or a nose grown in mice, shown below, attracted a lot of attention. Since then, numerous tissue-looking objects were made.

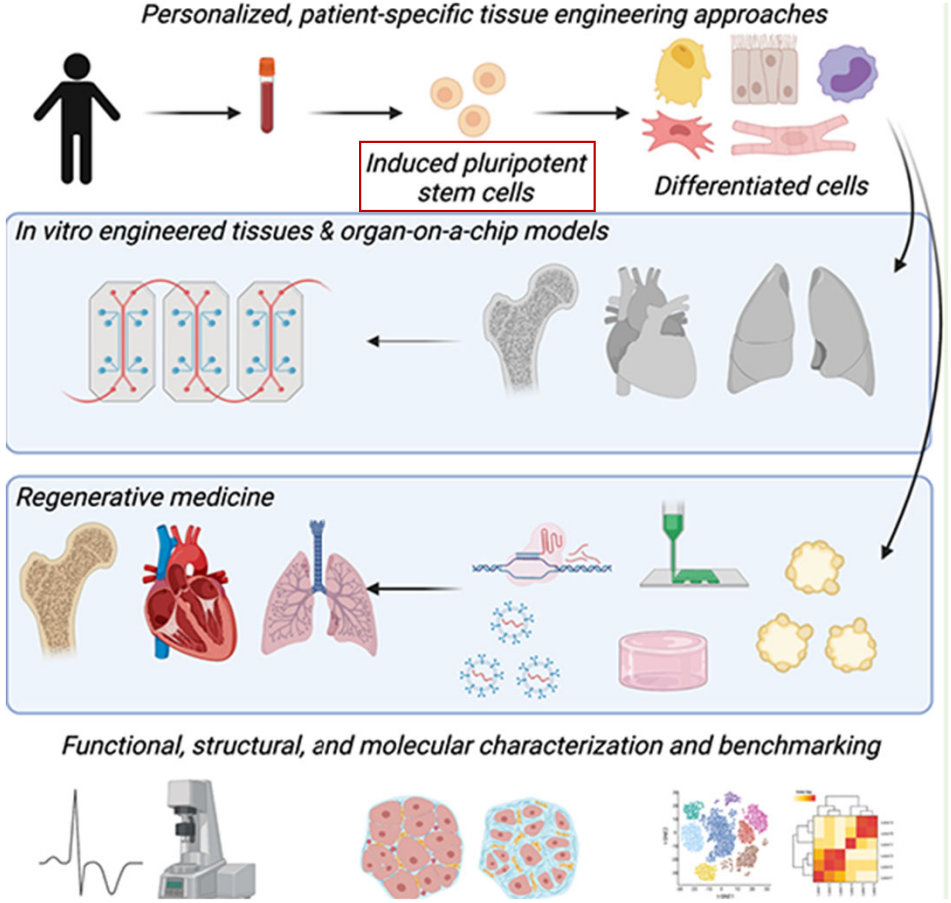
The difficulty here is that making something that looks like a human tissue or organ is one thing, but making it functional is entirely another.

Please remember that the whole purpose of tissue engineering is to make artificial tissues and organs. Until today, there are only a few products approved by the FDA, including artificial skins.

It is important to understand that after more than 4 decades of research on tissue engineering, the actual progress of developing artificial organs has been painfully slow. So, remember that a good idea is just an idea, unless it is translated into practical solution. There are many other good ideas, such as gene therapy, nanomedicine, etc., but all those still remain just ideas after decades of research. One could say that research is hard and takes time. Exactly. This is why you should not promote any particular research field from the outset, e.g., "We will develop a tissue-engineered heart in 10 years (a prominent scientist said this in 1986)" or "We will cure cancer using nanomedicine." Just do your research with conviction. You will be a better scientist.

Next Generation Tissue Engineers

The field of tissue engineering has evolved from its early days of engineering tissue substitutes to current efforts at building human tissues for regenerative medicine and mechanistic studies of tissue disease, injury, and regeneration. Advances in bioengineering, material science, and stem cell biology have enabled major developments in the field.



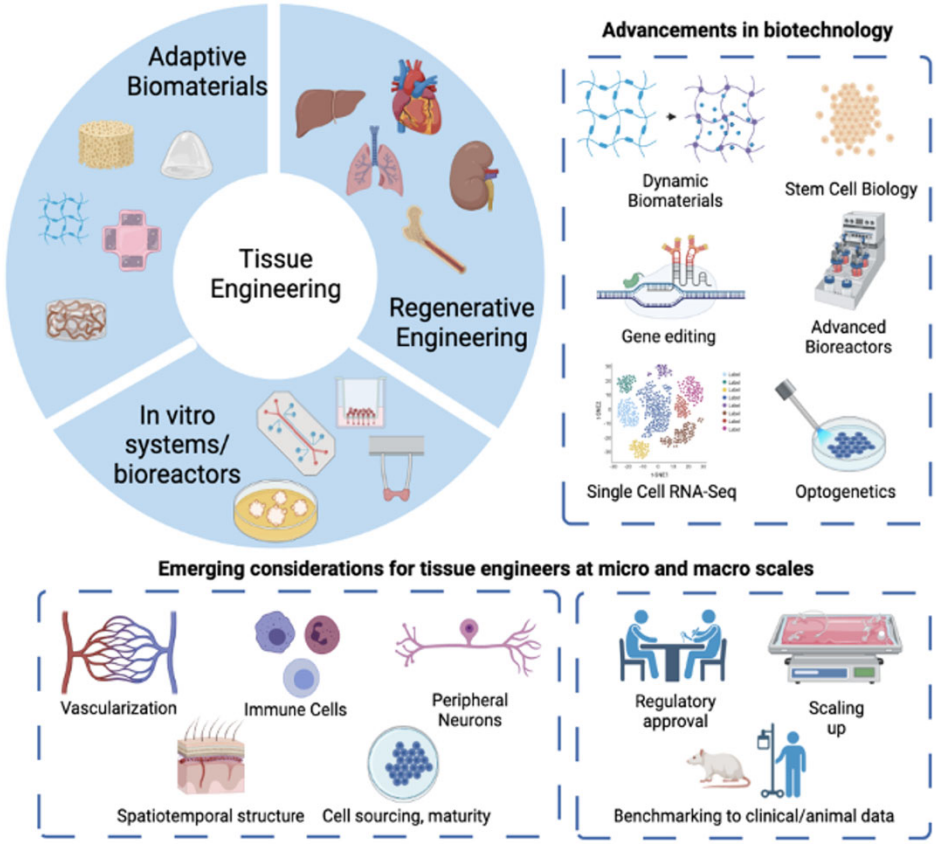


Figure 2. State of the field of tissue engineering. Innovations in biomaterials, regenerative engineering strategies, in vitro systems, and biotechnology have propelled the advancement of tissue engineering over the last 20 years. Emerging considerations for continued acceleration focus on building complexity into existing models and enabling translation to patients. Created with Biorender.com.

Tavakol 2021, Emerging Trajectories for Next Generation Tissue Engineers

Materials of Human Origin for Tissue Engineering

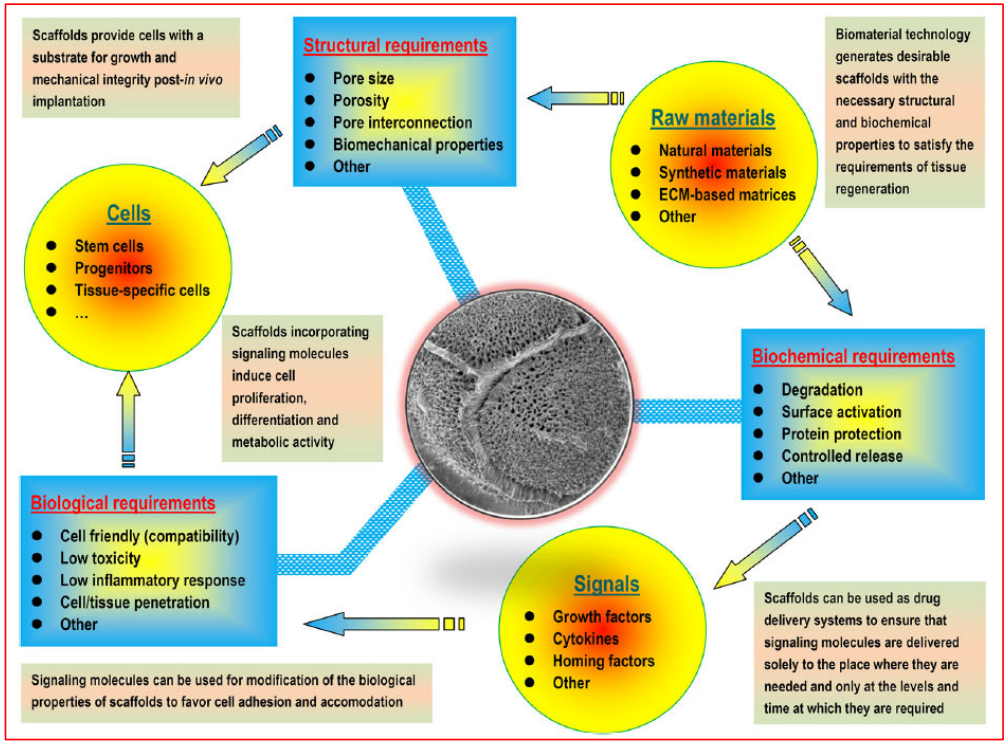


Fig. 2. Schematic representation of pivotal factors (structural, mechanical, biochemical and biological) involved in the design of biomaterials (templates) for tissue engineering that coax cells to behave in the same or a similar manner as their natural in vivo counterparts

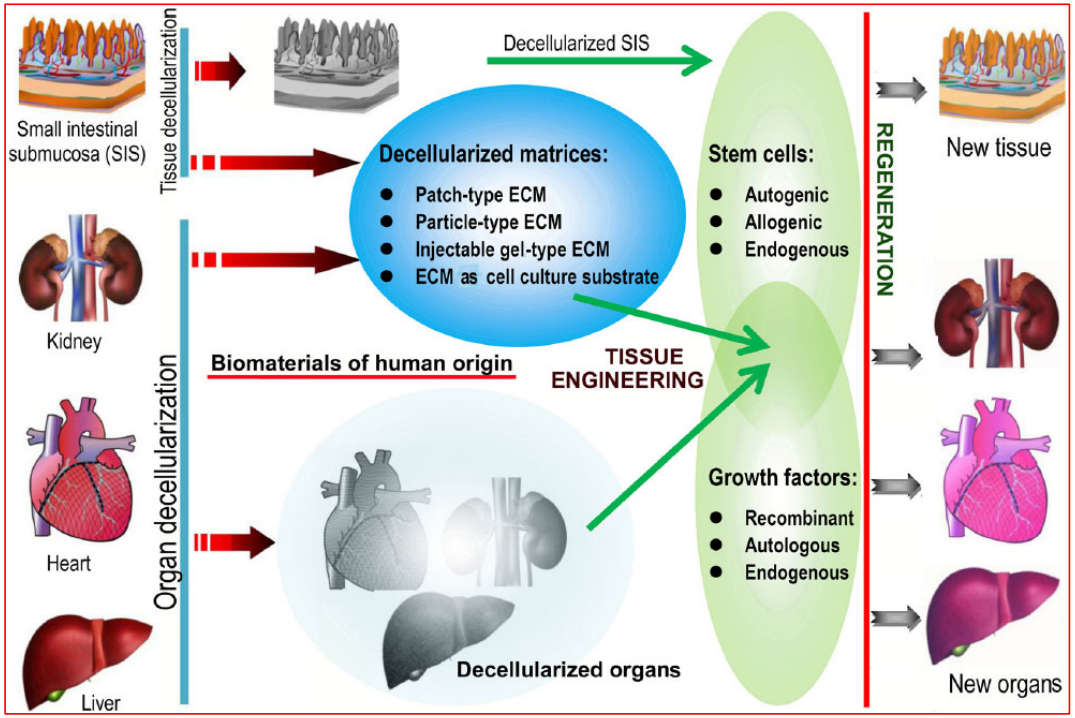


Fig. 12. Decellularized matrices from tissues (e.g., small intestinal submucosa (SIS)) or organs (e.g., kidney, heart and liver) that have native-like extra cellular matrix (ECM) microstructures, compositions and biomechanical properties (schematic is not to scale). These decellularized ECMs may maintain the shapes of the original tissues and organs when used as scaffolding materials in tissue engineering approaches for new tissue/organ regeneration. Alternatively, decellularized matrices derived from tissues and organs can be made into different types, such as a patch or particle, for tissue engineering scaffolding biomaterials or can be designed as an injectable gel for cell culture substrates [477].

Chen 2016, Advancing biomaterials of human origin for tissue engineering.
All references are available for you to download.

Synthetic & Natural Materials for Tissue Engineering

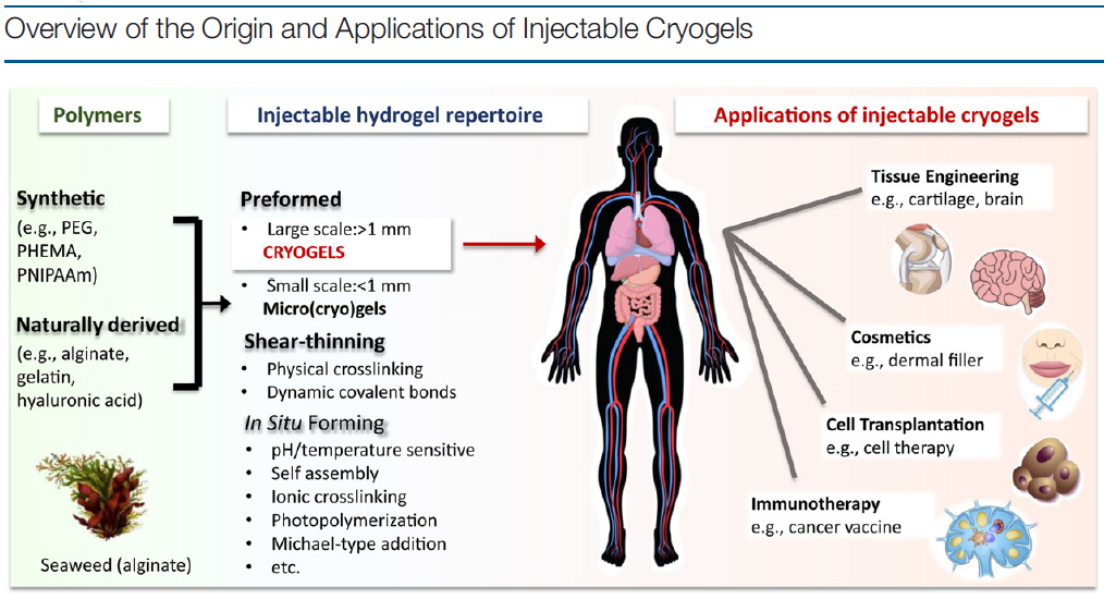


Fig. 1. Hydrogels can be fabricated using synthetic or naturally derived polymers. Within the injectable hydrogel repertoire, many in situ forming gels such as shear-thinning and small-scale gels have been developed. Cryogels are the only readily available large-scale, preformed, and injectable macroporous hydrogels with shape-memory properties, making them suitable for applications in tissue engineering, cosmetics, cell/drug delivery, and immunotherapy. Abbreviations: PEG, Polyethylene glycol; PHEMA, poly(2-hydroxyethyl methacrylate); PNIPAAm, poly(N-isopropylacrylamide).

Eggermont 2020, Injectable cryogels for biomedical applications

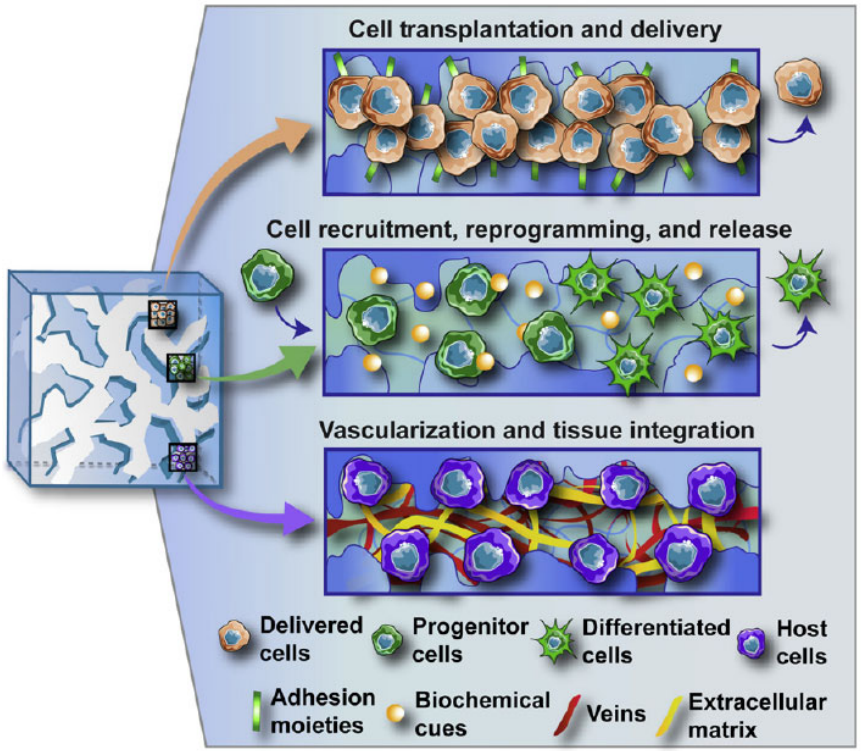


Figure 3.Cryogels Are Suitable Delivery Systems and Create a Confined Niche for Several Biomedical Applications. The Interconnected macropores are an ideal environment that allows cell attachment and protection during transplantation. Furthermore, the open macroporous structure facilitates recruitment and trafficking of cells. Finally, the inherent properties of cryogels promote neovascularization, stimulate native extracellular matrix formation, and facilitate tissue integration.

Gelatin Hydrogels for Cardiac Tissue Constructs

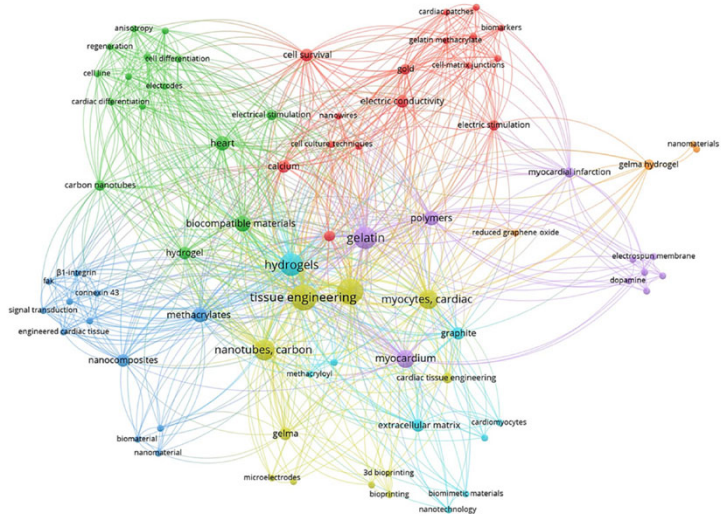


Fig. 1. Bibliometric map using PubMed data published between 2010 and 2023, searching for the keywords “GelMA” and “cardiac tissue” and “nanomaterial”, created with VOSviewer software version 1.6.16 [20].

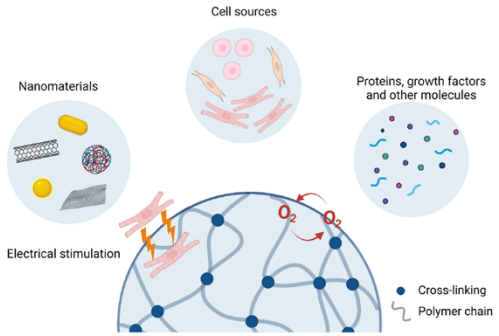


Fig. 3. Illustrative scheme identifying potential applications of hydrogels in tissue engineering. It is also possible to add growth factors and other molecules that will help the material’s communication and cell adhesion. Nanomaterials may also stimulate the electrical communication between cells, which is fundamental for tissues such as neural and cardiac.

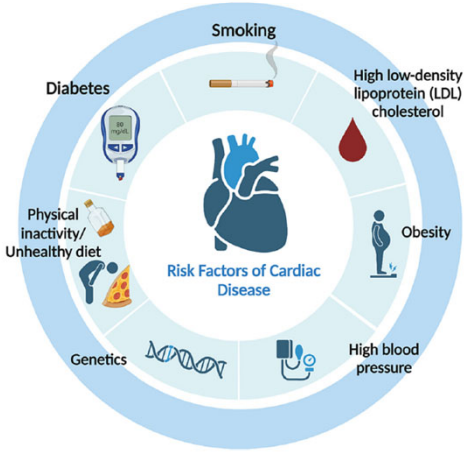


Fig. 2. Main cardiovascular disease risk factors: behavioral risk factors are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol and underlying determinants of CVDs as age, poverty, stress and hereditary factors.

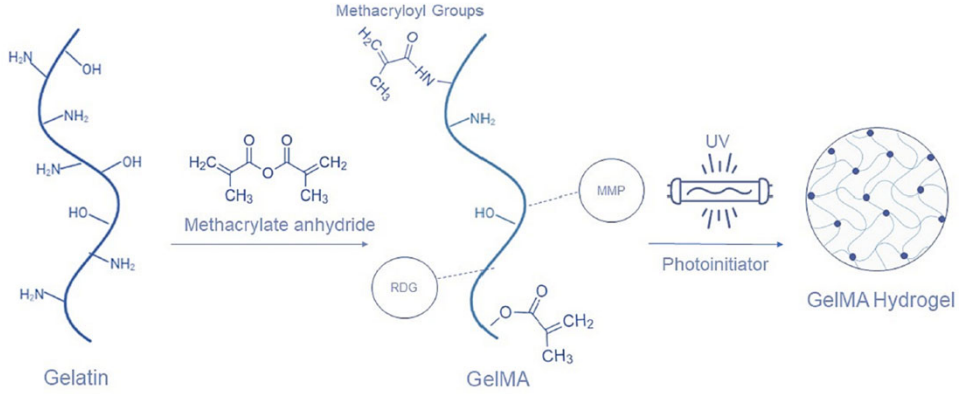


Fig. 4. Synthesis of methacrylated gelatin (GelMA). The primary amine groups of gelatin react with methacrylic anhydride (MA) to add methacryloyl pendant groups. Bioactives from gelatin, such as arginine-glycine-aspartic (RGD) and matrix metalloproteinases (MMP), are still kept in the GelMA chain (first reaction). To obtain the hydrogel, GelMA is crosslinked using UV irradiation in the presence of a photoinitiator (second reaction).

Porous Tissue Constructs using Marine Sponges

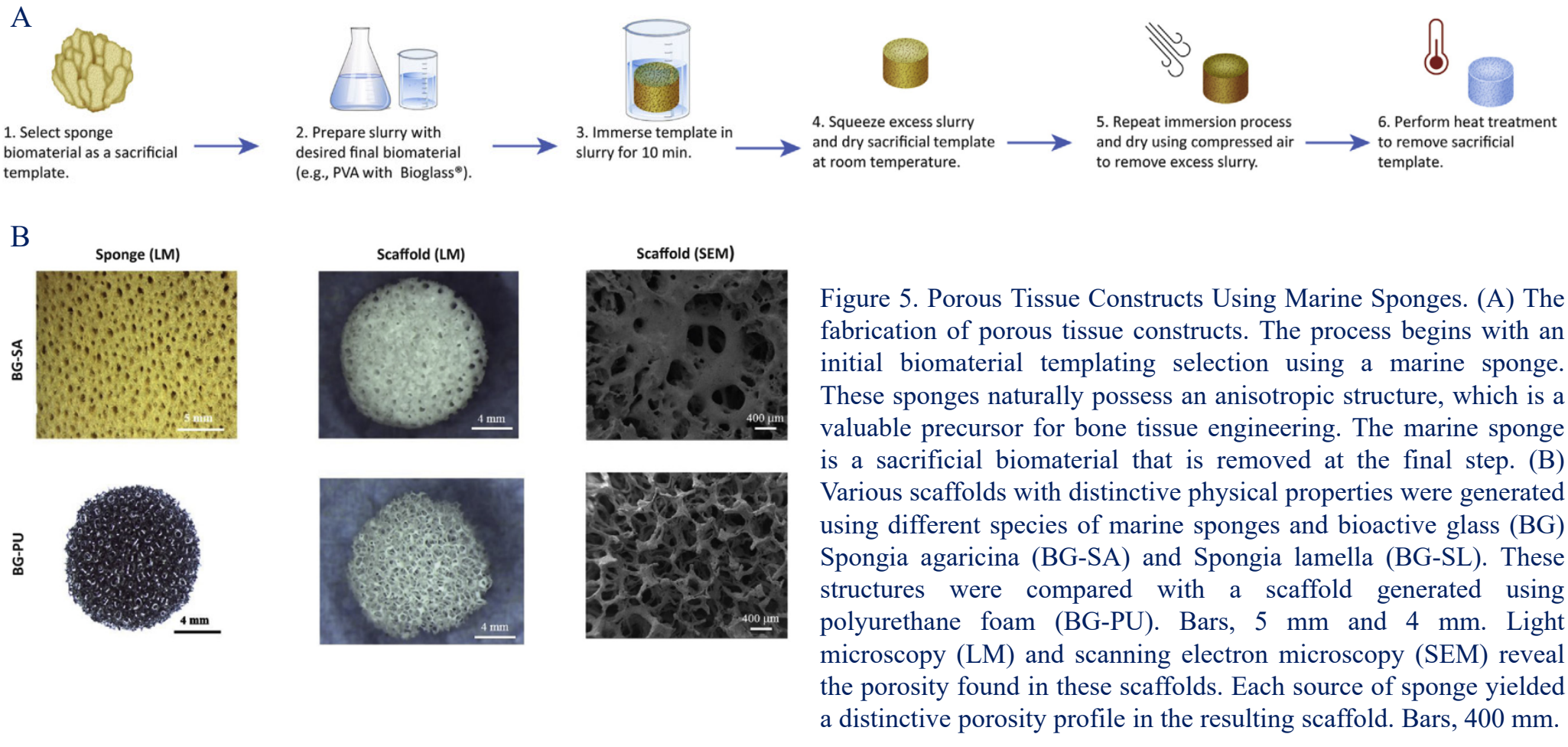
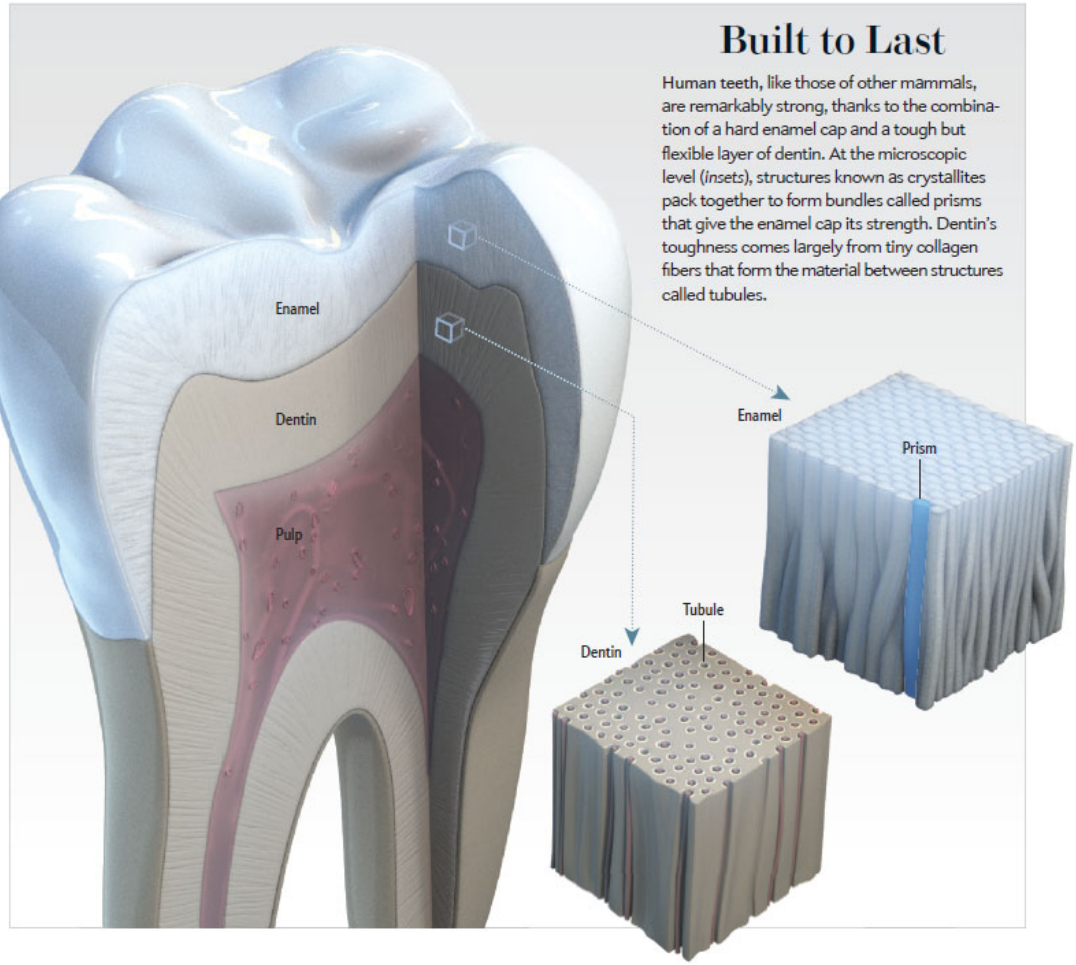


Figure 5. Porous Tissue Constructs Using Marine Sponges. (A) The fabrication of porous tissue constructs. The process begins with an initial biomaterial templating selection using a marine sponge. These sponges naturally possess an anisotropic structure, which is a valuable precursor for bone tissue engineering. The marine sponge is a sacrificial biomaterial that is removed at the final step. (B) Various scaffolds with distinctive physical properties were generated using different species of marine sponges and bioactive glass (BG) Spongia agaricina (BG-SA) and Spongia lamella (BG-SL). These structures were compared with a scaffold generated using polyurethane foam (BG-PU). Bars, 5 mm and 4 mm. Light microscopy (LM) and scanning electron microscopy (SEM) reveal the porosity found in these scaffolds. Each source of sponge yielded a distinctive porosity profile in the resulting scaffold. Bars, 400 mm.

Nguyen 2019, Unconventional tissue engineering materials in disguise

Human Teeth: Nature's Design



Ungr 2020, The trouble with teeth

Teeth figured heavily in the origin and early evolution of mammals because of their role in supporting warm-bloodedness (endothermy). Generating one's own body heat has a lot of advantages, such as enabling one to live in cooler climates and places with more variable temperatures; allowing one to sustain higher travel speeds to maintain larger territories; and providing stamina for foraging, predator avoidance and parental care. But endothermy comes with a cost: mammals burn 10 times as much energy at rest as reptiles of similar size do. Selective pressure to fuel the furnace has fallen on our teeth. Other vertebrates capture, contain and kill prey with their teeth. Mammalian teeth must wring more calories out of every bite. To do that, they must chew. Mammalian teeth guide chewing movements; direct and dissipate chewing forces; and position, hold, fracture and fragment food items. For teeth to function properly during chewing, their opposing surfaces must align to a fraction of a millimeter. The need for such precision explains why, unlike fishes and reptiles, most mammals do not just grow new teeth repeatedly throughout life when old ones wear out or break. Ancestral mammals lost that ability to facilitate chewing. **Enamel prisms** are part of the same adaptive package. Most researchers believe they **evolved to increase tooth strength to the level needed for chewing**. Whether the prisms evolved once or several times independently is a matter of some debate, but in any case, the basic mammalian tooth structure—a dentin crown capped by prismatic enamel—was in place in the Triassic period. The myriad forms of mammalian molars, including ours, followed as mere tweaks of the same general plan.

Hydroxyapatite-Binding Peptide

Understanding the Adhesion Mechanism of Hydroxyapatite-Binding Peptide

Tal Duanis-Assaf, Tan Hu, Maayan Lavie, Zhuo Zhang,* and Meital Reches*

Cite This: *Langmuir* 2022, 38, 968–978

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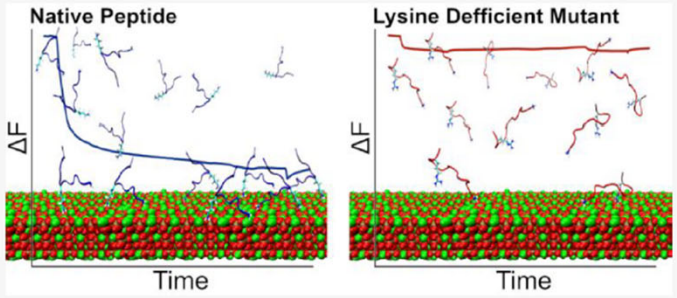
ABSTRACT: Understanding the interactions between the protein collagen and hydroxyapatite is of high importance for understanding biomineralization and bone formation. Here, we undertook a reductionist approach and studied the interactions between a short peptide and hydroxyapatite. The peptide was selected from a phage-display library for its high affinity to hydroxyapatite. To study its interactions with hydroxyapatite, we performed an alanine scan to determine the contribution of each residue. The interactions of the different peptide derivatives were studied using a quartz crystal microbalance with dissipation monitoring and with single-molecule force spectroscopy by atomic force microscopy. Our results suggest that the peptide binds via electrostatic interactions between cationic moieties of the peptide and the negatively charged groups on the crystal surface. Furthermore, our findings show that cationic residues have a crucial role in binding. Using molecular dynamics simulations, we show that the peptide structure is a contributing factor to the adhesion mechanism. These results suggest that even small conformational changes can have a significant effect on peptide adhesion. We suggest that a bent structure of the peptide allows it to strongly bind hydroxyapatite. The results presented in this study improve our understanding of peptide adhesion to hydroxyapatite. On top of physical interactions between the peptide and the surface, peptide structure contributes to adhesion. Unveiling these processes contributes to our understanding of more complex biological systems. Furthermore, it may help in the design of de novo peptides to be used as functional groups for modifying the surface of hydroxyapatite.



Duanis-Assaf 2022, Understanding the adhesion mechanism of hydroxyapatite-binding peptide

Understanding the interactions between the protein collagen and hydroxyapatite is of high importance for understanding biomineralization and bone formation. Here, we undertook a reductionist approach and studied the interactions between a short peptide and hydroxyapatite. The peptide was selected from a phagedisplay library for its high affinity to hydroxyapatite. To study its interactions with hydroxyapatite, we performed an alanine scan to determine the contribution of each residue. The interactions of the different peptide derivatives were studied using a quartz crystal microbalance with dissipation monitoring and with single-molecule force spectroscopy by atomic force microscopy. Our results suggest that the peptide binds via electrostatic interactions between cationic moieties of the peptide and the negatively charged groups on the crystal surface. Furthermore, our findings show that cationic residues have a crucial role in binding. Using molecular dynamics simulations, we show that the peptide structure is a contributing factor to the adhesion mechanism. These results suggest that even small conformational changes can have a significant effect on peptide adhesion. We suggest that a bent structure of the peptide allows it to strongly bind hydroxyapatite. The results presented in this study improve our understanding of peptide adhesion to hydroxyapatite. On top of physical interactions between the peptide and the surface, peptide structure contributes to adhesion. Unveiling these processes contributes to our understanding of more complex biological systems. Furthermore, it may help in the design of de novo peptides to be used as functional groups for modifying the surface of hydroxyapatite.

Scaffolds

Design Principles in Scaffolds

In tissue engineering, a central concept is the application of a temporary biomaterial scaffold at the defect site to facilitate healing that will provide some restoration of functionality. If this scaffold is used to carry precursor cells or other features that may induce a functional healing, the outcome potential may be further improved. In designing such solutions for tissue repair and replacement, the parameters that define the scaffold must be selected and optimized to provide the best possible outcome. If cells are to be used, this adds further design (and regulatory) complexity.

Given the mechanical support functions that are defined for a specific clinical application of a scaffold, together with the increasingly appreciated effect that mechanical parameters can have on host response and cell behavior, selecting materials with suitable mechanical parameters is usually the first step in scaffold design. Generally, metals and ceramics have high stiffness and strength (Fig. 30.2A and B), as do many composite materials containing these two major categories of biomaterials for scaffolding. Between the two, ceramics have the disadvantage of the risk for brittle fracture.

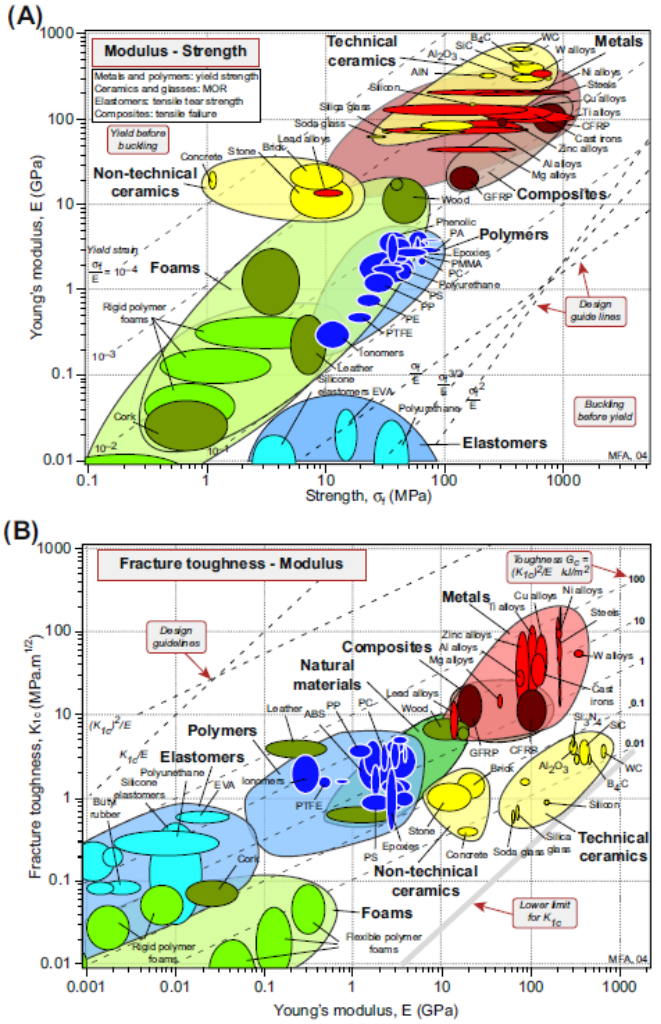


FIGURE 30.2 (A, B) Ashby chart of strength versus modulus. The "strength" for metals is the 0.2% offset yield strength. For polymers, it is the 1% yield strength. For ceramics and glasses, it is the compressive crushing strength, roughly 15 times larger than the tensile (fracture) strength. For composites, it is the tensile strength. For elastomers, it is the tear strength [22].

CFRP, carbon fiber-reinforced thermoplastic; EVA, ethylene(vinyl acetate); GFRP, glass fiber-reinforced plastic; MOR, modulus of rupture; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PMMA, poly(methyl methacrylate); PP, polypropylene; PS, phosphatidylserine; PTFE, polytetrafluoroethylene; WC, water cosolvent.

Zhu 2019, Design principles in biomaterials and scaffolds. Principles of Regenerative Medicine, Third Edition. https://doi.org/10.1016/B978-0-12-809880-6.00030-8

Design Principles in Scaffolds

In comparison, synthetic polymeric materials and naturally derived materials are usually weaker in all major aspects. However, polymers and naturally derived materials have lower densities; thus, the differences in specific modulus and specific strength between them and metals and ceramics are not as large. Because most human tissues have stiffness values below the gigapascal range (Fig. 30.2C) [21], metals and ceramics are commonly used in hard tissue replacements (bone and teeth) and applications in which smaller scaffold dimensions are desired (e.g., coronary stents), whereas polymers and naturally derived materials are more often used for soft tissue substitutes.

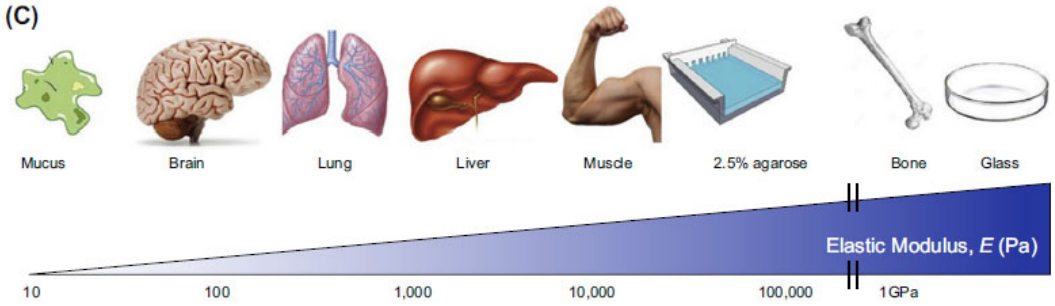


FIGURE 30.2 (C) Young's, or elastic, modulus of tissues [21].
CFRP, carbon fiber-reinforced thermoplastic; EVA, ethylene(vinyl acetate); GFRP, glass fiber-reinforced plastic; MOR, modulus of rupture; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PMMA, poly(methyl methacrylate); PP, polypropylene; PS, phosphatidylserine; PTFE, polytetrafluoroethylene; WC, water cosolvent.

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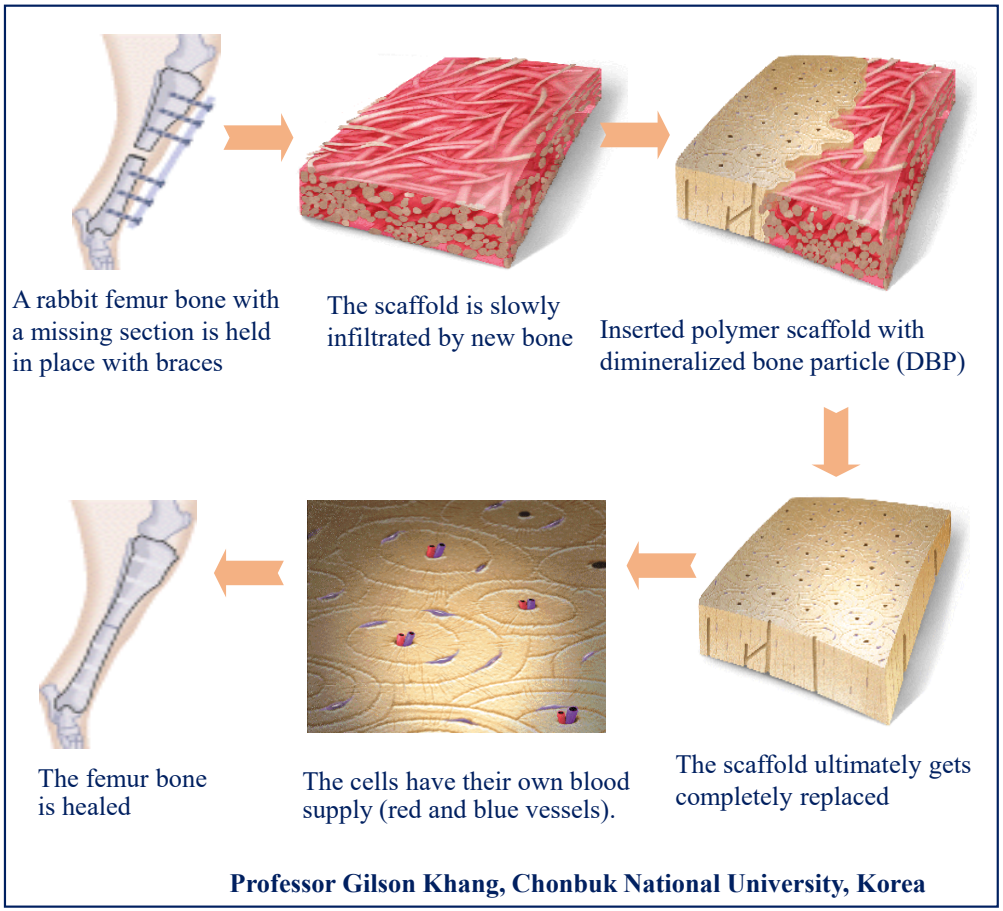


Injectable Hydrogels

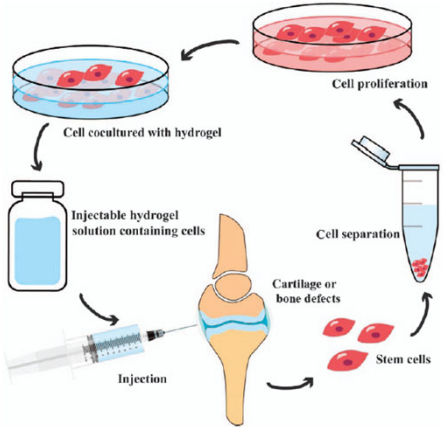


Figure 1. Schematic illustration of approaches to make injectable hydrogels for cartilage- and bone tissue-engineering applications.

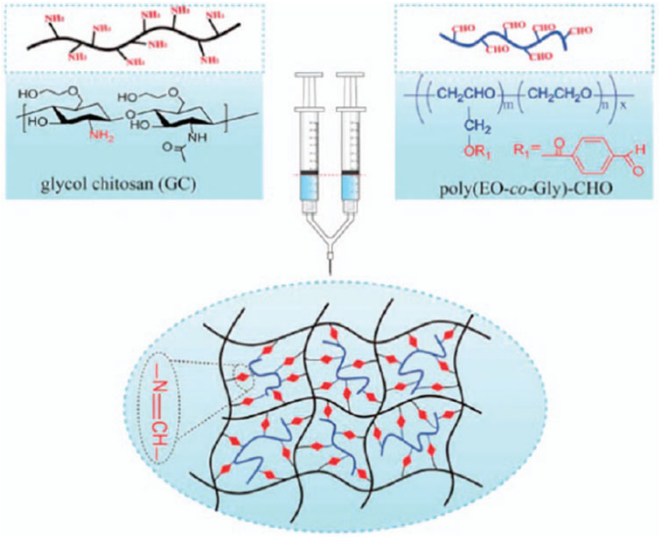


Figure 5. Schematic illustration of injectable hydrogels prepared by Schiff base cross-linking between aqueous solutions of GC and poly (EO-co-Gly)-CHO.230

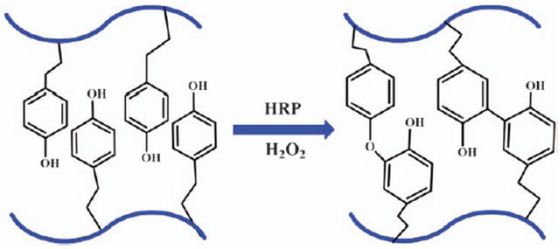


Figure 4. Schematic illustration of injectable hydrogels prepared by the enzymatic cross-linking method with horseradish peroxidase (HRP) and H_2O_2.

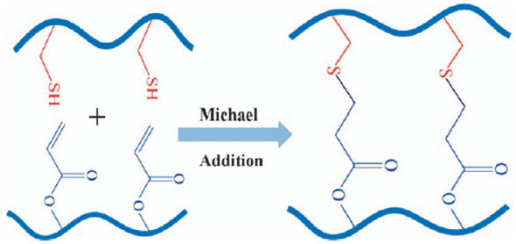


Figure 6. Schematic illustration of injectable hydrogels prepared by the Michael addition cross-linking method.

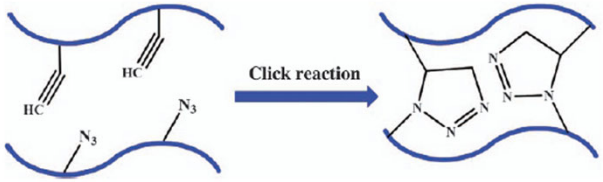


Figure 7. Schematic illustration of injectable hydrogels prepared by click chemistry.

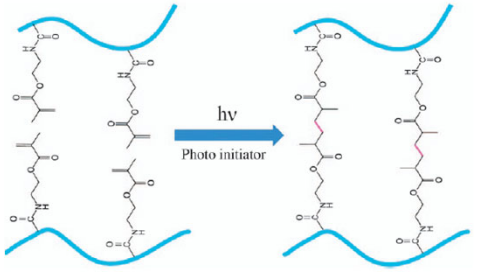


Figure 8. Schematic illustration of injectable hydrogels prepared bythe photo-cross-linking method.

Liu 2017, Injectable hydrogels for cartilage and bone tissue engineering

Functional Semi-Interpenetrating Polymeric Materials

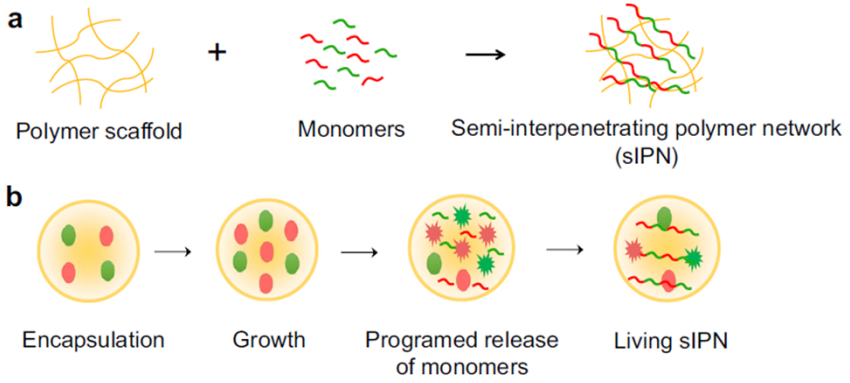


Fig. 1 Conventional and living fabrication of semi-IPN. a A semi-interpenetrating polymer network (sIPN) is defined as a linear or branched polymer in the presence of another cross-linked polymer. sIPN can be assembled by dissolving monomers (for the 2nd component) in a crosslinked scaffold (the 1st component), before initiating polymerization. b Living fabrication of sIPN using engineered bacteria. When encapsulated inside a polymeric microcapsule (the 1st component, cross-linked), the engineered bacteria can undergo autonomous lysis at a high local density. Lysis releases protein monomers that can react to form the polymerized protein (the 2nd component, polymerized but not necessarily fully crosslinked) inside the polymeric scaffold.

Dai 2021, Living fabrication of functional semi-interpenetrating polymeric materials

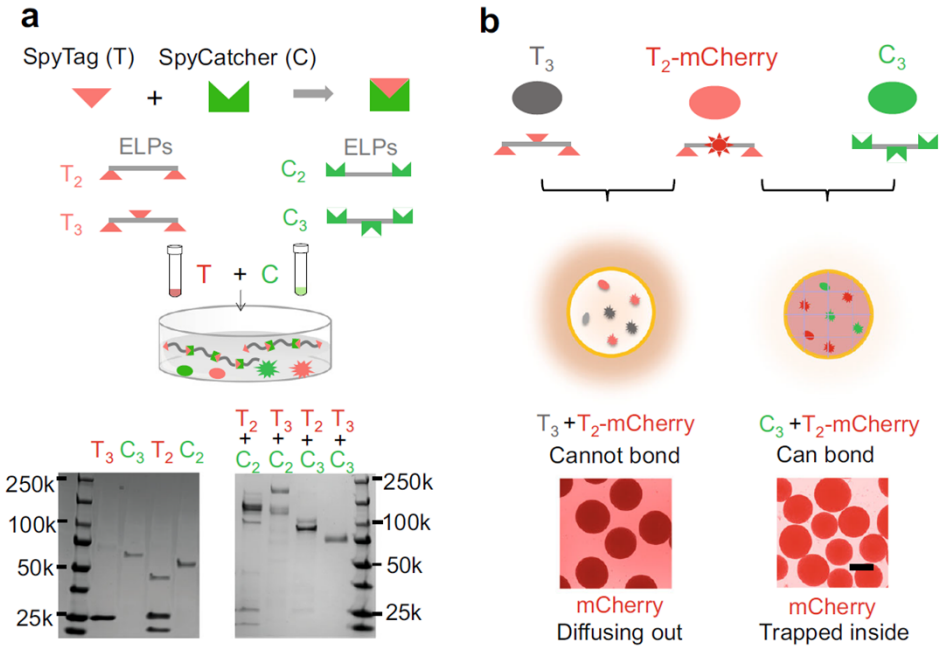


Fig. 2 Formation of living sIPN.

a Formation of protein complexes by elastin-like polypeptides (ELPs) monomers containing SpyTags and SpyCatchers released from engineered bacteria (top: schematic; bottom: experimental data). ELPs decorated with multiple SpyTags or SpyCatchers released by the cocultured bacteria reacted to form covalent bonds in the supernatant. Overnight culture of bacteria expressing proteins with multiple SpyTags (T, MC(T_2- mCherry) or MC(T_3)) and bacteria expressing proteins with multiple SpyCatchers (C, MC(C_2) or MC(C_3)) were co-cultured in M9 medium containing 1 mM IPTG. After 24 h, the supernatant was harvested, purified by His-tag affinity resins and tested by SDS–PAGE. Compared with the monomers (left), all four combinations between SpyTag and SpyCatcher generated protein complexes with higher molecular weight, indicating reaction occurred between monomers. The experiment was repeated more than three times independently with similar results.

b Stronger mCherry signal inside living sIPN capsules containing compatible monomers released by engineered bacteria (top: schematic; bottom: experimental data). MC(T_2-mCherry) and MC (C_3) were mixed, pelleted and encapsulated with chitosan. As a control, MC(C_3) was replaced with MC(T_3). The capsules were cultured in M9 medium containing 1 mM IPTG. Due to the sIPN formation, the mCherry protein was immobilized inside the capsules, leading to strong fluorescence of the capsules but little in the surrounding medium (right). In the control group, the mCherry protein was not trapped due to the lack of sIPN formation, and diffused out to the surrounding medium (left). The scale bar is 200 μm and the photos were taken at 24 h. The experiment was repeated more than three times independently with similar results.

Unconventional Tissue Engineering Materials

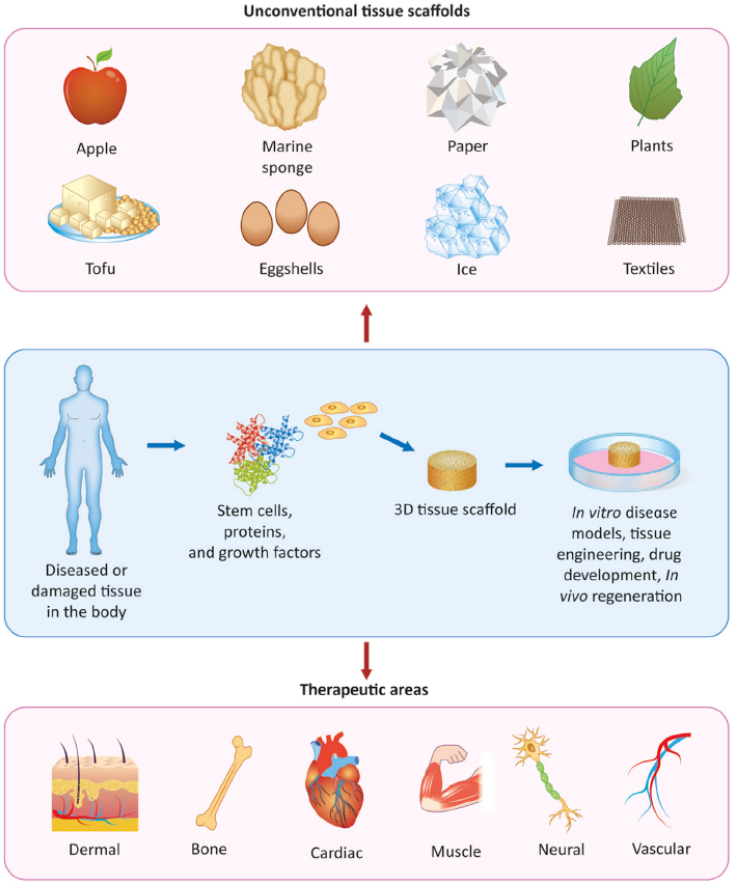


Figure 1. These approaches can cover a plethora of therapeutic and research areas including dermal, bone, cardiac, muscle, neural, and vascular tissues.

Nguyen 2019, Unconventional tissue engineering materials in disguise

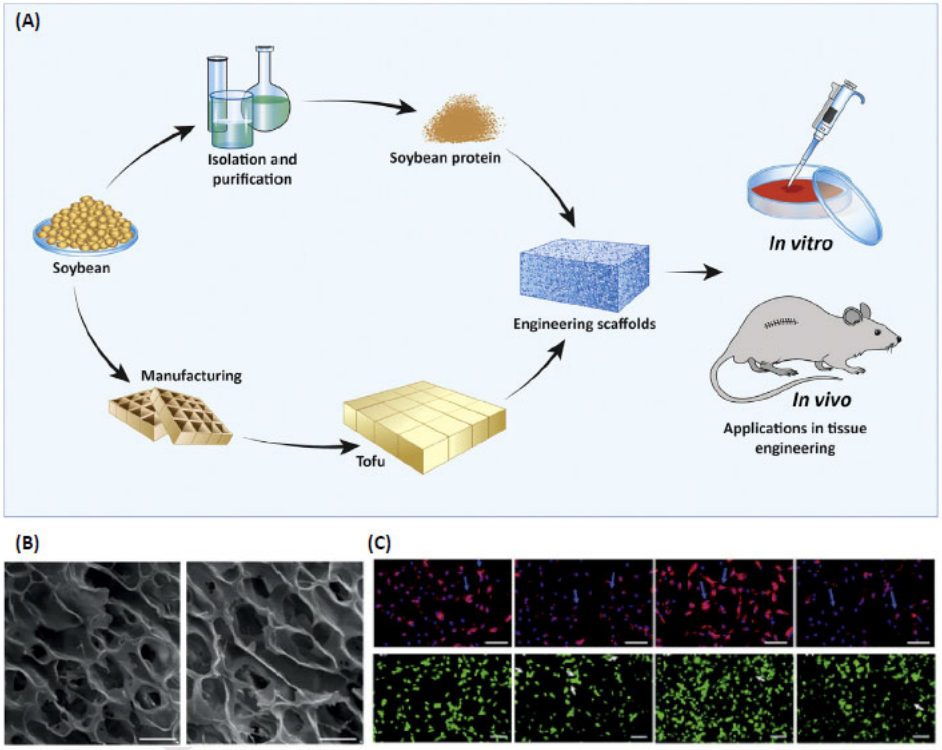


Figure 6. Biomimetic Protein Scaffolds from Tofu. (A) A schematic diagram illustrating the fabrication process from soybean to tofu-based engineered scaffold. The scaffold was seeded with 3T3 cells and cultured in vitro. The 3D construct was also implanted into mice for in vivo testing, which did not elicit any significant immune response, demonstrating biocompatibility with the host organism. (B) Scanning electron microscopy images that reveal the porosity achieved in tofu and soybean protein scaffolds. As shown, similar physical properties were achieved using two different soy-based derivatives. Bars, 20 μm. (C) A 40,6-diamidino-2-phenylindole (DAPI) staining of 3T3 cells seeded on these scaffolds and live/dead staining of these tissue constructs. Blue arrows indicate good cell morphology and white arrows point to dead cells shown in the assay after various time points in culture. High cell viability and compatibility were achieved using these scaffolds. Bars, 50 μm. (A–C) Adapted, with permission, from [54].

Conductive Biomaterials

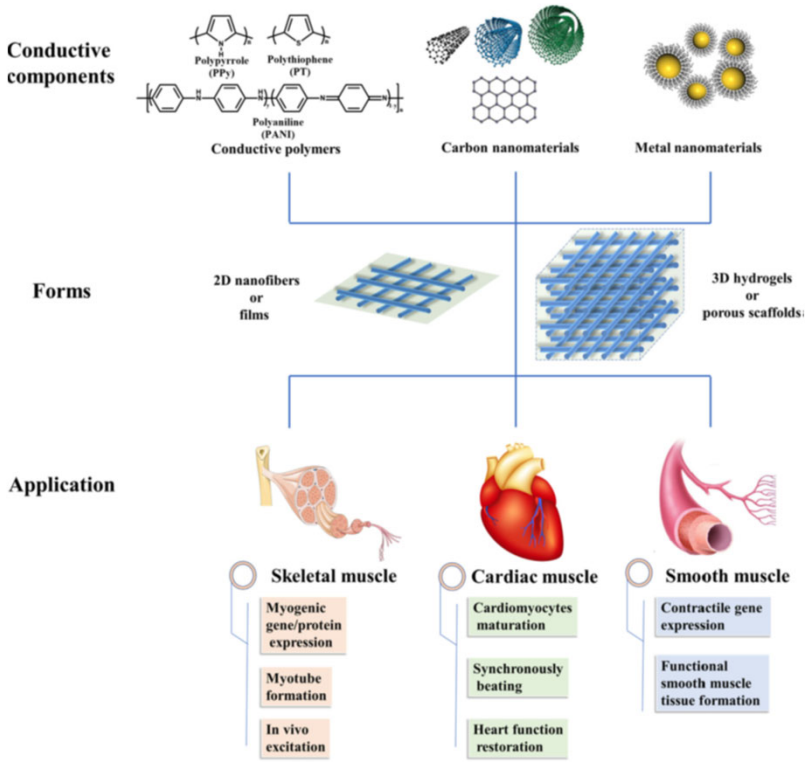


Fig. 1. Schematic illustration of conductive biomaterials' fabrication, forms and their application in muscle tissue repair.

Dong 2020, Conductive biomaterials for muscle tissue engineering

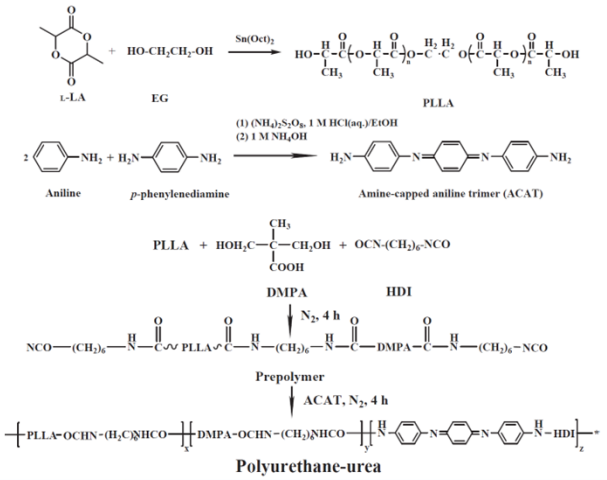


Fig. 3. Synthetic methods of PLLA, ACAT, and PUU copolymer.

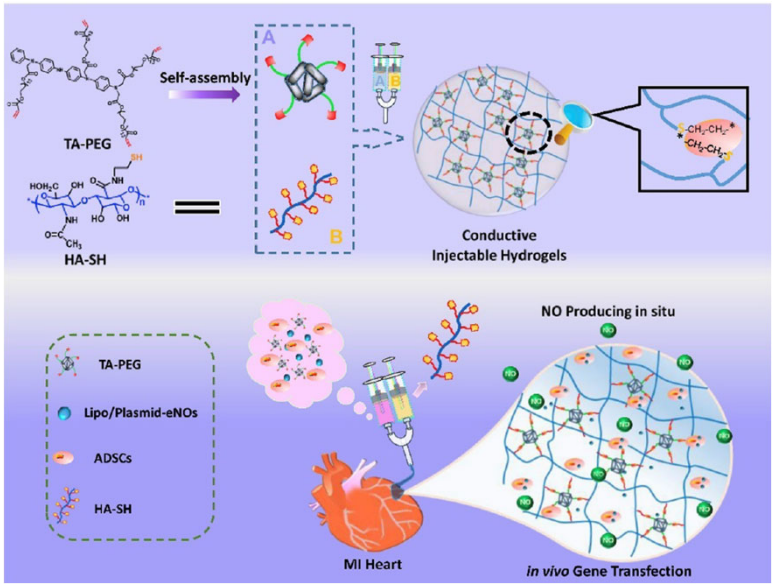


Fig. 4. Schematic sketch showing how to construct the conductive injectable hydrogel. TA-PEG is soluble in water and the hydrogel can form in aqueous solution. DNA-eNOs nanoparticles and ADSCs are encapsulated inside this hydrogel to treat myocardial infarction.

Lignin

Lignin forms key structural materials in the support tissues of most plants.[1] Lignins are particularly important in forming cell walls, especially in wood and bark, because of their rigidity and stability. Lignin is an amorphous biomacromolecule with a highly complex structure. It is composed of three phenylpropane units of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H). These structural units of lignin are cross-linked mainly via aryl ether linkages viz. β−O−4′ and carbon−carbon linkages such as β−5′, β−β′, and 5−5′.

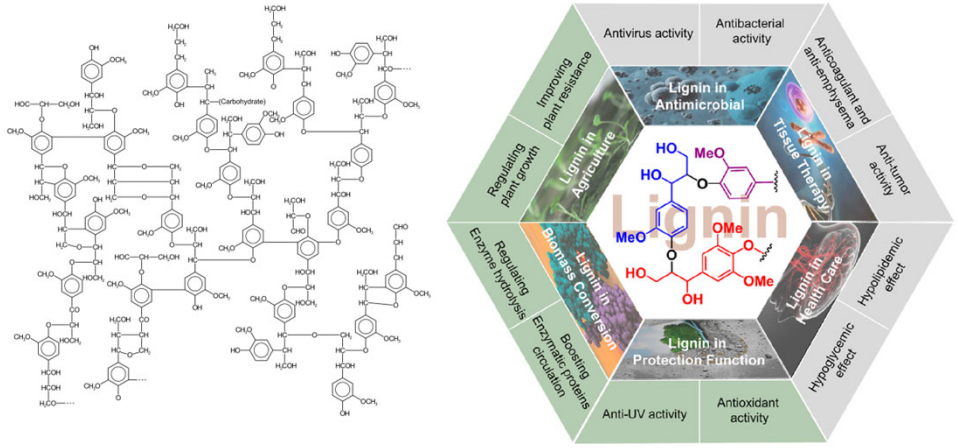


Figure 1. Biological activities and effective utilizations of lignin.

Lignin

- Antibacterial Activity, Antiviral Activity
- Antitumor Activity
- Anticoagulant and Antiemphysema Activity of LMW Lignin
- Hypoglycemic Effect
- Hypolipidemic Effect.

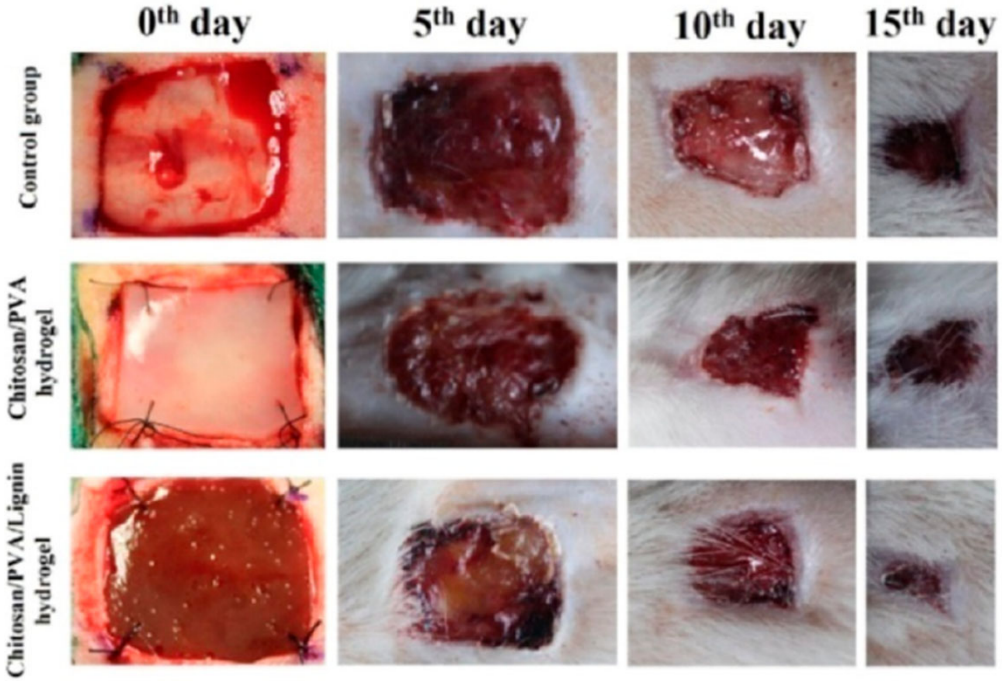


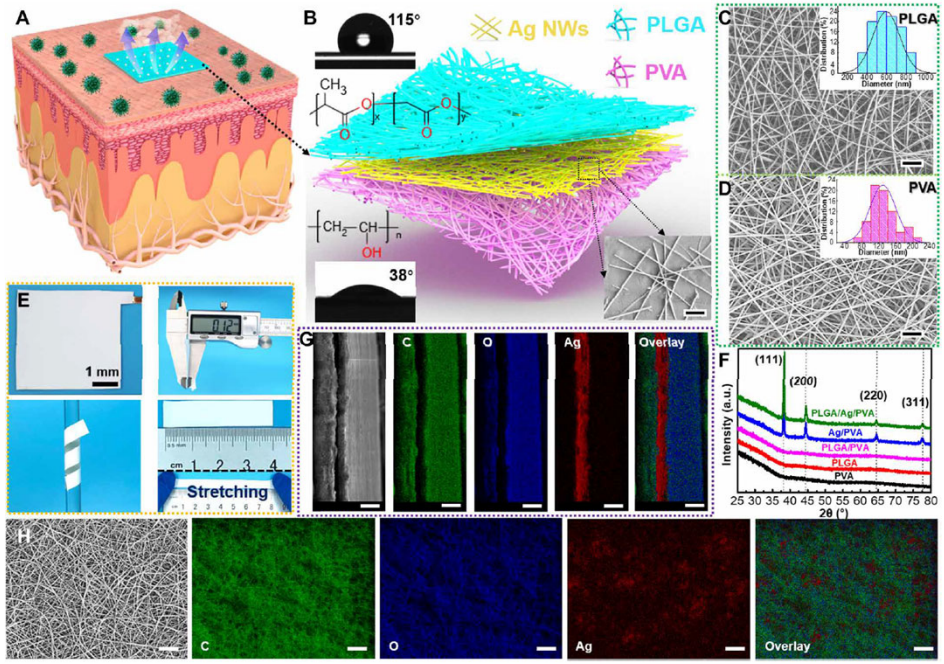
Figure 3. (a) Wound healing status of a blank control group, chitosan−PVA hydrogel, and chitosan−PVA−lignin hydrogel (lignin 10 wt %) at days 0, 5, 10, and 15.

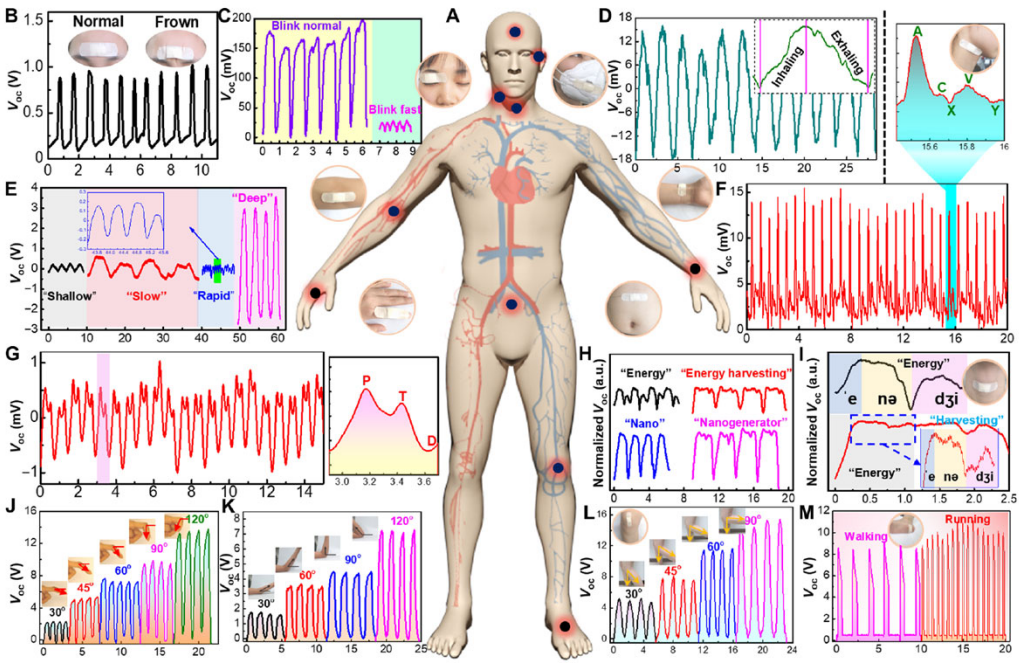
Shu 2021, Biological activities and emerging roles of lignin and lignin-based products

Self-powered Electronic Skin

A breathable, biodegradable, antibacterial, and self-powered electronic skin based on all-nanofiber triboelectric nanogenerators

Mimicking the comprehensive functions of human sensing via electronic skins (e-skins) is highly interesting for the development of human-machine interactions and artificial intelligences. Some e-skins with high sensitivity and stability were developed; however, little attention is paid to their comfortability, environmental friendliness, and antibacterial activity. Here, we report a breathable, biodegradable, and antibacterial e-skin based on all-nanofiber triboelectric nanogenerators, which is fabricated by sandwiching silver nanowire (Ag NW) between polylactic-co-glycolic acid (PLGA) and polyvinyl alcohol (PVA). With micro-to-nano hierarchical porous structure, the e-skin has high specific surface area for contact electrification and numerous capillary channels for thermal-moisture transfer. Through adjusting the concentration of Ag NW and the selection of PVA and PLGA, the antibacterial and biodegradable capability of e-skins can be tuned, respectively. Our e-skin can achieve real-time and self-powered monitoring of whole-body physiological signal and joint movement. This work provides a previously unexplored strategy for multifunctional e-skins with excellent practicability.

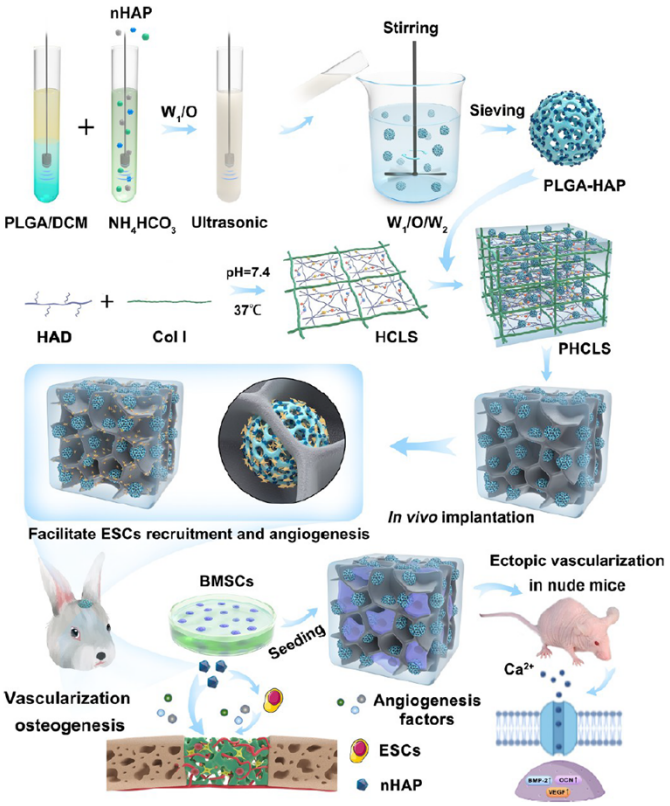




Peng 2020, A breathable, biodegradable, antibacterial, and self-powered electronic skin

Microporous Hydrogel Scaffolds

Hybrid cross-linked hierarchical microporous hydrogel scaffold



Scheme 1. Illustration of a schematic for the creation of a hybrid cross-linked hierarchical microporous hydrogel scaffold (PHCLS) using poly(lactic-co-glycolic)/nanohydroxyapatite (PLGA-HAP) open porous microspheres and an interconnected hybrid cross-link network made of multireactive polyphenols that serve as links between organic and inorganic componentsa[a]

[a] The hybrid cross-linked hierarchical microporous structure within the PHCLS could not only enhance mechanical strength and structural stability of the scaffold but also promote osteogenesis by facilitating ESC recruitment and angiogenesis. Additionally, the in vivo investigation revealed that the PHCLS might speed up bone repair following implantation into the critical-sized skull defect model in rabbits and the subcutaneous model of mice.

Lu 20213, Polysaccharide-based composite hydrogel with hierarchical microstructure

Adhesive hydrogels

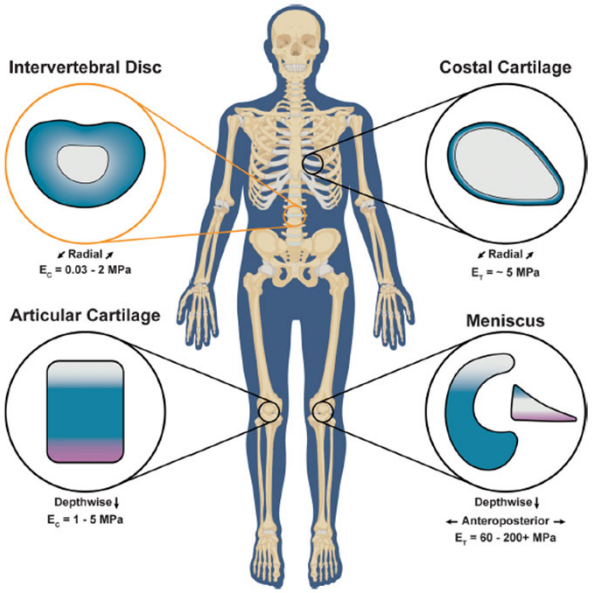


Figure 1. Cartilage tissues throughout the body exhibit regional properties (e.g., depthwise and radial). These regional properties give rise to unique mechanical functions to support articulation and load bearing. Representative intervertebral disc (IVD)-like construct illustrated in this work (orange). *EC* = compressive modulus and E_T = tensile modulus.

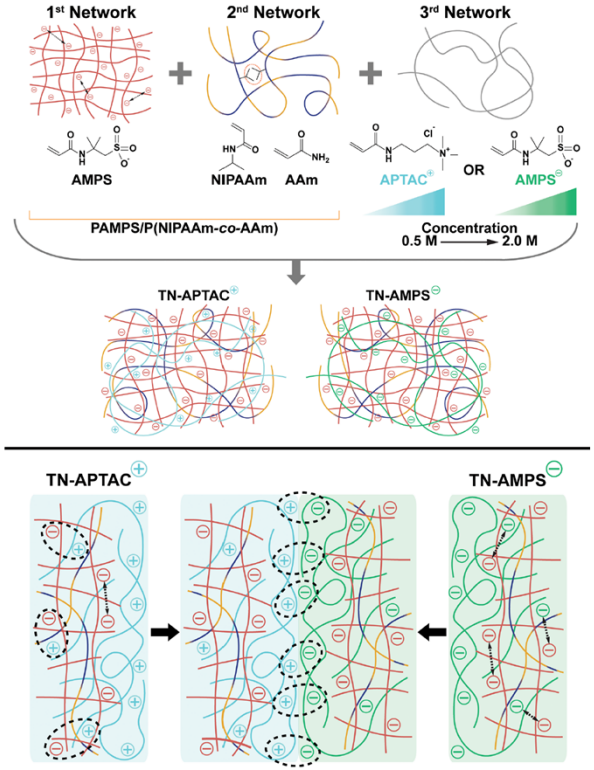


Figure 2. Top: Triple network (TN) hydrogels were fabricated with either a cationic TN- poly((3-acrylamidopropyl)-trimethylammonium chloride) (PAPTAC) or anionic (TN-AMPS) 3rd network, wherein the concentration of APTAC or poly(2-acrylamido-2-methylpropane sulfonic acid) (AMPS) was tuned (0.5−2.0 M). Bottom: the 3rd network in TN hydrogels drives surface charge, enabling adhesion between the two types via electrostatic attractive forces.

Demott 2023, Adhesive hydrogel building blocks to reconstruct complex cartilage tissues

3D & 4D Bioprinting

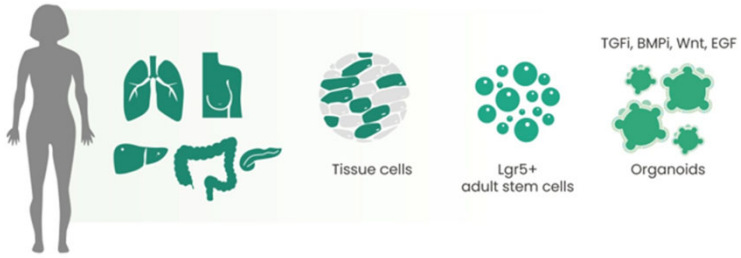
Exploring New Frontiers: Organoids in Cancer Research

In the field of in-vitro 3D culture technologies, specifically organoids, recent advances have revolutionised the development of more physiologically relevant human cancer models. These models are crucial for translating basic cancer research into effective treatment strategies for patients with cancer. One significant advantage of organoids is that they can be cultured from healthy tumor tissues obtained from individual patients. This approach enables physicians to conduct patient-specific drug testing, the results of which support development of personalised treatment regimens.

Traditionally, cancer-drug screening has relied on two-dimensional cell-culture models and patient-derived tumor xenograft (PDX) models. Although these models have their advantages, they fail to capture the complexity and heterogeneity of tumors in a three-dimensional context. Moreover, PDX models are expensive and time-consuming, which limits their utilisation. The development of tumor organoids has revolutionised cancer research by providing more physiological and personalised models for cancer research. Given the ability of these models to mimic the tumor microenvironment, their potential for patient-specific drug testing, and their advantages over traditional models, organoids have become a promising approach to improved cancer-treatment strategies.

Breakthroughs for the Use of Organoid Models

In 2009, Sato et al. described the ability to indefinitely culture 3D organoids in Corning Matrigel matrix for organoid culture (Corning Inc., US) from single Lgr5-positive intestinal stem cells under defined culture conditions that artificially provide niche factors (e.g., R-spondin, noggin, epidermal growth factors). Intestinal organoids were successfully established, and the next five years saw the establishment of numerous organoid culture protocols, such as for the colon and small intestine, retina, brain, liver, stomach, and breast. In 2014, the research of Gao et al. and Li et al. led to breakthroughs for using organoid models in cancer research. The researchers performed comprehensive genomic analysis, which showed that the organoids closely recapitulated in vivo prostate cancers in copy-number alterations, gene-expression subtypes, and histological patterns. Subsequently, researchers successfully constructed colorectal cancer, gastric cancer, prostate cancer, bladder tumors, esophageal cancer, endometrial cancer, and other tumor organoid models.



Establishment of numerous organoid culture methods from various organs.

Cell-Culture Models

Compared with traditional tumor research models, tumor organoids can more accurately reflect the tumor microenvironment. They not only can intuitively show the tumor growth process, but also reflect individual differences in patients. Other advantages include closer to physiological cells, stable genome, and suitability for biological transfection and high-throughput screening (Table 1). Overall, tumor organoids provide an organ-level research system that is intuitive, reliable, efficient, and avoids ethical disputes.

Table 1: Comparison of three major models

	Animal Models	2D Cell Culture	3D Organoids
High-throughput	Limited	Yes	Yes
Manipulability	Limited	Excellent	Good, but may have experimental variability
Biobanking	Only at the cellular level	Yes	Yes
Gene editing	Requires generation of embryonic stem cells	Yes	Yes
Modeling organogenesis	Limited by complex tissue environment	Lack cell-cell and cell-matrix interactions	Suitable for studying of cell-cell communication; reduced complexity
Modeling human development and disease	Yes	Poor	Yes

Cell-Culture Models

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Importance of Cytokines in Organoid Culture

Successful culture of organoids requires specific items, such as Matrigel matrix and culture media, as well as essential factor cytokines. The categories of these cytokines mainly include activators, inhibitors, and hormones that promote cell growth, differentiation, and signaling pathways; cytokines that promote cell proliferation; and cytokines added to improve the success rate of organoid culture. Different organoid cultures survive in medium supplemented with growth factors relevant to the organ of interest. For example, mouse small-intestinal organoid cultures survive in medium supplemented with the growth factors noggin, R-spondin 1 or R-spondin 3, and epidermal growth factor (EGF), and the addition of exogenous Wnt is often not needed because Paneth cells in culture produce sufficient Wnt for organoid proliferation.

https://www.scientistlive.com/content/exploring-new-frontiers-organoids-cancer-research-0?dm_i=371,8ISXC,BN3KXL,Z947Q,1 23rd January 2024

3D Bioprinting

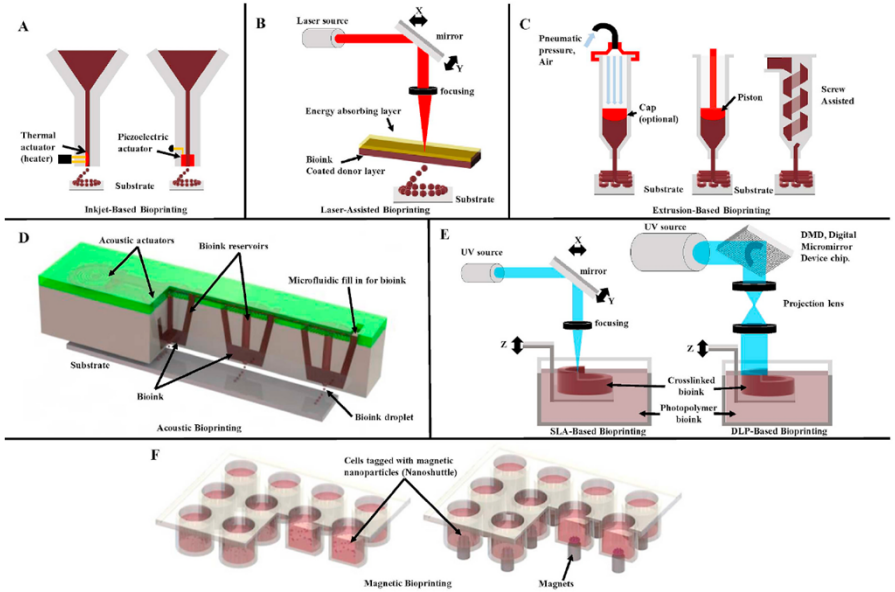
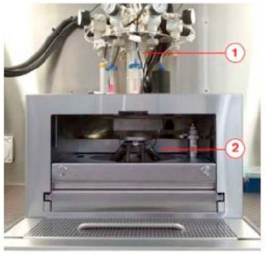


Fig. 1. Schematic figures showing the Bioprinting modalities. (A) Inkjet Bioprinting systems including Thermal and Piezoelectric Drop-On-Demand (DOD) based mechanisms. (B) Laser-Assisted Bioprinting system; Laser-Induced Forward Transfer mechanism. (C) Extrusion-based Bioprinting systems including Pneumatic pressure, Piston and Screw assisted mechanisms. (D) Acoustic Bioprinting system; a type of DOD mechanism. (E) Stereolithography Bioprinting systems including SLA and DLP (Digital Light Processing) Laser-based mechanisms. (F) Magnetic Bioprinting system; cells are shown within the culture media.

Fig. 2. In situ skin bioprinting prototype and gross examination of printed skin in murine full-thickness excision wound repair.



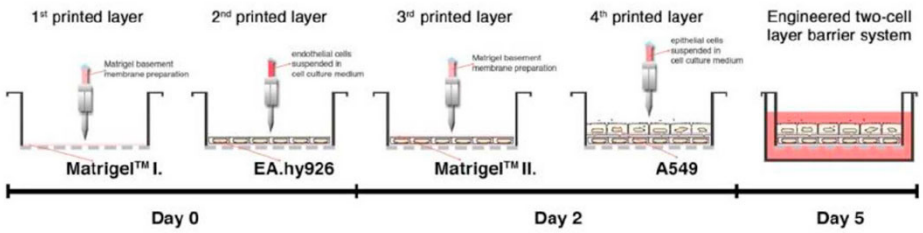


Fig. 7. Biofabrication of the 3D in vitro air-blood tissue barrier and examination of functional-structural relation of the bioprinted constructs. (A) Main process unit of bioprinter- BioFactory®, tool changer (1) with multiple print heads and a building platform (2). (B) Schematic for the bioprinting of two-cell layer barrier system at predefined times. For the manual co-culture assembly, a similar timeline is followed, however made manually, i.e. by manually pipetting the ECM/cell layers.

Matai 2020, Progress in 3D bioprinting technology for tissue-organ regenerative engineering

3D Jet Writing of Mechanically Actuated Tandem Scaffolds

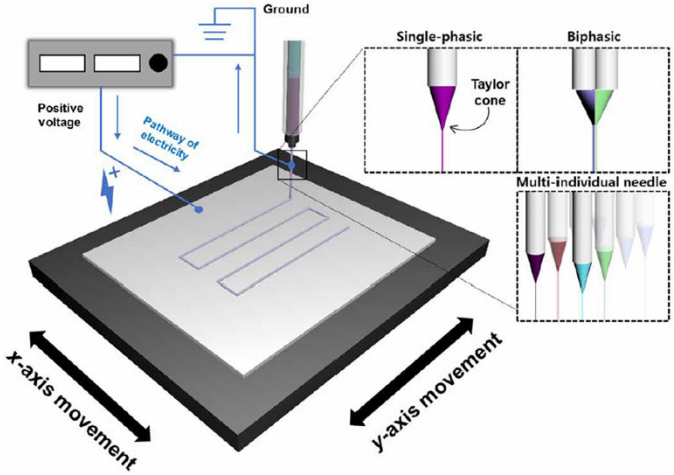


Fig. 1. Schematic diagram of the CREW system. The CREW system for fabrication of a fiber-based scaffold using the moving stage. The positive voltages are applied to the conductive collector. The blue lines represent pathway of electricity.

Fig. 2. Mechanism of a straight jet. Digital images of Taylor cones for (A) a single-phasic microfiber and (B) a biphasic microfiber. PLGA was used for the single-phasic microfiber; PLGA and TPU were used for the biphasic microfiber. Schematic diagrams of the forces applied to the polymeric jet for (C) the conventional jetting system and (E) the CREW system. SEM images of grid patterns that are produced by (D) the conventional jetting system and (F) the CREW system. Scale bars, 300 μm.

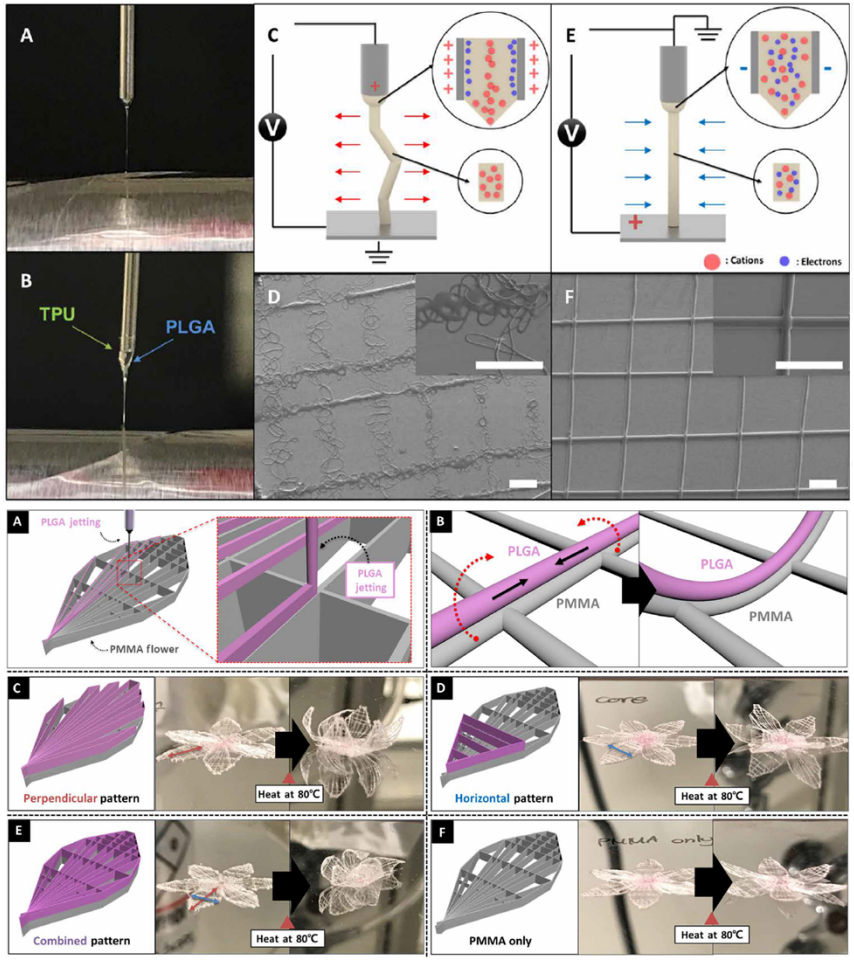


Fig. 5. Tandem scaffold. (**A**) Schematic diagram of the design of tandem scaffolds using PMMA and PLGA, where the PLGA was precisely positioned onto a PMMA pattern; (**B**) schematic diagram of the mechanism of bending motion of a PLGA/PMMA bilayered pattern at elevated temperature (here, 80°C); schematic diagram and digital photo of before and after heat treatment depending on its PLGA pattern, (**C**) perpendicular pattern, (**D**) horizontal pattern, (**E**) combined pattern, and (**F**) PMMA only. (Photo credit: Seongjun Moon, Chungnam National University.)

The need for high-precision microprinting processes that are controllable, scalable, and compatible with different materials persists throughout a range of biomedical fields. Electrospinning techniques offer scalability and compatibility with a wide arsenal of polymers, but typically lack precise three-dimensional (3D) control. Charge reversal during 3D jet writing can enable the high-throughput production of precisely engineered 3D structures. The trajectory of the jet is governed by a balance of destabilizing charge-charge repulsion and restorative viscoelastic forces. The reversal of the voltage polarity lowers the net surface potential carried by the jet and thus dampens the occurrence of bending instabilities typically observed during conventional electrospinning. In the absence of bending instabilities, precise deposition of polymer fibers becomes attainable. The same principles can be applied to 3D jet writing using an array of needles resulting in complex composite materials that undergo reversible shape transitions due to their unprecedented structural control.

Moon 2021, 3D jet writing of mechanically actuated tandem scaffolds

3D Printable Elastomers

3D printing elastomers enables the fabrication of many technologically important structures and devices such as tissue scaffolds, sensors, actuators, and soft robots. However, conventional 3D printable elastomers are intrinsically stiff; moreover, the process of printing often requires external mechanical support and/or post-treatment. The self-assembly of a responsive linear-bottlebrush-linear triblock copolymer can create stimuli-reversible, extremely soft, and stretchable elastomers for their applicability as inks for in situ direct-write printing 3D structures without the aid of external mechanical support or post-treatment. The elastomers are thermostable and remain to be solid up to 180 °C, yet they are 100% solvent-reprocessable. Their extreme softness, stretchability, thermostability, and solvent-reprocessability bode well for future applications.

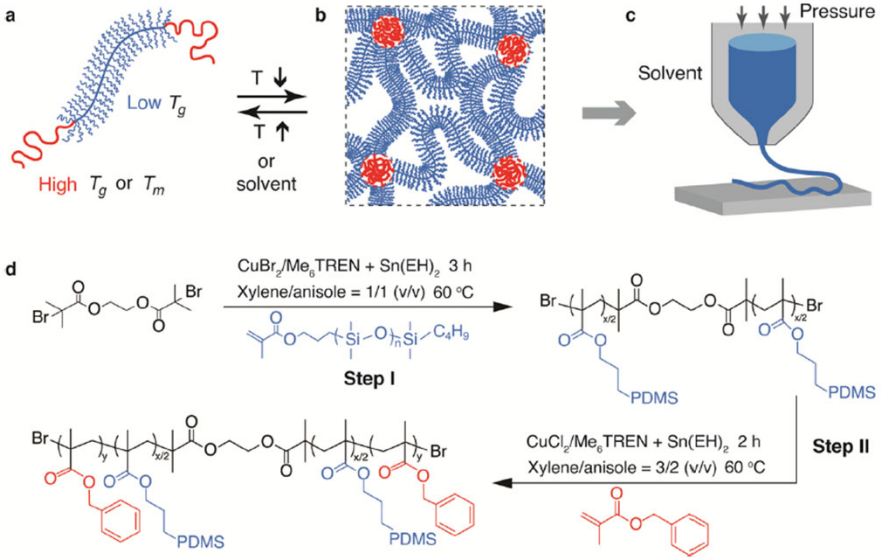


Figure 1. Design concept and synthesis of 3D printable, reversible, ultrasoft, and stretchable elastomers. (a) Schematic of a responsive linearbottlebrush- linear triblock copolymer. (b) At low temperature, the middle bottlebrush blocks (blue) act as elastic network strands, whereas the high Tg end linear blocks aggregate to form spherical glassy domains. (c) Glassy domains dissociate at high temperature or in the presence of solvents, resulting in a solid-to-liquid transition of the network. The stimuli-triggered reversibility allows the elastomers for direct-write 3D printing. (d) Synthesis of linear-bottlebrush-linear triblock copolymers using ARGET ATRP. The side chain of the middle bottlebrush block is linear polydimethylsiloxane (PDMS), whereas the end blocks are linear poly(benzyl methacrylate) (PBnMA). A bottlebrush-based triblock polymer is denoted as BnMAy-b- PDMSx w-b-BnMAy, in which y is the number of repeating BnMA units, x is the number of PDMS side chains per bottlebrush, and w represents theMW of PDMS side chains in kg/mol. The weight fraction of the end blocks in the triblock copolymer is kept below 6% to ensure that the bottlebrush-based ABA triblock copolymers self-assemble to a sphere phase.

Solvent-reprocessable elastomers were developed by exploiting the self-assembly of linear-bottlebrush-linear (LBBL) triblock copolymers. In an LBBL triblock copolymer, the linear blocks are a polymer of relatively high glass transition temperature T_g, whereas the middle block is a bottlebrush polymer with a linear backbone densely grafted by low T_g linear polymers (Figure 1a). At room temperature, the triblock copolymer microphase separates to a network structure, in which cross-links are spherical hard glassy domains formed by the high T_g end blocks, whereas the soft elastic network strands are the low T_g bottlebrush polymer (Figure 1b). Above the melting point of the end blocks or in the presence of solvents, the glassy domains can dissociate such that the solid network becomes liquid-like. Therefore, it is expected that such a stimuli-triggered solid-to-liquid transition allows the physically crosslinked elastomers for direct-write 3D printing (Figure 1c). Polydimethylsiloxane (PDMS) and poly(benzyl methacrylate) (PBnMA) were used as the two polymer species to create the linear-bottlebrush-linear ABA triblock copolymer (Figure 1d).

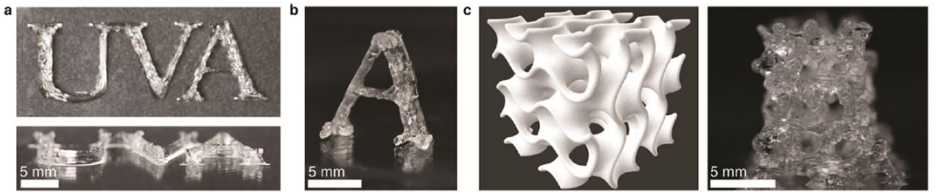
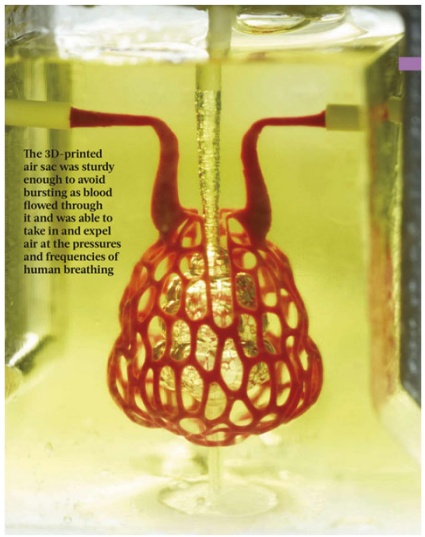


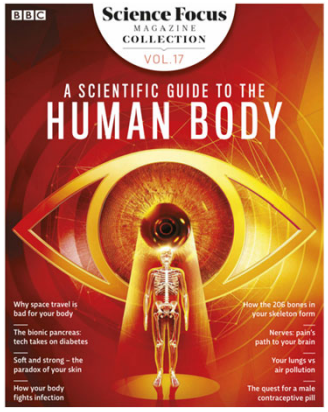
Figure 3. Direct-write printing soft elastomers to create deformable 3D structures. (a) 3D printed UVA initials with a stack thickness of 2 mm. Upper: bird's eye view; lower: side view. (b) Free-standing, 3D printed letter "A". (c) 3D rendering of a cubic gyroid (left) and the corresponding printed product with dimensions 10 × 10 × 10mm3 (right).

Nian 2021, Three-dimensional printable, extremely soft, stretchable, and reversible elastomers from molecular architecture-directed assembly

3D-Printed Tissue Engineering: Print Your Own Body Parts



The 3D-printed model of a lung-mimicking air sac



Print your own body parts

Synthetic organs suitable for transplant could be ready in as little as two decades

words by JASON GOODYER

The image on the left shows a 3D-printed model of a lung-mimicking air sac complete with airways capable of delivering oxygen to surrounding blood vessels. It was made by a team of researchers in the US by gradually building up layers of hydrogel, a synthetic, jelly-like material that shares many features with human tissue.

The same technique could be used for creating complex, entangled vascular networks that mimic the body's passageways for blood and other vital fluids, potentially opening up a means of bioprinting human organs for transplant, the researchers say.

The work was led by Rice University's Jordan Miller and the University of Washington's Kelly Stevens, along with collaborators from Duke University, Rowan University and Nervous System, a design firm in Somerville, Massachusetts. Dubbed 'Stereolithography Apparatus for Tissue Engineering', or SLATE, Miller and Stevens's technique works by building up layers of a liquid pre-hydrogel solution that become solid when exposed to blue light. It can produce soft, 3D structures made from water-based, biocompatible gels with intricate internal architecture in minutes.

In tests, the 3D-printed air sac was sturdy enough to avoid bursting as blood flowed through it and was able to take in and expel air at the pressures and frequencies of human breathing. It was also found that red blood cells could take up oxygen as they flowed through a network of blood vessels surrounding the air sac – a process similar to the gas exchange that occurs in the lungs.

FROM THE LUNGS TO THE LIVER

The researchers are already using the new technique to explore more complex structures and have successfully transplanted 3D-printed tissues loaded with primary liver cells into mice with chronic liver injury.

"The liver is especially interesting because it performs 500 functions – second only to the brain," Stevens said. "The liver's complexity means there is currently no machine or therapy that can replace all its functions when it fails. Bioprinted human organs might someday supply that therapy."

There are currently around 6,000 people waiting for organ transplants in the UK alone. Bioprinted organs could not only help meet this need but, as they can be printed using a patient's own cells, they could also greatly reduce the possibility of organ rejection. "We envision bioprinting becoming a major component of medicine within the next two decades," Miller said.

by JASON GOODYER

Jason is BBC Science Focus Magazine's commissioning editor.

BBC SCIENCE FOCUS MAGAZINE COLLECTION 91

Bagrat Grigoryan, a bioengineer at Rice University, oversaw the development of a 3D-printing technique that's capable of building functioning vascular structures

RESPIRATORY SYSTEM

Synthetic organs suitable for transplant could be ready in as little as two decades (JASON GOODYER)

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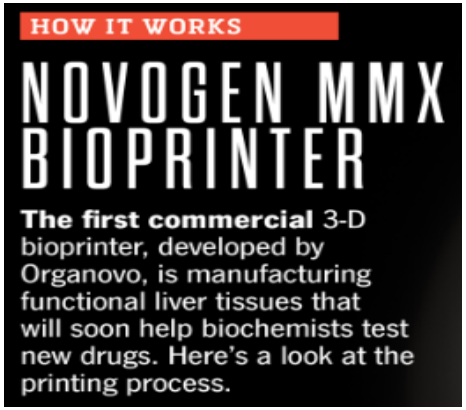
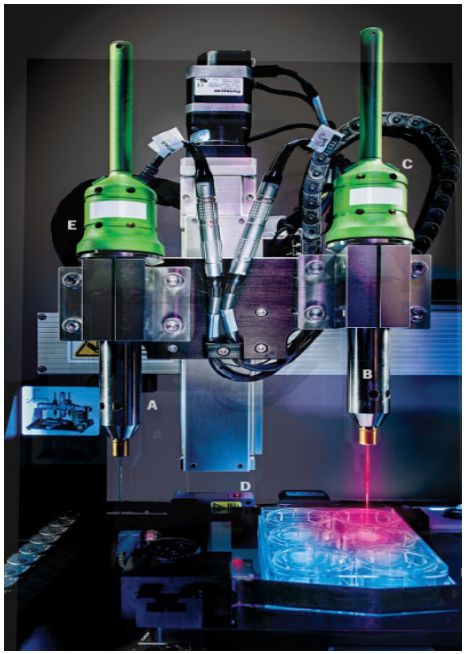
3D-Printed Tissue Engineering

With the dawning of 3D printing, tissue engineering also moved to exploiting the technology. 3D printing devices are relatively cheap, and readily available. It is a great tool to make tailor-made tissues and organs.





STEP 1: Engineers load one **syringe with a bio-ink (A)** made up of spheroids that each contain tens of thousands of parenchymal liver cells and a second **syringe with a bio-ink (B)** containing non-parenchymal liver cells that bolster cellular development and a hydrogel that helps with extrusion.

STEP 2: Software on a PC wired to the bioprinter instructs a stepper motor attached to the robotic arm to move and lower the **pump head (C)** with the second syringe, which begins printing a mold. The mold looks like three hexagons arranged in a honeycomb pattern.

STEP 3: A matchbox-size **triangulation sensor (D)** sitting beside the printing surface tracks the tip of each syringe as it moves along the x-, y-, and z- axes. Based on this precise location data, the software determines where the first syringe should be positioned.

STEP 4: The robotic arm lowers the **pump head (E)** with the first syringe, which fills the honeycomb with parenchymal cells.

STEP 5: Engineers remove the **well plate (F)**—which contains up to 24 completed microtissues, each approximately 250 microns thick—and place it in an incubator. There, the cells continue fusing to form the complex matrix of a liver tissue.

Intraoperative Bioprinting

Intraoperative bioprinting (IOB): a bioprinting process that is performed on a live subject during the course of a surgical operation, in which defect imaging, data processing, process planning, and bioprinting are performed consecutively in a single process.

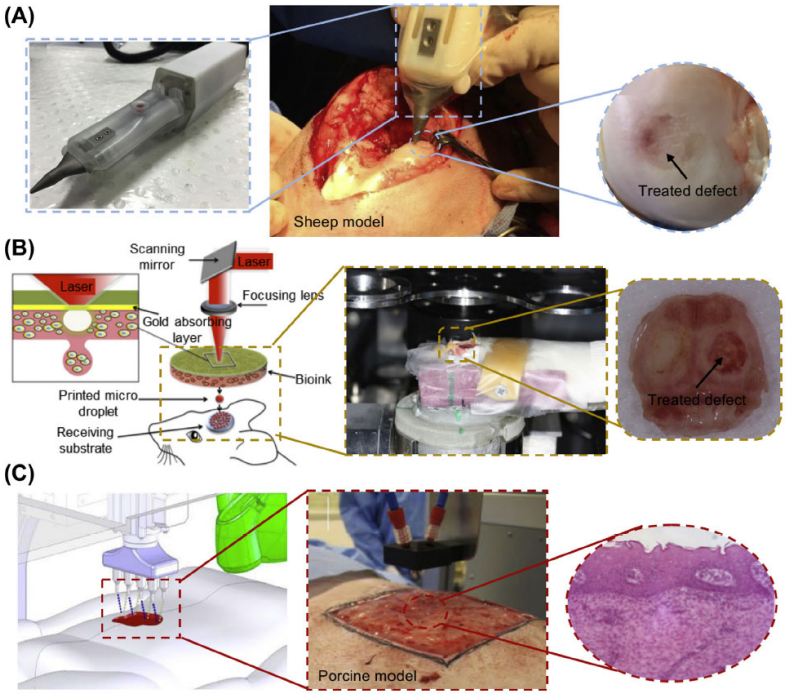


Figure 1. Examples for Intraoperative Bioprinting (IOB).
(A) IOB using the biopen for treatment of a full-thickness chondral defect in a sheep. (B) Laser-based IOB for skull repair in a mouse calvarial defect. (C) Droplet-based IOB for skin repair in a porcine model.

Wu 2020, Intraoperative bioprinting

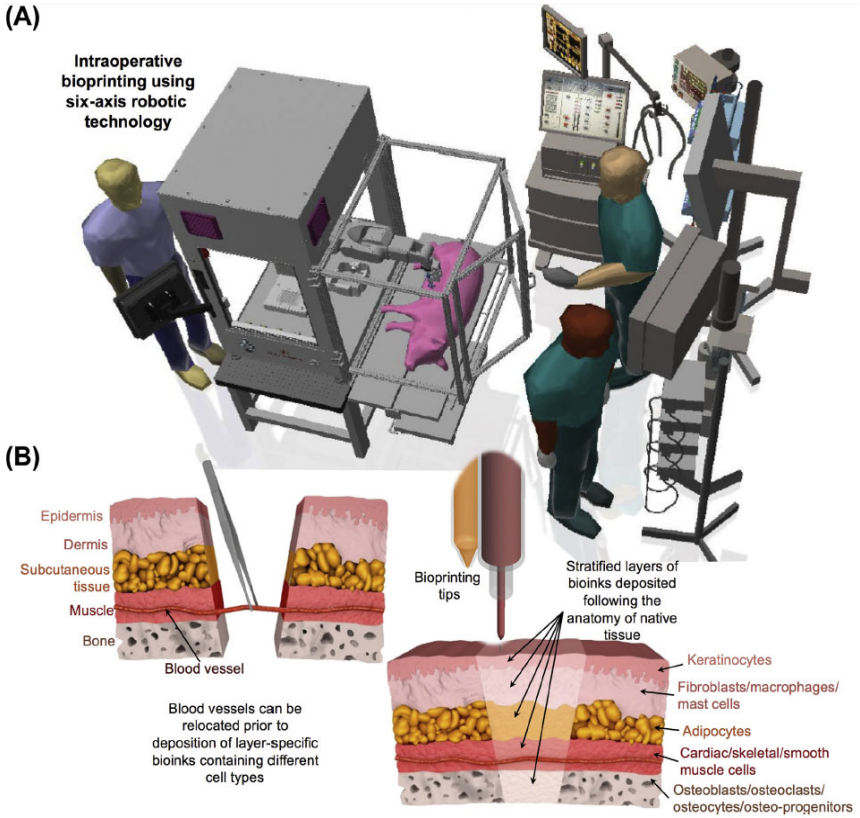


Figure 2. Conceptual schematic diagram of (A) IOB in a surgical setting, and (B) the regeneration of composite tissues in a stratified manner. In the case where blood vessels are retained in host tissue; they can be relocated (left) prior to the deposition of layer-specified bioinks containing different cell types (right).

Stimuli-responsive Polymeric Materials

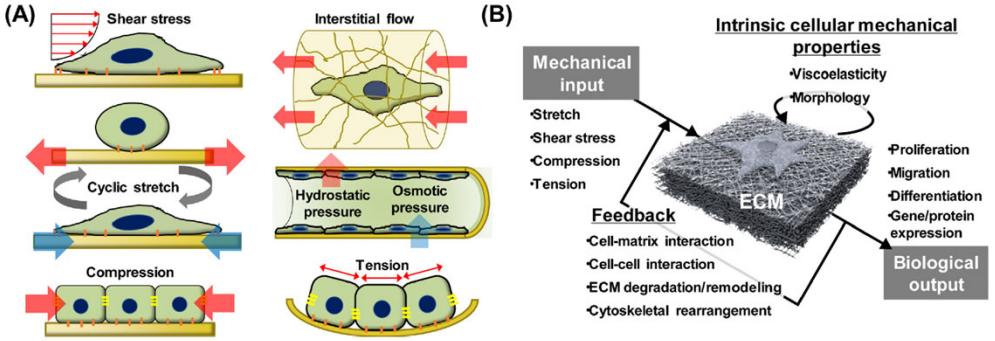


Fig. 1. Mechanical forces in our body and their transduction process into biological output. (A) Mechanical stimuli found at the cell, tissue, and organ levelinside the body. (B) Mechanotransduction is the process by which cells convert mechanical inputs into biological responses. Mechanotransduction often involves a feedback process, and their mechanical environment is dynamic and complex [36].

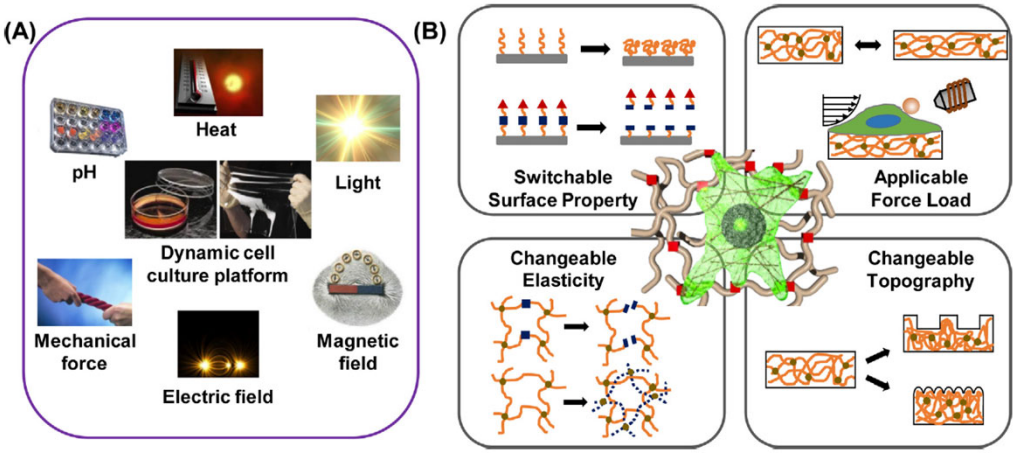
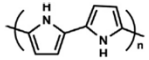
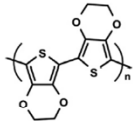
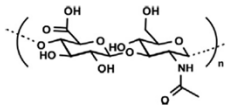
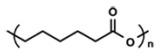


Fig. 3. Stimuli-responsive polymeric materials for dynamic cell culture platforms. (A) Various external stimuli can be utilized for dynamic biomaterials. By modulating chemical, physicochemical and mechano-structural properties, dynamic cell culture platforms can be generated using these materials.(B) Schematic illustration of dynamic cell culture platforms with: tunable matrix stiffness, variable caging-mediated ligand presentation, cleavage- or desorption-mediated ligand presentation, and applicable forces or switchable topography [22].

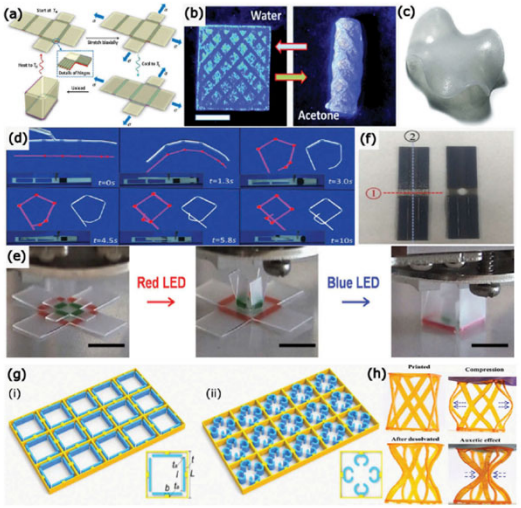
Table 1
Overview of commonly used polymers for the designing of dynamic cell culture platforms.

Polymers	Chemical structures	Characteristics	Application
Poly(*N*-isopropylacrylamide) (PNIPAAm)		Temperature responsive ability Reversibly changeable hydrophilic-ity/hydrophobicity or surface energy Functionalizable	Adhesion-detachment manipulation Cell sheet engineering 3D tissue fabrication
Poly(ethylene glycol) (PEG)		Bioinert and biocompatible	Non-adhesive surface 3D and 4D tissue culture
Polypyrrole		Electro-responsive ability electroconductivity Changeable charge (polarity)	Adhesion-detachment manipulation
Poly(3,4-ethylenedioxythiophene)		Electro-responsive ability electroconductivity	Adhesion-detachment manipulation
Poly(dimethyl siloxane) (PDMS)		Mechanically-responsive ability Transparent, processability Tunable Stiffness (~MPa range)	Cell assay device Organ-on-chip
Polyacrylamide (PA)		Bioinert nature Tunable stiffness (~kPa range)	2D cell culture Cell–material interaction assay
Hyaluronic acid (HA)		Natural ECM Tissue-like stiffness Biocompatible, biodegradable Modificable	Tissue engineering 3D and 4D tissue culture
Poly(ε-caprolactone) (PCL)		Biocompatibility and biodegradability Semicrystalline nature shape-memory property Changeable shape and stiffness	Tissue engineering Dynamic cell manipulation (orientation, movement)

Uto 2017, Dynamically tunable cell culture platforms for tissue engineering and mechanobiology

4D-Printing

Recent advances in additive manufacturing (AM), commonly known as three-dimensional (3D)-printing, have allowed researchers to create complex shapes previously impossible using traditional fabrication methods. A research branch that originated from 3D-printing called four-dimensional (4D)-printing involves printing with **smart materials that can respond to external stimuli**. 4D-printing permits the creation of on-demand dynamically controllable shapes by integrating the dimension of time.



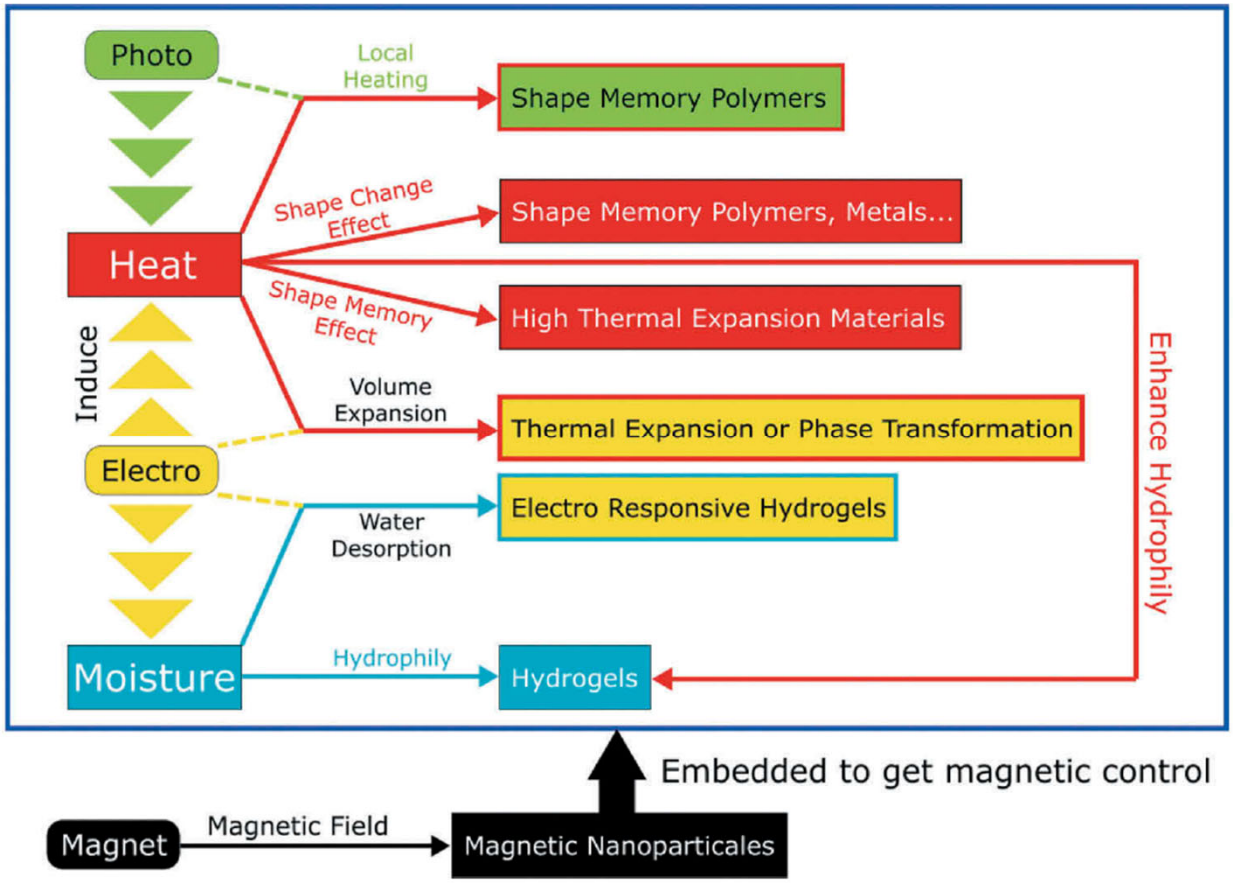
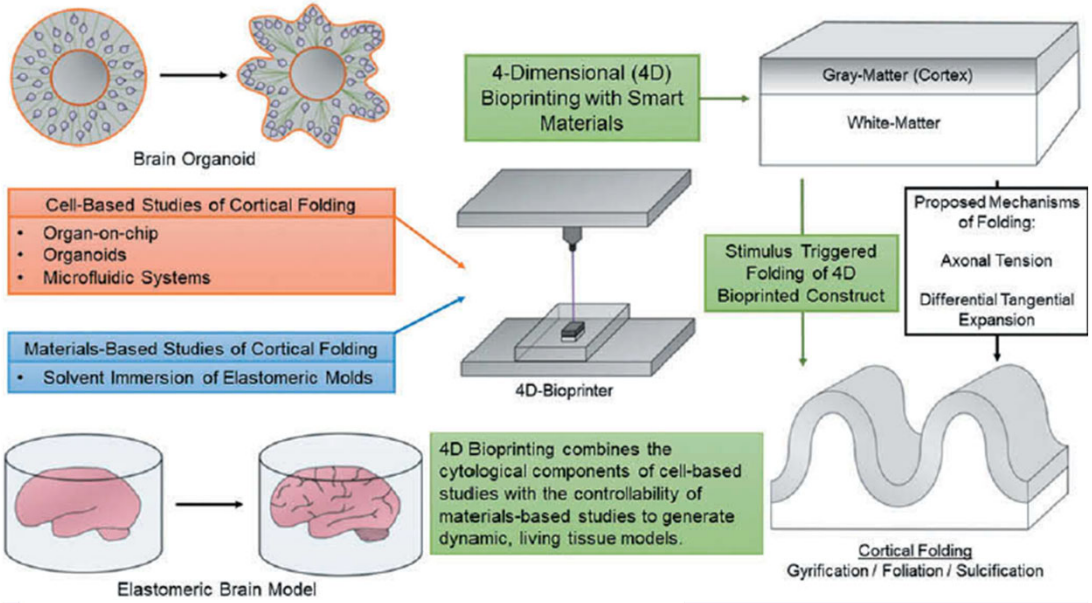


Figure 2. Classes of smart materials that can respond to different types of stimulus including heat, moisture, light, electricity, and magnetic fields.

Zhang 2019, Developments in 4D-printing- A review on current smart materials, technologies, and applications

4D-Bioprinting Technologies



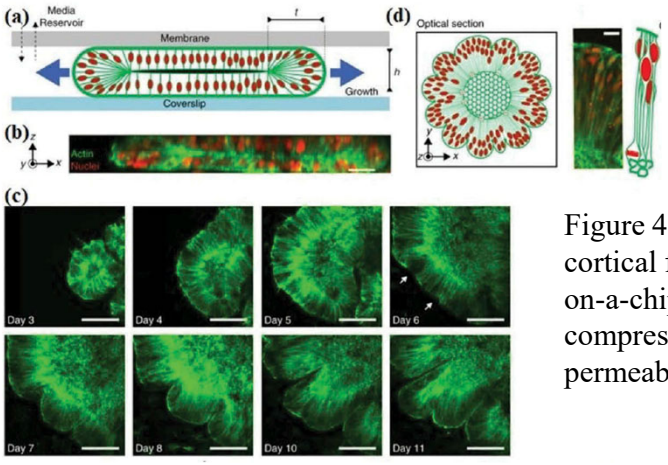


Figure 4. Brain organoid-on-a-chip model of cortical folding. (a) Schematic of neural organoid-on-a-chip system where the organoid is compressed between a coverslip and a media permeable membrane.

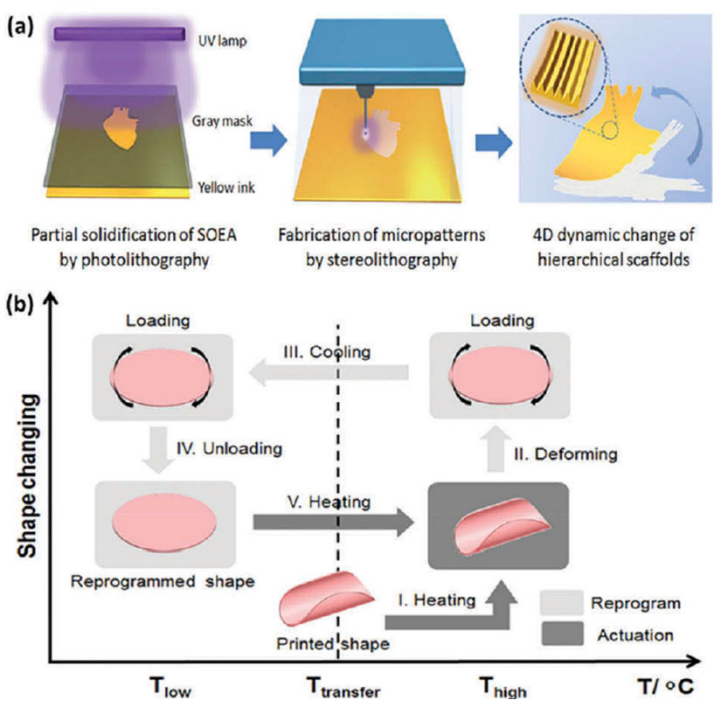


Figure 5. A 4D printed thermally sensitive natural soybean oil epoxidized acrylate (SOEA) constructs developed in our lab. (a) A tandem photolithography-stereolithography process to fabricate heart-shaped constructs from novel soybean oil epoxidized acrylate. (b) Schematic illustration of the 4D shape memory process triggered by temperature

Esworthy 2019, Advanced 4D-bioprinting technologies for brain tissue modeling and study

4D-Bioprinting Technologies

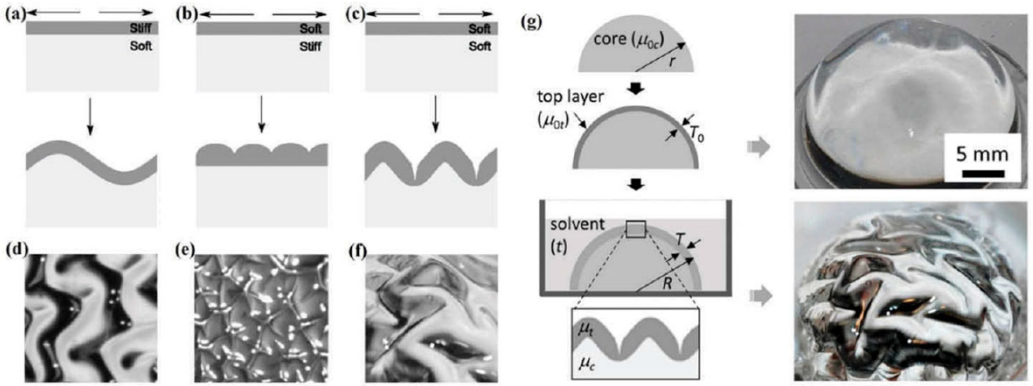


Figure 2. Surface swellability leads to different cortical folding patterns. (a) Sinusoidal folding occurs when the upper layer (gray matter) is stiffer than the lower layer (white matter) of a growing bi-layer cortical model. (b) Cupsed (cusped?) folding occurs when the upper layer is softer than the lower layer. (c) Distinctive gyri and sulci arise when both the upper and lower layers have similar stiffnesses. (d-f) show the folding patterns of bi-layer gels which arise from differential swelling. The resultant patterns demonstrate the sinusoidal, cupsed, and gyri/sulci folding predicted by (a)-(c) respectively. (g) Elastomer model of the brain folding constructed by making a core hemisphere of radius (r) which has a shear modulus of (μ_{0c}). The hemispheric core is coated in a thin layer of polymer with a thickness (T_0) which exhibits a shear modulus of (μ_{0t}). The top and core (or upper and lower) layers have a combined radius (R). The completed model is then submeregd in solvent and allowed to swell for time (t). When the moduli of both the top and core layers are similar (moduli ratio $\mu_t/\mu_c \approx 1$) the distinct gyri/sulci folding pattern from (c) arises.

Esworthy 2019, Advanced 4D-bioprinting technologies for brain tissue modeling and study

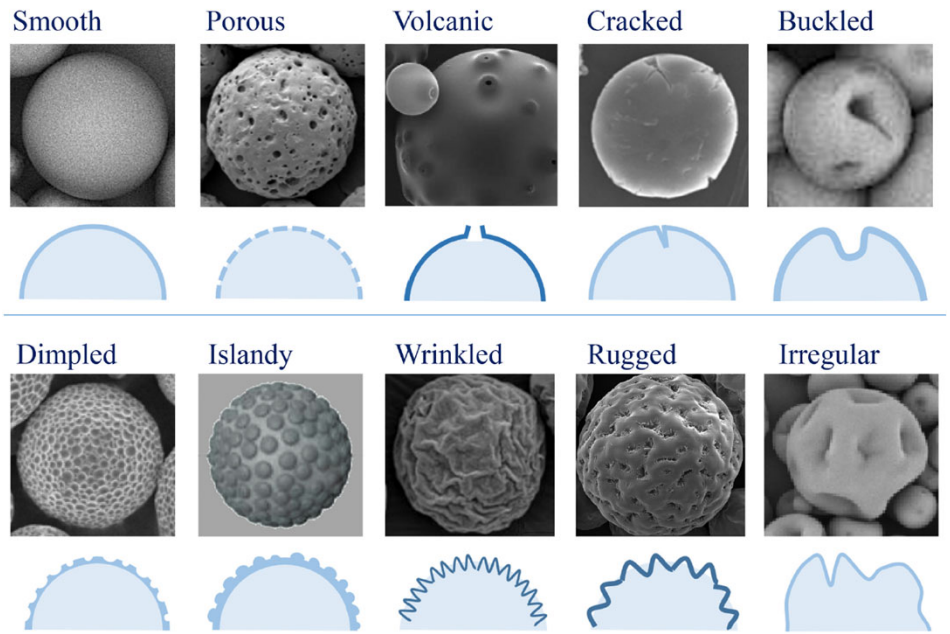


Fig. 4. Examples of different types of surface morphologies observed on microparticles depending on the formulation and/or processing parameters.

Park 2021, Formulation composition, manufacturing process, and characterization of PLGA microparticles

Cell Encapsulation & Cell Therapy

Cell Delivery (Encapsulated Cell Transplantation)

"A living drug delivery system to replace a single cell type or to produce appropriate amounts of a particular biochemical product"

Target Diseases

Islets for diabetes treatment

Parathyroid tissue for hypoparathyroidism

Growth hormone secretion for the treatment of pituitary dwarfism

Hepatocytes for liver disease

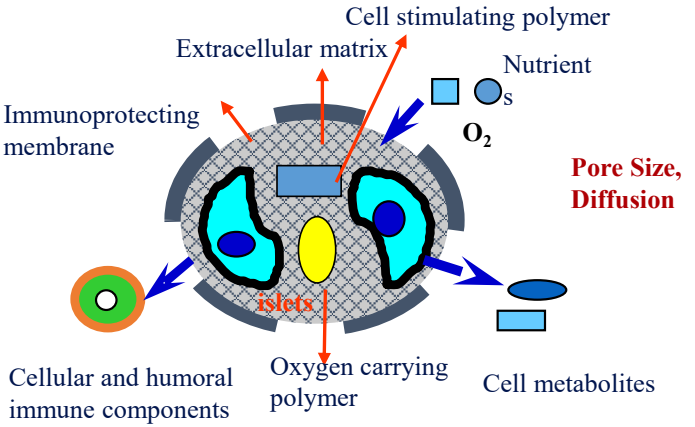
Bovine adrenal chromaffin cells for the treatment of chronic pain

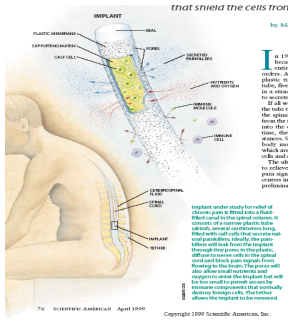
Factor IX secretion for the treatment of hemophilia B

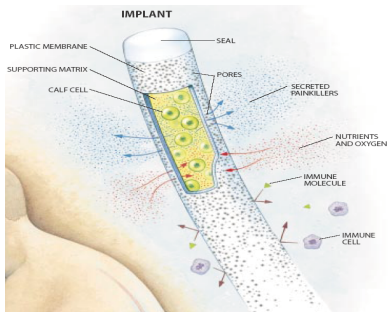
PC12 cells and bovine chromaffin cells for Parkinson's disease

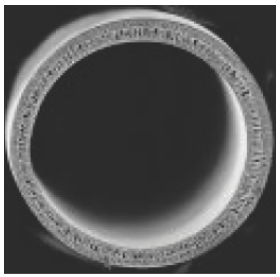
Genetically modified rat fibroblast or baby hamster kidney cells to release nerve growth factor for the treatment of Alzheimer's disease and Huntington's disease

Genetically modified baby hamster kidney cells to secrete glial cell-line-derived neurotropic factor

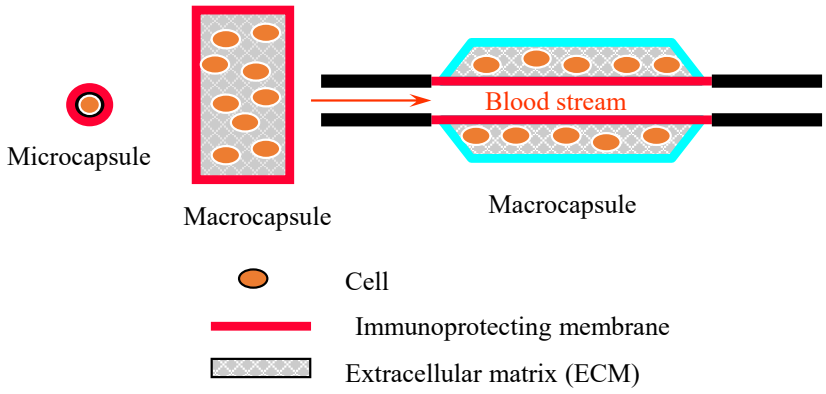








Shielding the cell from immune attack by plastic membrane
(Michael J. Lysaght and Patrick Acbischer)



Cell Macroencapsulation

Type 1 diabetes (T1D) is characterized by the autoimmune destruction of pancreatic β cells and it burdens millions worldwide. T1D patients typically require the life-long administration of insulin. Islet transplantation for type 1 diabetes treatment has been limited by the need for lifelong immunosuppression regimens. This challenge has prompted the development of macroencapsulation devices (MEDs) to immunoprotect the transplanted islets. A macroencapsulation device (MED) acts as a bioartificial pancreas and can immunoprotect encapsulated β cells. While promising, conventional MEDs are faced with insufficient transport of oxygen, glucose, and insulin because of the reliance on passive diffusion. Hence, these devices are constrained to two-dimensional, wafer-like geometries with limited loading capacity to maintain cells within a distance of passive diffusion. However, conventional MEDs suffer from limited, cell-loading capacity and slow, glucose-stimulated insulin secretion (GSIS) because of the sole reliance on diffusion.

A convection-enhanced MED (ceMED) was developed to afford 3D capsule geometry for maximized cell loading and faster GSIS driven by convection. Convective transport improves nutrient delivery throughout the device and affords a three-dimensional capsule geometry that encapsulates 9.7-fold-more cells than conventional MEDs. Transplantation of a convection-enhanced MED (ceMED) containing insulin-secreting β cells into immunocompetent, hyperglycemic rats demonstrated a rapid, vascular-independent, and glucose-stimulated insulin response, resulting in early amelioration of hyperglycemia, improved glucose tolerance, and reduced fibrosis.

In order to validate the effectiveness of a flow-based convection at a controlled flow rate, a syringe pump was used.

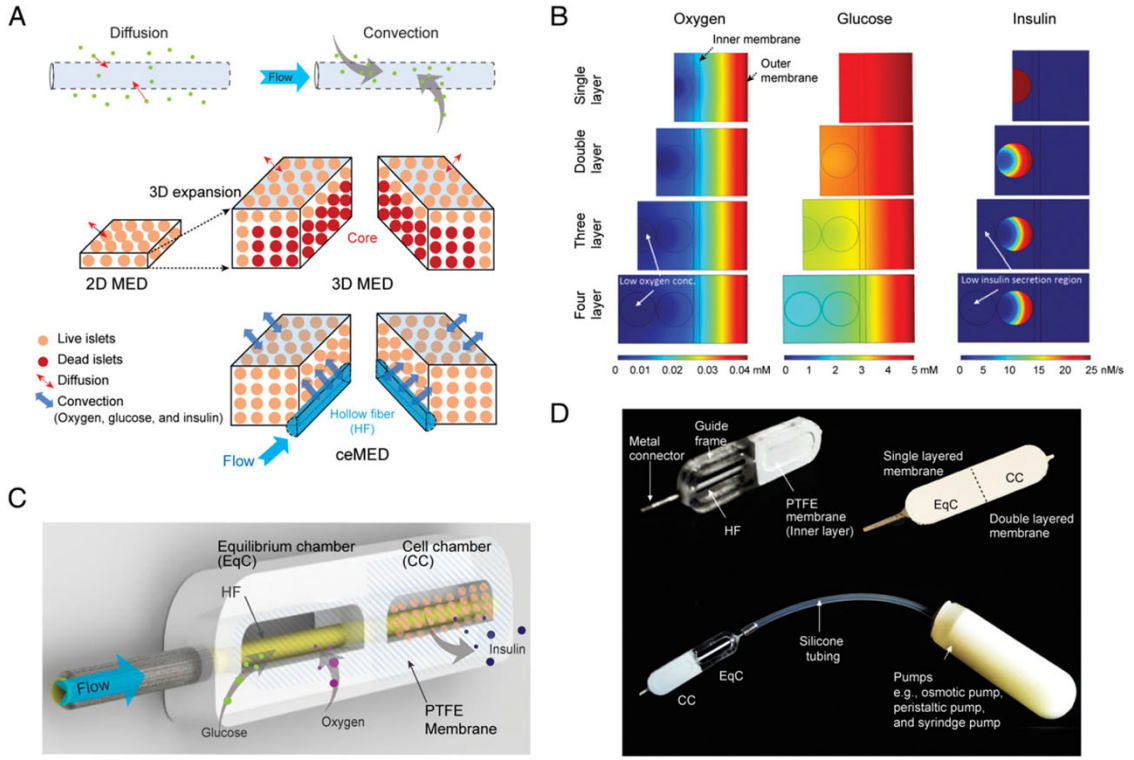


Fig. 1. Design of a ceMED for increasing mass transport, β cell viability, and insulin secretion sensitivity. (*A*) Illustration comparing diffusion-based versus convection-enhanced approaches. Expanding MEDs from a typical two-dimensional (2D) wafer static system to a 3D MED brings forth mass transport limitations and cell death. These limitations motivate the introduction of a HF in a 3D expanded MED to allow increased nutrient delivery by perfused flow in the ceMED. (*B*) Simulation showing the gradient of oxygen (millimolar), glucose concentration (millimolar), and insulin secretion rate (nanomolar per second) as a function of position inside a static macroencapsulation with multiple layers of islets. The color bars indicate the concentration of each variable. White arrows indicate the hypoxic regions in the islet due to diffusion-limited transport of the oxygen in the device. (*C*) Scheme of the ceMED, consisting of an EqC, a CC, and a connecting HF. EqC captures glucose and oxygen from the surroundings; HF transports these solutes to the encapsulated cells in the CC. Inside the CC, positive pressure facilitates flow and improved mass transport to and from the encapsulated cells. The CC is enclosed by a PTFE membrane for protection from immune attack while allowing for nutrient transfer. (*D*) Gross view of a fully assembled, transplantable ceMED and its components. The ceMED can be connected to various pump systems, exemplified here by an osmotic pump.

Yang 2021, A therapeutic convection–enhanced macroencapsulation device for enhancing β cell viability and insulin secretion

Microfabrication by Simultaneous Two PDMS Molds Replication

ABSTRACT: Not very far away, “tissue engineering” will become one of the most important branches of medical science for curing many types of diseases. This branch needs the cooperation of a wide range of sciences like medicine, chemistry, cellular biology, and genetic and mechanical engineering. Different parameters affect the final produced tissue, but the most important one is the quality and biocompatibility of the scaffold with the desired tissue which can provide the functionality of “native ECM” as well. The quality of the scaffold is directly dependent on its materials, design, and method of fabrication. As to the design and fabrication, there are two main categories: (a) **random microporosity such as phase separation, electrospinning, and fused deposition modeling (3D printing)** and (b) **designed microporosity mostly achievable by stereo lithography and soft lithography**. The method of fabrication implemented in this research is **a novel method** in soft lithography employing a type of “replica molding” with one pair of polydimethylsiloxane (PDMS) molds in contrast to traditional replica molding with just one single mold. In this operation, the solution of **polycaprolactone in chloroform** is initially prepared, and one droplet of the solution is placed between the molds while a preset pressure is applied to maintain the molds tightly together during the solidification of the polymer layer and vaporization of the solvent. Thus, a perfect **warp and woof pattern** (threads running lengthwise and threads running crosswise) is created. In this research, it has been approved that this is a feasible method for creating complex patterns and simple straight fiber patterns with different spacings and pore sizes. Cell attachment and migration was studied to find the optimum pore size. It was shown that the small pore size improves the cells’ adhesion while reducing cell migration capability within the scaffold.

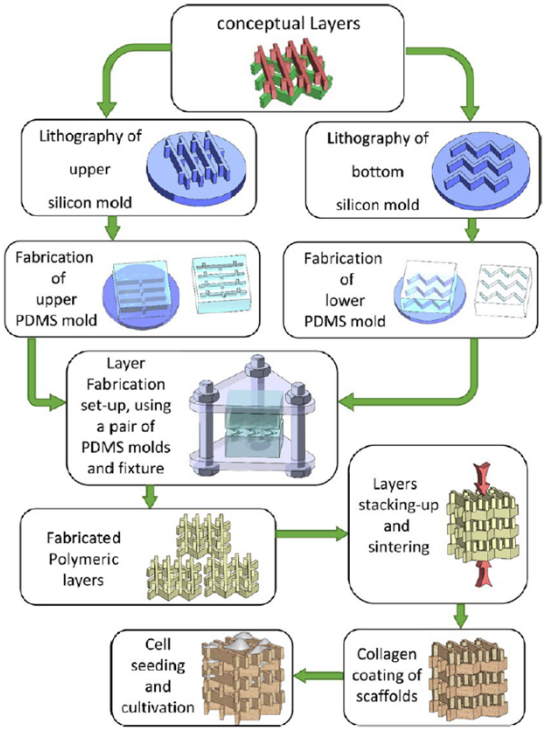


Figure 4. Manufacturing process chart from design to cell cultivation.

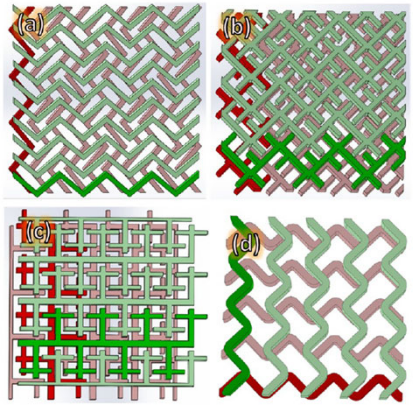


Figure 6. Schematic of the designed scaffold structure with special fiber forms and overall dimension of 20 × 20 mm. Green fibers represent the top level woofs, and the red one represent warps of one polymeric layer. The highlighted woof and warp in each picture can help to understand the pattern design concept. (a) Simple zig-zag fibers. (b) Branched zig-zag fibers. (c) Straight dendritic fibers. (d) Modified zig-zag pattern for HDF cell culturing.

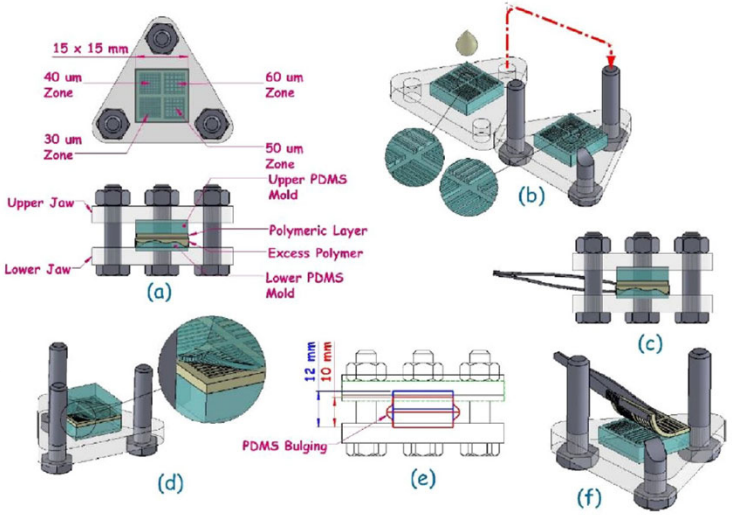
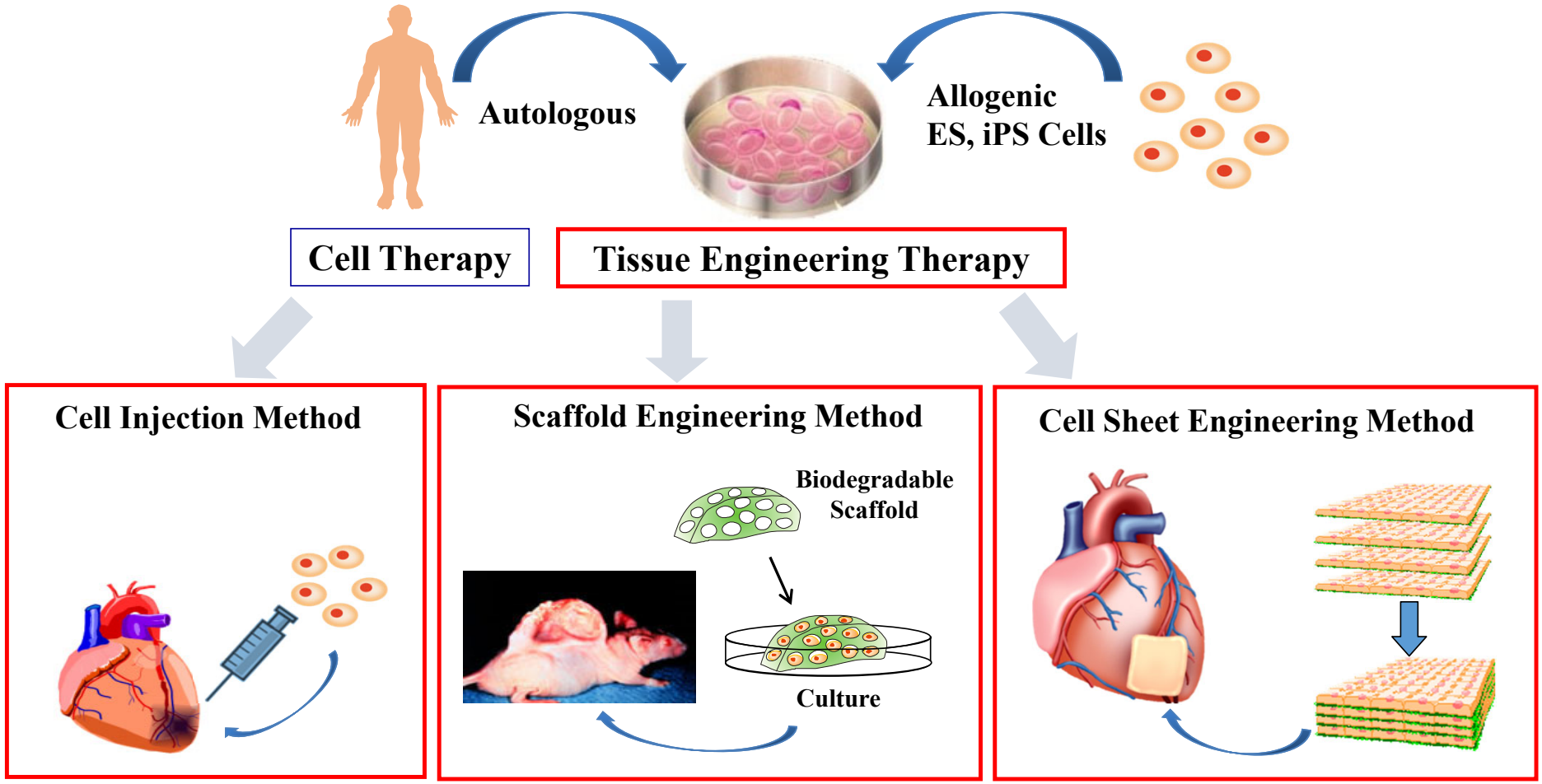


Figure 10. Process of replica molding of the polymeric layer between the two segment PDMS mold including the designed fixture. (a) Drying fixture top and side views. (b) Pouring the polymer solution and closing the fixture. (c) Removing excess polymer. (d) Removing the top PDMS platen starting form one corner. (e) Applying a 2 mm squeeze to pressurize the PDMS segments. (f) Peeling off the casted polymeric layer.

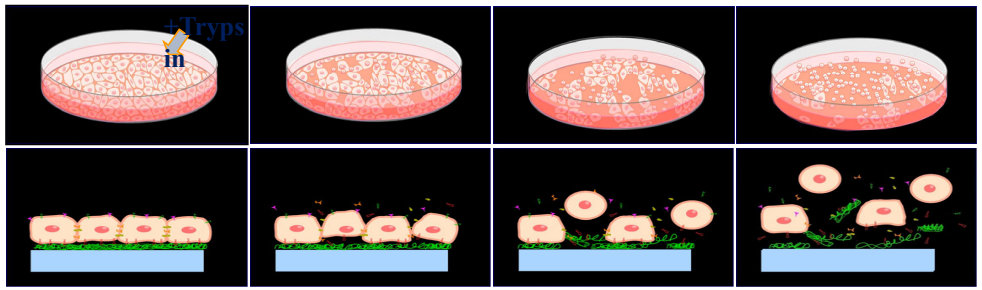
Najafi sani 2021, A microfabrication method of PCL scaffolds for tissue engineering by simultaneous two PDMS molds replication

Cell Therapy / Tissue Engineering Therapy

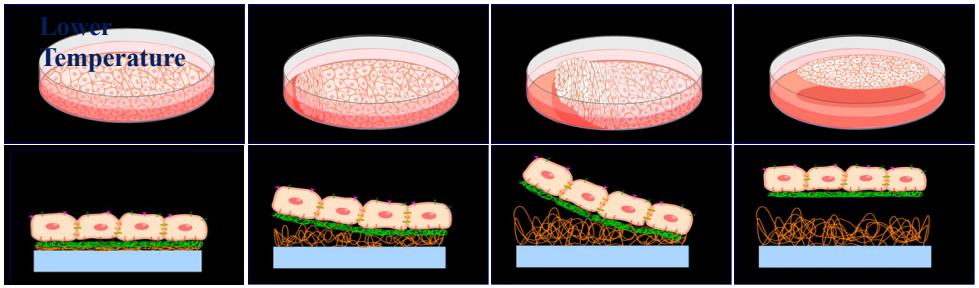


Cell Sheet Tissue Engineering

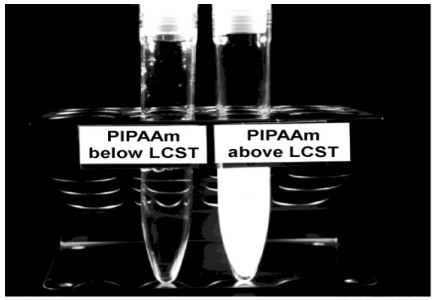
Conventional Cell Harvest with Proteolytic Enzymes

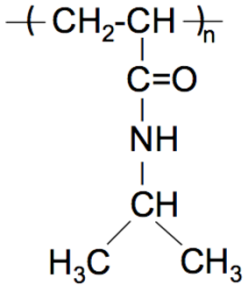


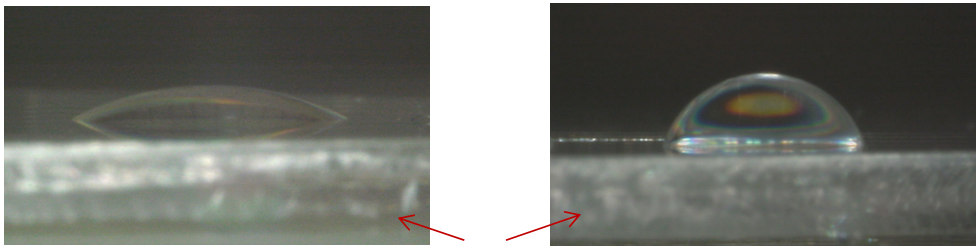
Non-Invasive Cell Sheet Harvest by Reducing Temperature



Poly(N-isopropylacrylamide)
LCST ~32 °C

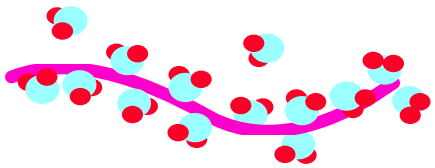




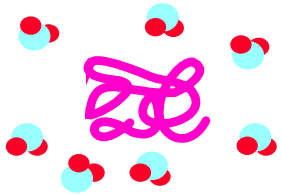


PIPAAm-grafted Surface

Hydrophilic surface at <32 °C



Hydrophobic surface at <32 °C



Teruo Okano, Ph.D., Tokyo Women's Medical University
Institute of Advanced Biomedical Engineering and ScienceTokyo, Japan

Temperature-Responsive Polymer-Grafted Surfaces

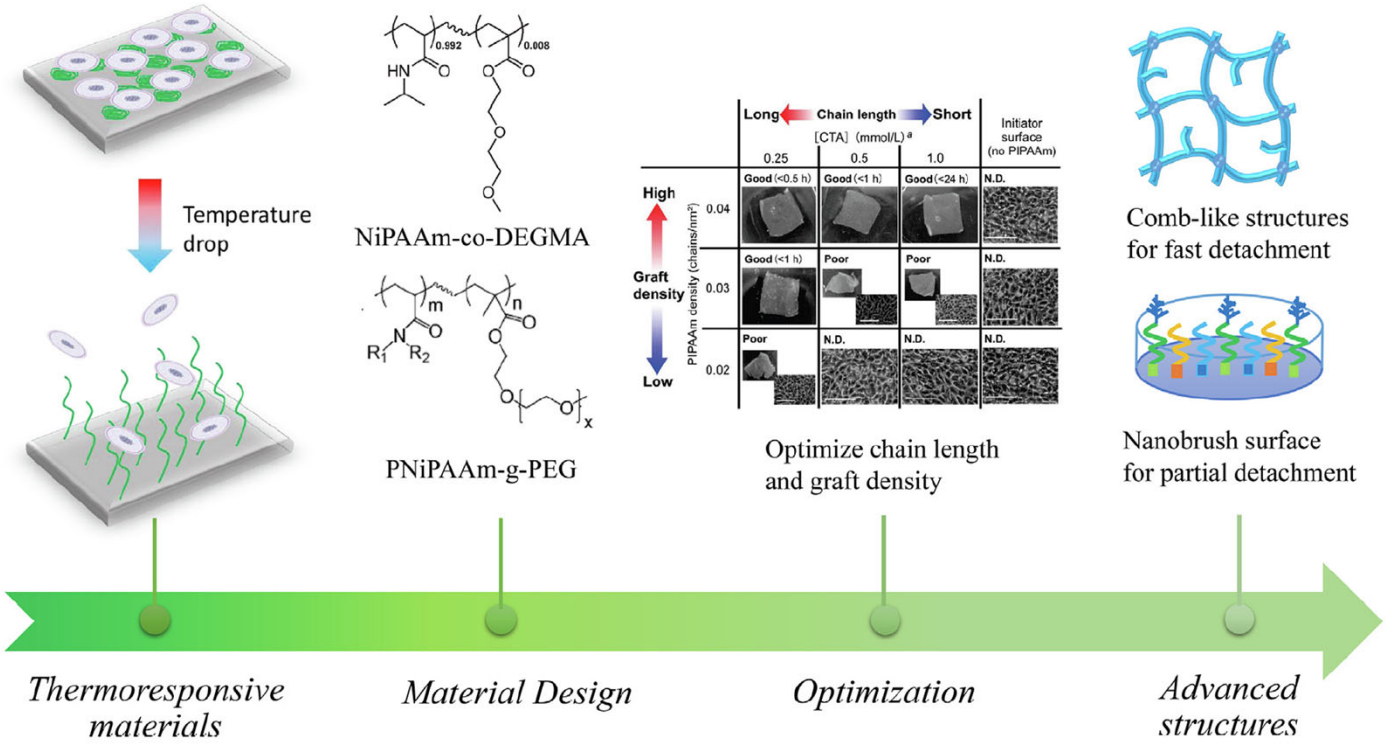


Fig. 3. Thermoresponsive materials for harvesting cells without cell damage. Thermoresponsive materials release cells when temperature is reduced. These materials are designed using copolymers to tune the LCST or other physical properties.

Chen 2020, Biomaterial-assisted scalable cell production for cell therapy

Vascularization of Tissue-Engineered Skeletal Muscle Constructs

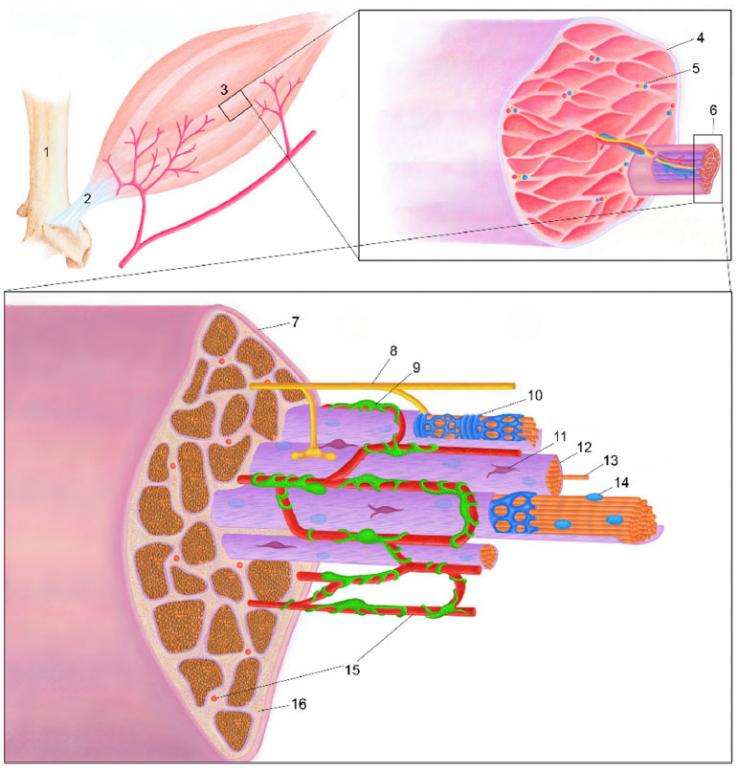


Fig. 1. Skeletal muscle structure. 1. Bone, 2. Tendon, 3. Muscle, 4. Epimysium, 5. Artery (red), vein (blue), nerve (yellow), 6. Fascicle, 7. Perimysium, 8. Nerve, 9. Pericyte 10. Sarcoplasmic reticulum, 11. Satelite cell, 12. Sarcolemma, 13. Myofibril, 14. Muscle cell nucleus 15. Capillary 16. Endomysium [46].

Gholobova 2020, Vascularization of tissue-engineered skeletal muscle constructs

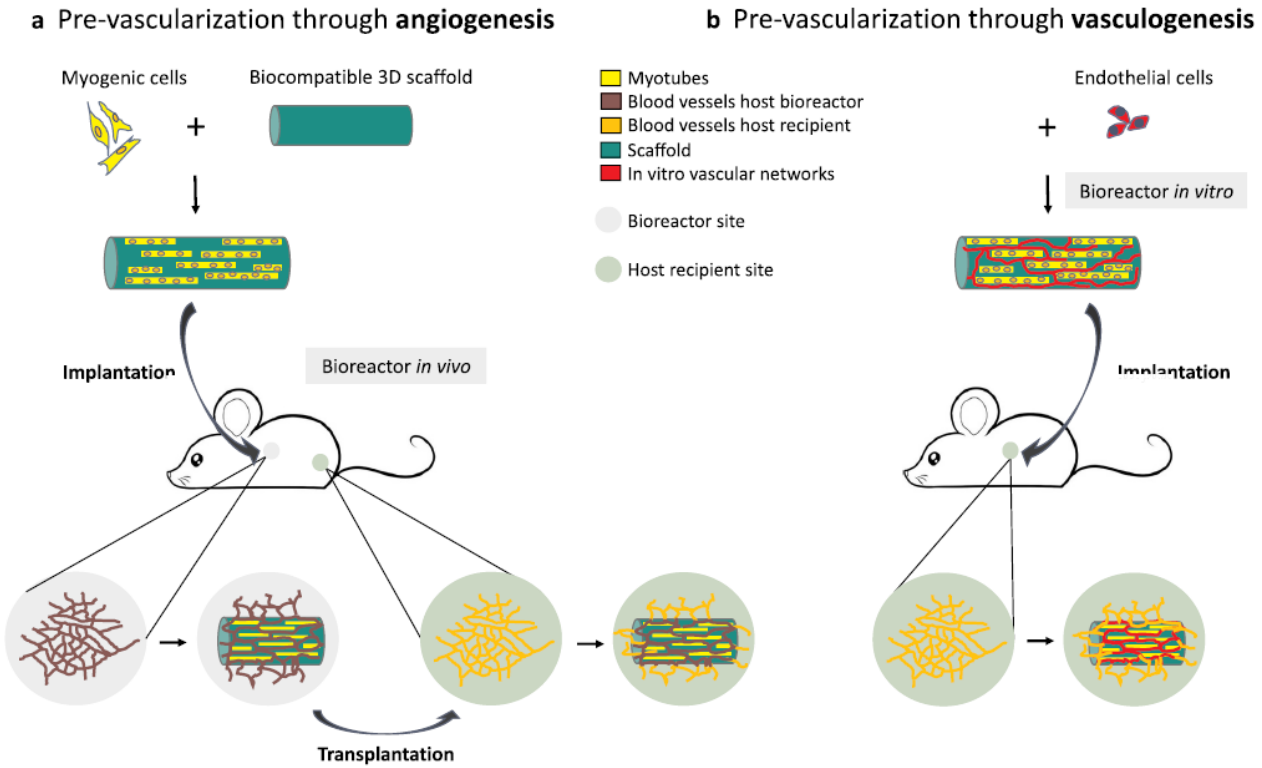


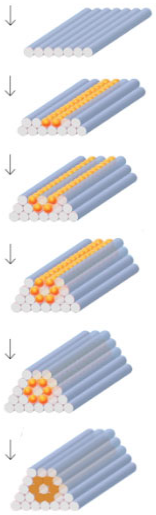
Fig. 2. Pre-vascularization strategies of tissue-engineered constructs. (a) In the pre-vascularization through angiogenesis, a construct with target cells (yellow) is implanted in a bioreactor site in vivo allowing for angiogenesis. Through angiogenesis and ingrowth of host vessels (dark red) the construct is provided by blood vessels. Upon transplantation of the prevascularized construct in a host recipient site, a new implantation site or animal, connection between the preformed blood vessels (dark red) and host vessels (orange) takes place. (b) In the pre-vascularization through vasculogenesis, vascular networks are formed in vitro by de novo formation of vascular networks by endothelial cells (bright red). Upon implantation in the host recipient site, preformed networks (bright red) connect with host vessels (orange), providing perfusion through the whole construct.

How to Grow Blood Vessels

TISSUE

BUILDING BLOCKS

In order to form tubular structures, the basis for blood vessels, scientists at Organovo use a bioprinter to deposit layers of hydrogel rods (blue) and a bio-ink made of spheres or cylinders that contain thousands of human cells (yellow). After printing, the bio-ink fuses into a tube, and the hydrogel can be removed to leave behind the vessel. Vascular grafts could be combined with liver, lung, or cardiac tissue to eventually build complex organs.



The difficulty of providing a blood supply has always limited the size of engineered tissues.

Building Blood Vessels

Living tissues quickly starve without the oxygen and nutrients delivered to cells by blood, so an engineered tissue construct that is more than a few cells thick usually requires an integrated vasculature. Endothelial cells form tiny capillaries and the interior lining of larger vessels within natural tissues, but coaxing endothelial cells to build a vascular network that penetrates an engineered tissue has been a major challenge. Nanoscale and microfabrication technologies borrowed from other fields, such as the semiconductor industry, are now allowing tissue engineers to control the cells' behavior and placement with unprecedented precision.

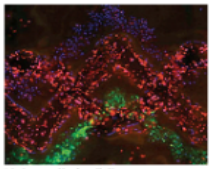
By embedding wormlike, 3-D-printed casts in a gel (left), then removing them, Harvard's Ali Khademhosseini and his team built rudimentary blood vessels (right).

67

Blood Vessels Via Printer

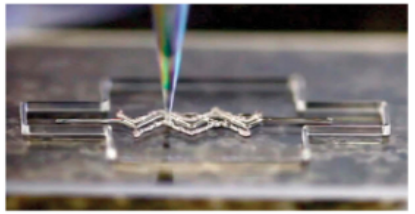
Could tomorrow's surgeons create customized replacement tissue for patients just by hitting print? Two teams of Harvard bioengineers made big strides toward that goal in 2014, reporting two new 3-D-printing methods that help construct rudimentary blood vessels.

Each year, thousands of people die waiting for tissue and organ donations, which are scarce. For that reason, tissue engineers are working to build replacements, and they've managed to do it for sheetlike tissues, like the skin and bladder. But replacement tissues for solid organs, such as the liver, heart or kidney, are tougher to construct because cells inside those tissues rely on a network of blood vessels that are difficult to replicate.



Living cells (red) line a rudimentary blood vessel created by Lewis' method.

In February, Jennifer Lewis' team reported printing tissue pieces infused with the beginnings of blood vessels. They used a custom-built 3-D printer and special inks containing extracellular matrix — the naturally derived material that the body uses to knit cells into tissues. The printer builds tissue, layer by layer. As the print heads move, they squeeze out inks like toothpaste from a tube. Those inks solidify into worm-shaped gels, some of which contain living cells. To print blood vessels, the researchers made the worm-shaped gels from a special ink that, strangely, melts as it cools. This allowed them to suction out the resulting liquid, leaving tunnels that they lined with other cells to form rudimentary blood vessels.



A 3-D printer built by Jennifer Lewis of Harvard and colleagues layers strand upon strand of biocompatible material, some containing cells, that they hope will form living tissue.

In May, a second team, led by Ali Khademhosseini, reported building tiny blood vessels that branch or merge in three dimensions, as blood vessels do in human organs. First, they 3-D-printed wormlike strands of a gel called agarose, each serving as a cast of a tiny blood vessel. Around those casts, they poured a cell-rich liquid that solidifies into a biocompatible gel. Then they carefully tugged or suctioned away the agarose casts, leaving channels that they lined with cells to create simple blood vessels.

For now, Lewis' team is working to create kidney and bone tissue mimics for drug safety screening. Khademhosseini seeks to hone his 3-D-printing process to make replacement blood vessels for individual patients. —LACY SCHLEY

FROM TOP: KHADEMHOSSEINI LAB; D. KOLESKY AND J. LEWIS, HARVARD UNIVERSITY/WYSS INSTITUTE/SEAS (2)

Discover, Jan/Feb 2015

How to Grow Blood Vessels

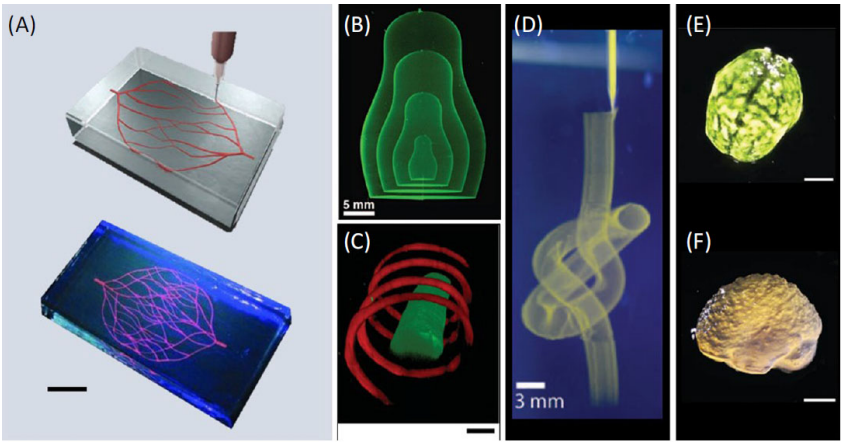


Figure 2. Advancement of Omnidirectional 3D Printing: From Yield Stress Fluids to Suspension Media. (A) Omnidirectional printing in a non–self-healing yield stress fluid. (Top) Schematic for 3D-printed vascular network using a removable ink. (Bottom) A fluorescence image of a 3D microvascular network fabricated via omnidirectional printing of a fugitive ink (dyed red) within a photopolymerizable Pluronic F-127–diacrylate matrix. Scale bar, 10 mm. Reproduced from [13]. (B–F). Omnidirectional printing in suspension media. (B) Miniature Russian dolls printed in a granular suspension medium [2]. Reproduced from the indicated reference, licensed under Creative Commons 4.0 (CC 4.0) series. (C) Printed filament of a fluorescein-labeled ink (in green) surrounded by a continuous spiral structure (in red), printed with a rhodamine-labeled ink [5]. Scale bar, 200 μm. (D) Continuous knot written with fluorescent microspheres in a granular suspension medium. Reproduced from [2]. (E and F). Human brain model 3D-printed in an alginate suspension media. Scale bar, 1 cm. [3]. Reproduced from the original, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0).

McCormack 2019, 3D printing in suspension baths

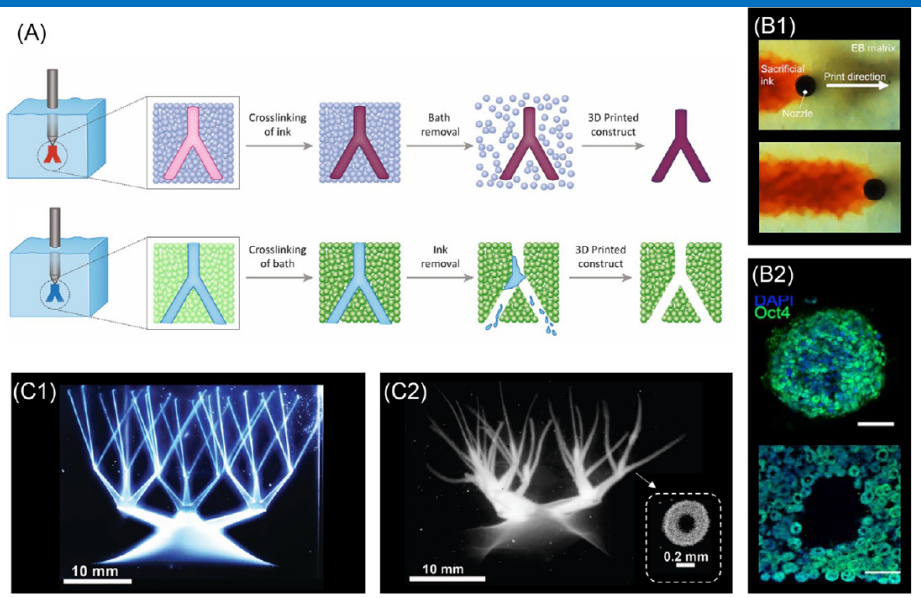


Figure 3. Suspension Media Used as a Strategy to Aid Better Biomimicry in the 3D-Bioprinting Field. (A) Overview of the 3D-printing pathways that can be followed when printing in a suspension medium. (Top) Pathway is defined by removal of the medium after printing. Suspension medium provides mechanical stability to printed ink while crosslinking of the ink takes place (e.g., by exposing the embedded ink to ultraviolet light). The medium is subsequently removed in order to extract the printed construct. (Bottom) Pathway is defined by retention of the medium after printing. Following deposition of a sacrificial ink, the medium is crosslinked to form a single construct. In this crosslinked state, the medium has lost its ability to flow. The sacrificial ink can then be extracted, such as with a syringe needle, leaving behind hollow channels embedded within the construct. (B1) Writing of sacrificial ink within an embryoid body suspension medium [27]. Reproduced from the original, licensed under Creative Commons Attribution 4.0 International (CC BY 4.0). (B2) Row 1: Singular organ building block. Scale bar, 50 μm. Row 2: Cross-section of channel printed in an embryoid body suspension medium following removal of the sacrificial printed ink. Scale bar, 500 μm [27]. Reproduced from the original, licensed under CC BY 4.0. (C1 and C2) Example of a highly branched tubular network printed in a Carbopol suspension medium where (C2) is the structure freed from the media [2]. Reproduced from the original, licensed under CC BY-NC 4.0.

Brain Organoid Reservoir Computing

Brain-inspired computing hardware aims to emulate the structure and working principles of the brain and could be used to address current limitations in artificial intelligence technologies. However, brain-inspired silicon chips are still limited in their ability to fully mimic brain function as most examples are built on digital electronic principles. Here we report an artificial intelligence hardware approach that uses adaptive reservoir computation of biological neural networks in a brain organoid. In this approach-which is termed Brainoware-computation is performed by sending and receiving information from the brain organoid using a high-density multielectrode array. By applying spatiotemporal electrical stimulation, nonlinear dynamics and fading memory properties are achieved, as well as unsupervised learning from training data by reshaping the organoid functional connectivity. We illustrate the practical potential of this technique by using it for speech recognition and nonlinear equation prediction in a reservoir computing framework.

A team at Indiana University has successfully grown their own nanoscale “brain organoid” in a Petri dish using human stem cells. After connecting the organoid to a silicon chip, the new biocomputer (dubbed “Brainoware”) was quickly trained to accurately recognize speech patterns, as well as perform certain complex math predictions.
Researchers treated their Brainoware as what’s known as an “adaptive living reservoir” capable of responding to electrical inputs in a “nonlinear fashion,” while also ensuring it possessed at least some memory. Simply put, the lab-grown brain cells within the silicon-organic chip function as an information transmitter capable of both receiving and transmitting electrical signals. While these feats in no way imply any kind of awareness or consciousness on Brainoware’s part, they do provide enough computational power for some interesting results.

To test out Brainoware’s capabilities, the team converted 240 audio clips of adult male Japanese speakers into electrical signals, and then sent them to the organoid chip. Within two days, the neural network system partially powered by Brainoware could accurately differentiate between the 8 speakers 78 percent of the time using just a single vowel sound. Next, researchers experimented with their creation’s mathematical knowledge. After a relatively short training time, Brainoware could predict a Hénon map. While one of the most studied examples of dynamical systems exhibiting chaotic behavior, Hénon maps are a lot more complicated than simple arithmetic, to say the least.
In the end, Brainoware’s designers believe such human brain organoid chips can underpin neural network technology, and possibly do so faster, cheaper, and less energy intensive than existing options. There are still a number of hurdles—both logistical and ethical—to clear, but although general biocomputing systems may be years down the line, researchers think such advances are “likely to generate foundational insights into the mechanisms of learning, neural development and the cognitive implications of neurodegenerative diseases.”

https://www.popsci.com/technology/brainoware-brain-organoid-chip/

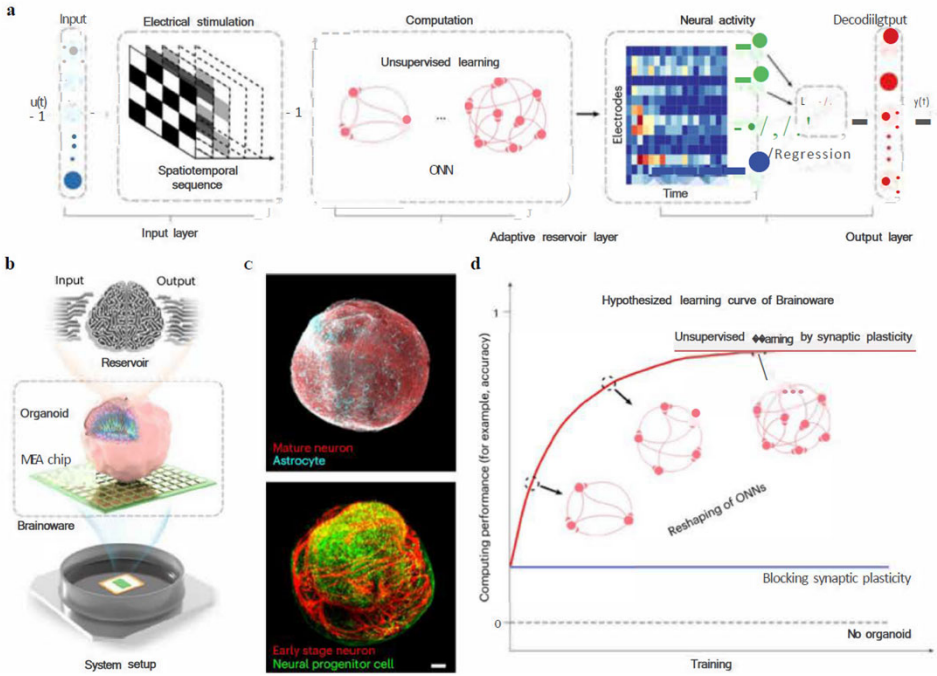
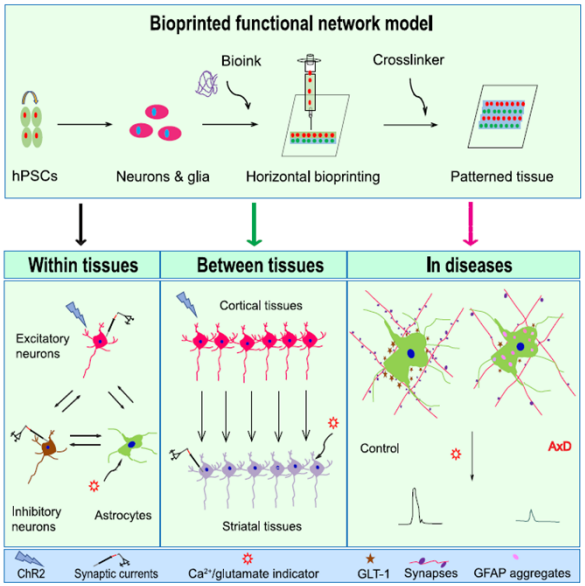


Fig. I. Brainoware with unsupervised learning for Alcomputing. a, Schematic of an adaptive reservoir computing framework using Brainoware. b, Schematic of the paradigm of Brainoware setup that mounts a single brain organoid onto a high-density MEA for receiving inputs and sending outputs. c, Whole-mount immunostaining of cortical organoids showing complex three-dimensional neuronal networks with various brain cell identities (for example, mature neuron, MAP2; astrocyte GFAP; neurons of early differentiation stage, Tujl; neural progenitor cells, SOX2). d, Schematic demonstrating the hypothesized, unsupervised learning of Brainoware by reshaping the BNN during training, and the inhibition of unsupervised learning after synaptic plasticity is blocked. Scale bar, 100 μm.

Cai 2023, Brain organoid reservoir computing artificial intelligence

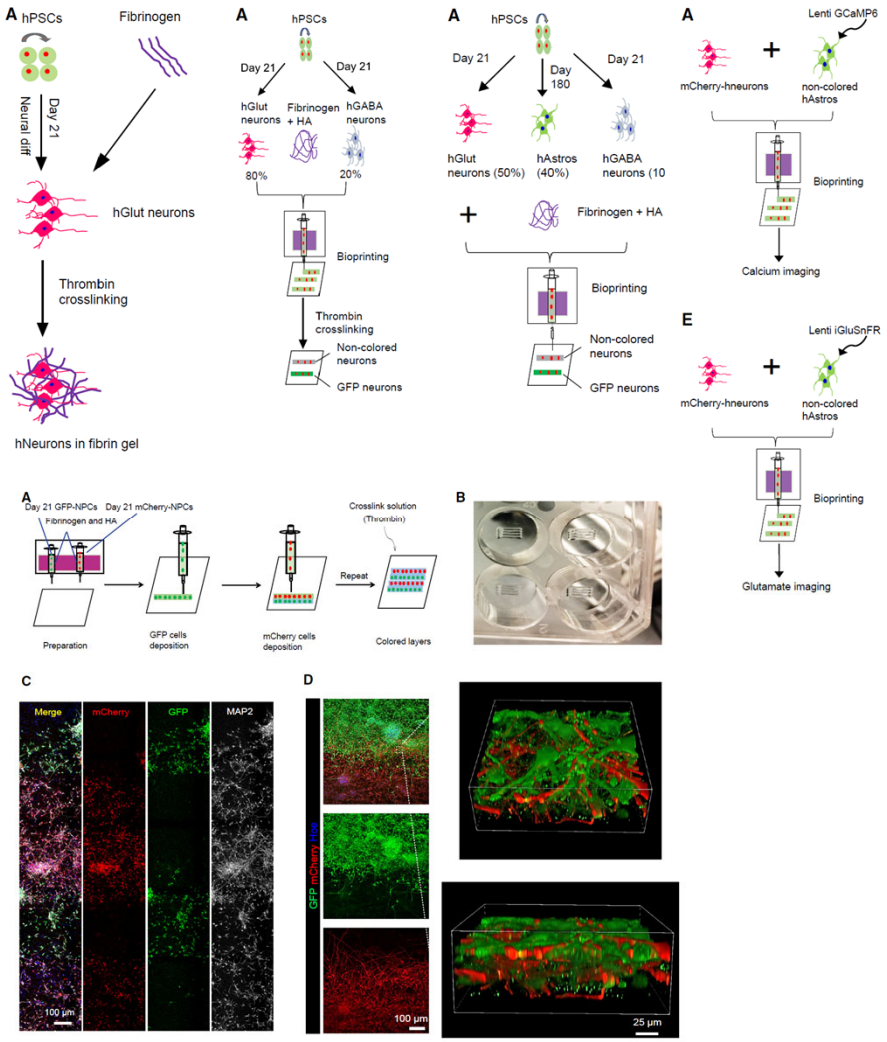
3D Bioprinting of Human Neural Tissues



Yan et al. generate 3D bioprinted human brain tissues that form functional neural networks within and between tissues, providing an effective tool for modeling network activity under physiological and pathological conditions.

Human pluripotent stem cells (hPSCs), including induced pluripotent stem cells (iPSCs) and human embryonic stem cells (hESCs),

Yan 2024, 3D bioprinting of human neural tissues with functional connectivity



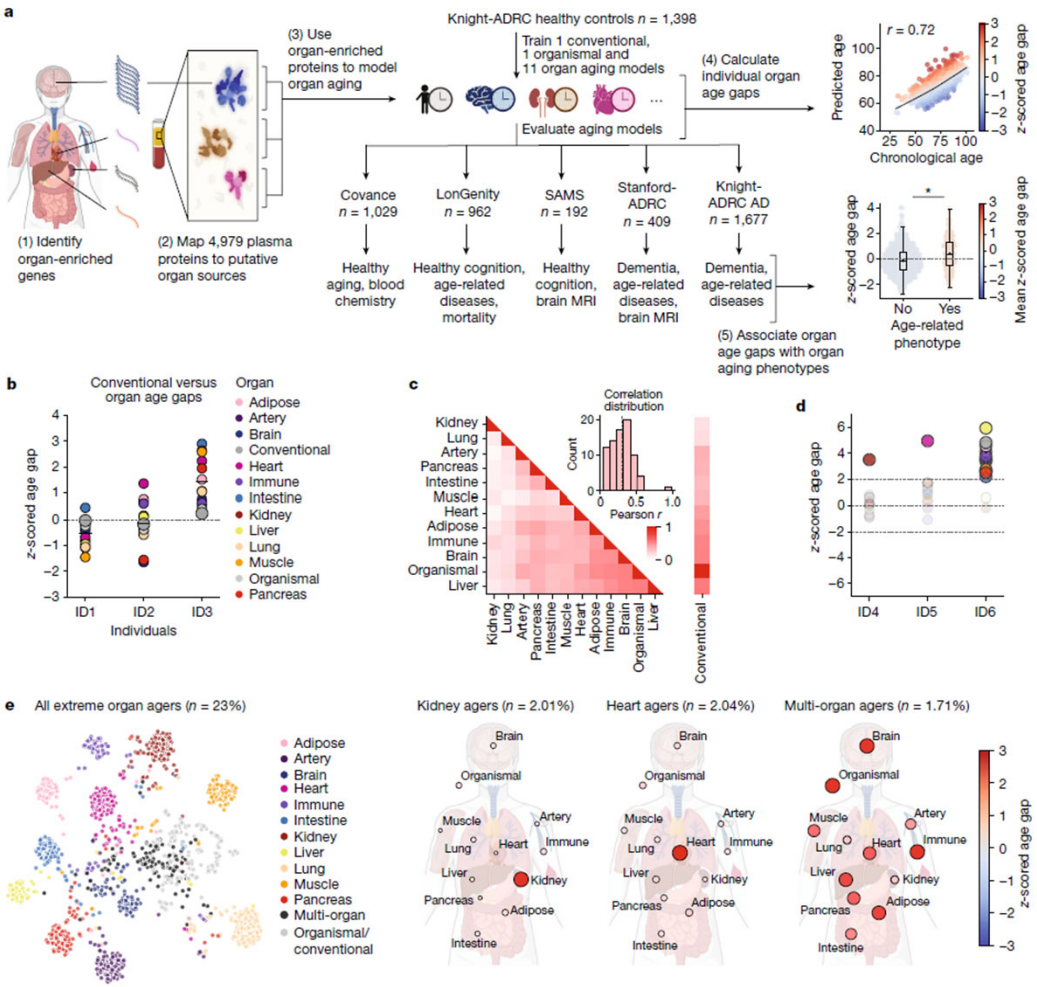
Limitations

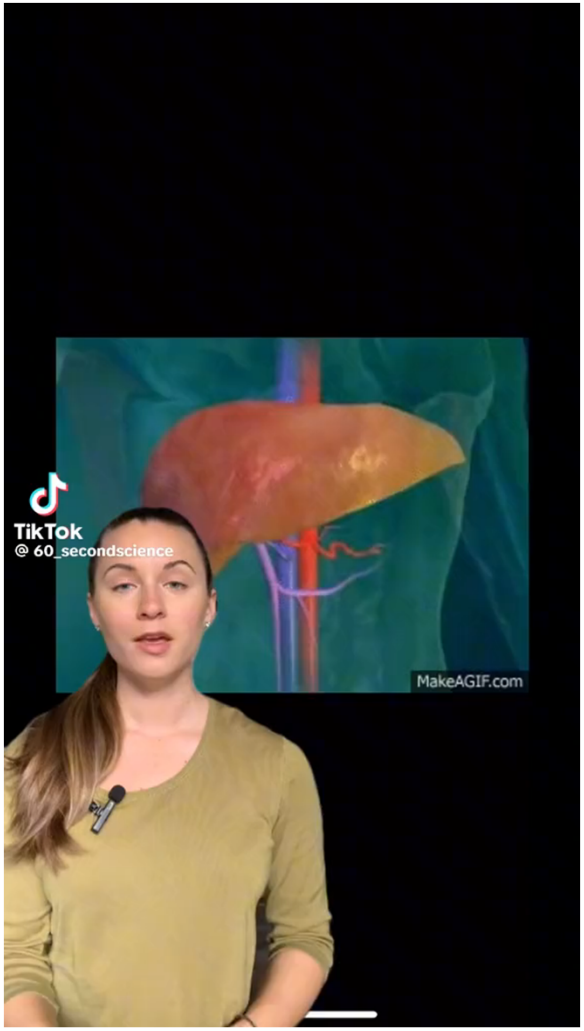
Our prototype 3D bioprinting has weaknesses. Due to the softness of the gel, our bioink does not support multiple-layer printing vertically. We also limited the thickness of the printed tissues to about 50 µm to maximize the formation of functional neural networks. Additionally, our current printing technology does not enable the orientation of the mature neurons although many neurons exhibit a pyramidal morphology. Since the tissue is assembled by design, the printed tissue lacks the intrinsic structural organization of brain organoids. Interestingly, however, some of the intrinsic properties, including the anatomical and functional neuronal connections, are retained in the printed tissue, as evidenced by the uni-directional axonal projection from the cortical tissue to the striatal tissue. On the other hand, the cells in the printed brain tissue mature rapidly and in a synchronized manner, thus complementing the existing organoids and offering a defined platform for examining human neural networks under physiological and pathological conditions. It is expected that with the advancement of bioprinting technology, more sophisticated human neural tissues may be produced with defined cellular compositions and orientation, tissue organization, and tissue assembly. Given that many neuronal subtypes can now be generated from hPSCs,[50] our platform allows printing neural tissues with a defined composition of neural types at a particular ratio, potentially enabling assessment of the biophysical nature of the human neural circuits. As exemplified by the dynamic interaction between the neuronal activation and altered response from AxD astrocytes in the printed neural tissues, the platform also provides a promising tool for studying interactions between specific neural cell types and neural circuits under pathological conditions. The defined dimensions and cellular compositions make the platform amenable to throughput analysis and drug development.

Tissue Regeneration

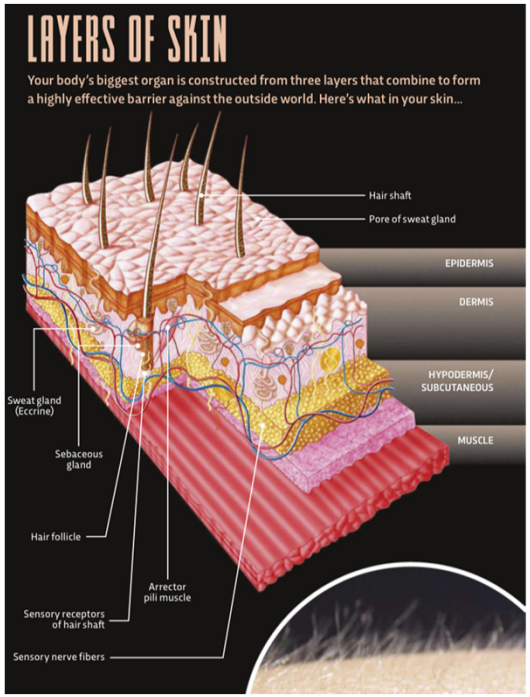
Organ Aging

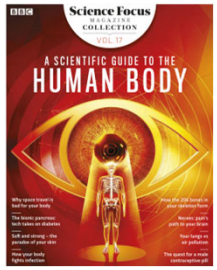
Oh 2023, Organ aging signatures in the plasma proteome track health and disease

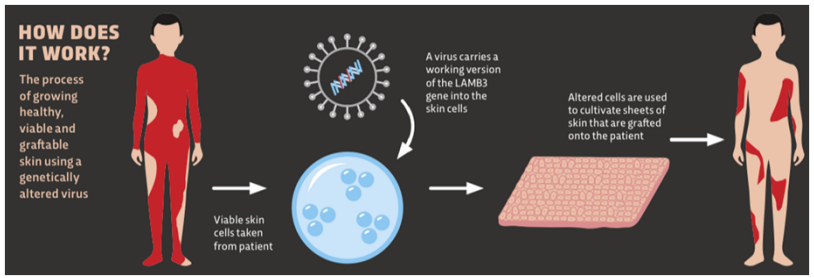




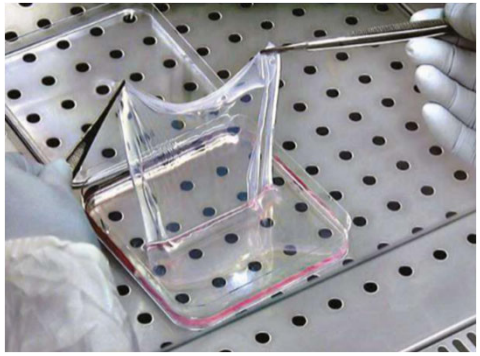
Fixing Broken Skin







The process of growing healthy, viable and gradable skin using a genetically altered virus.
A virus carries a working version of the LAMB3 gene into the skin cells.
Altered cells are used to cultivate sheets of skin that are grafted onto the patient.
Viable skin cells taken from patient.



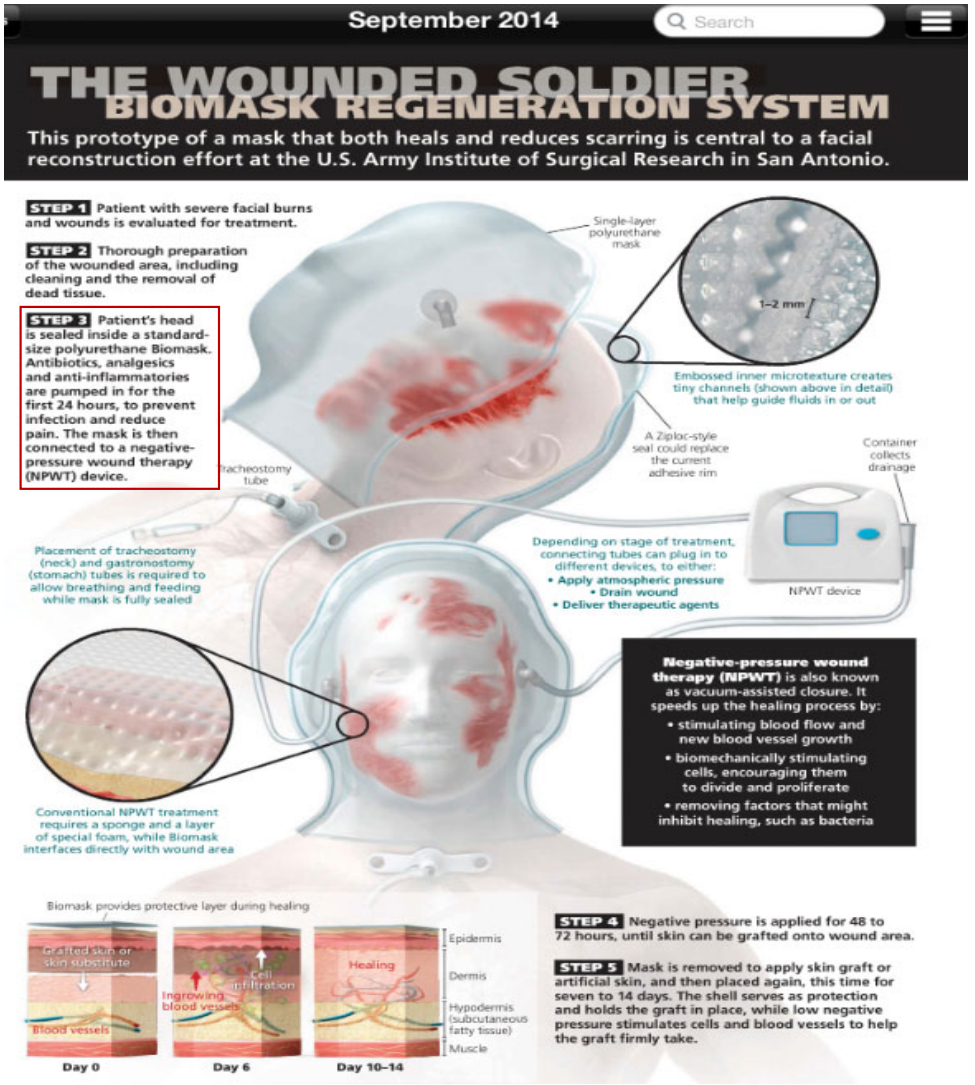
Sheets of genetically modified skin cells can be cultivated to match a patient and bypass the problem of their body rejecting the graft.

New medical techniques can help mend our outer membranes
Skin is at the forefront of many revolutions in modern medicine. New medications containing antibodies that target specific molecules – known as monoclonal antibodies – are incredibly ffective at treating autoimmune and in ammatory diseases. These have transformed the treatment of moderate to severe psoriasis, which soon may be a thing of the past, and new monoclonal antibodies for eczema are looking very promising. Recent leaps in the understanding of our skin's microbiome are also opening up new avenues of treatment, such as the research that suggests underarm bacterial transplants could be the cure for body odour.
Remarkably, one recent case involving a seven-year-old Syrian immigrant called Hassan, living in Germany, shows the skin as the laboratory for two emerging fields poised to revolutionise medicine: stem cell therapy and gene therapy. Hassan was born with a genetic condition called epidermolysis bullosa, caused by a mutation in the LAMB3 gene, in which the proteins that tightly anchor the epidermis to the dermis are missing. A shearing force as light as twisting a door handle would rip o the epidermis of his hand, causing immense pain and breaking the all-important barrier, le ing water out and microbes in.

The only viable skin left on Hassan's body clung to his face, left thigh and a few patches on his trunk. In this state, he didn't have long to live. Almost half of the children with this condition never make it to adolescence. But in 2015 a team from University of Modena and Reggio Emilia, Italy, took some of his skin cells, infected them with a virus that contained a healthy version of the LAMB3 gene, and grew nine square feet of this renewed skin in the lab. They replaced his skin in two operations and, amazingly, when the study was published, two years a er this experimental operation, Hassan's skin was still completely intact. The stem cells incorporated in the new skin were producing fresh, healthy skin cells for perpetuity.
BBC SCIENCE FOCUS MAGAZINE COLLECTION p. 41

Immortal Man by Year 2045

Biomask Regeneration System

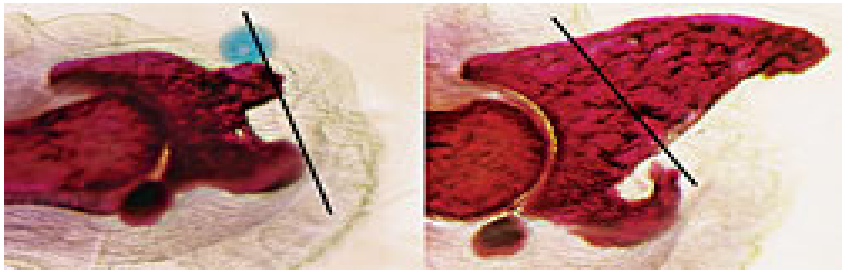


Recipes for Limb Renewal

Salamanders and other creatures that regrow lost body parts provide clues for ways to regenerate human limbs (Sophie L. Rovner)

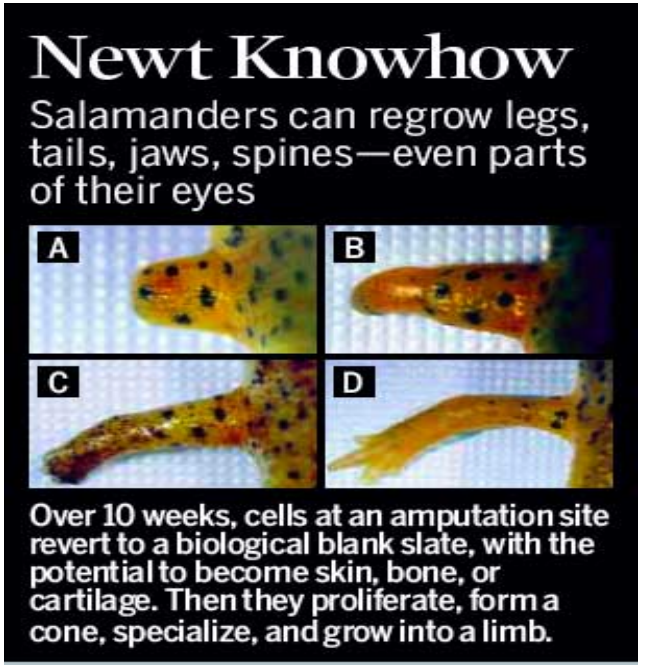
BODY ELECTRIC By inducing expression of a particular potassium ion channel in this tadpole, Levin's team altered its bioelectrical signaling, enabling it to grow multiple arms.

REGENERATION CHAMPION Salamanders, including this Notophthalmus viridescens specimen, can regrow lost limbs multiple times.

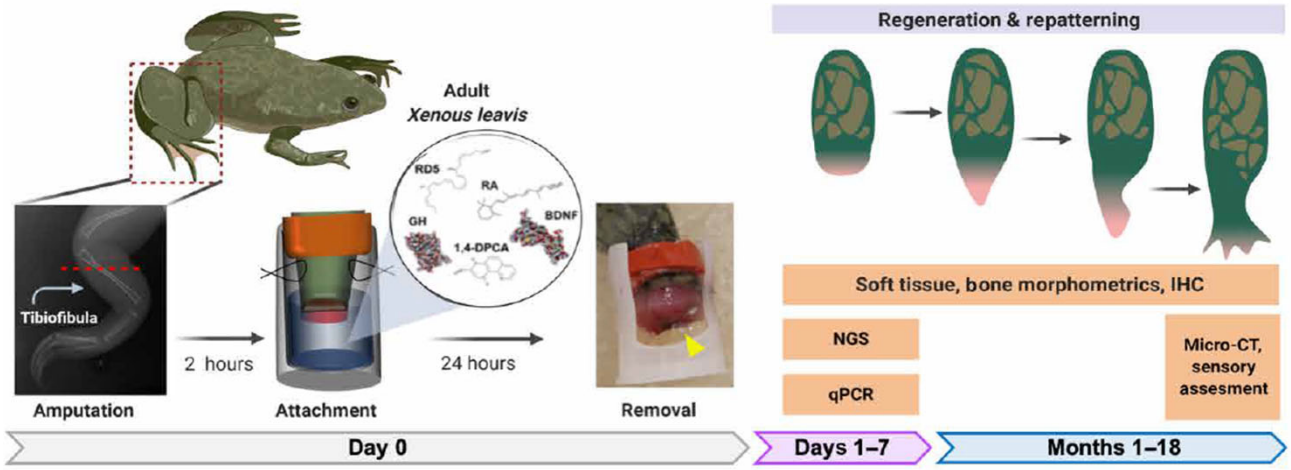


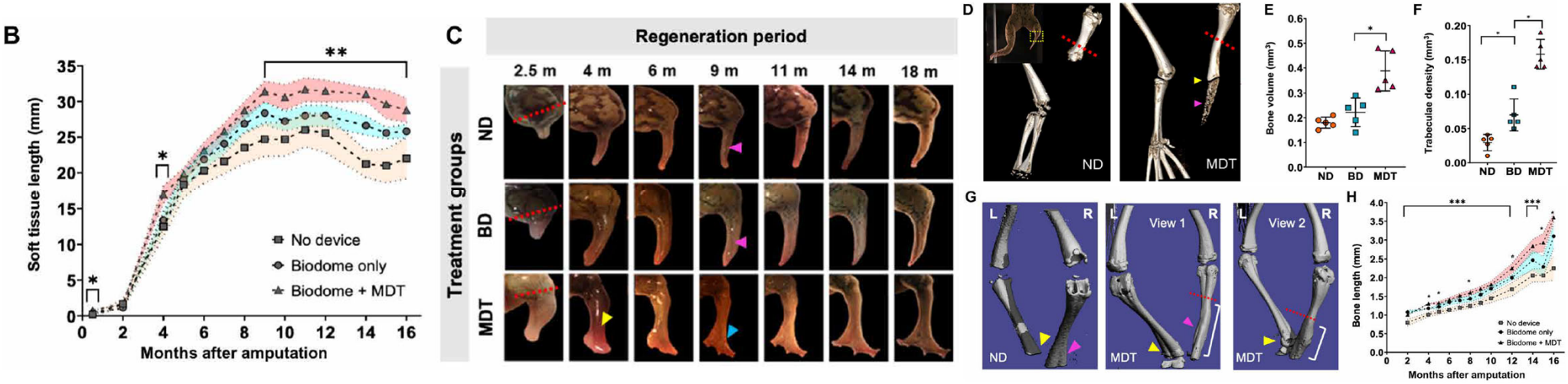
REGENERATION FERTILIZER A mouse can regrow an amputated digit tip, but if enough of the digit is removed—as shown in the image at left, in which the black bar indicates the line of amputation—the digit won't grow back. However, Muneoka found that treating such an amputation site with bone morphogenetic proteins enables a mouse to grow a replacement tip (right). In these images, captured with a dissecting microscope, bones are stained red and surrounding tissue appears clear. The triangular bone on the right is 1–2 mm long.

http://pubs.acs.org/cen/science/88/8831sci1.html



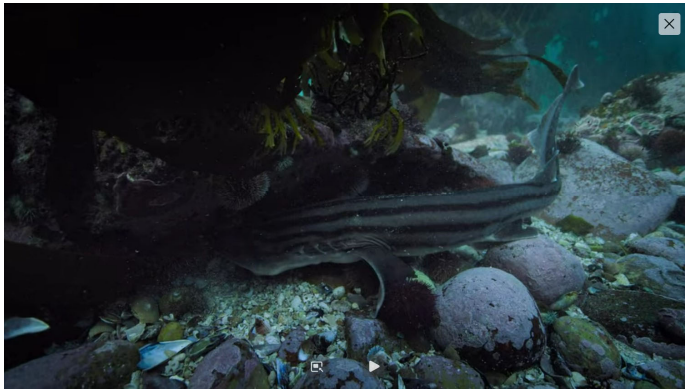
Long-term Limb Regeneration and Functional Recovery





Murugan 2022. Acute multidrug delivery via a wearable bioreactor facilitates long-term limb regeneration and functional recovery in adult Xenopus laevi

Fully Regrown after 100 Days





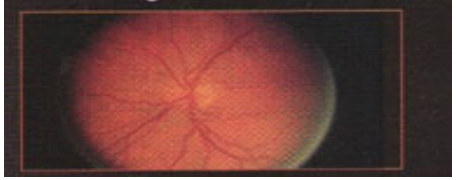

My Octopus Teacher (Netflix 2020)
A filmmaker forges an unusual friendship with an octopus living in a South African kelp forest, learning as the animal shares the mystery of her world (1:05:34)

The Two Faces of Angiogenesis

Vessel overgrowth can contribute to a variety of diseases that could be treatable with angiogenic inhibitors.

Problems of Extra Blood Vessels

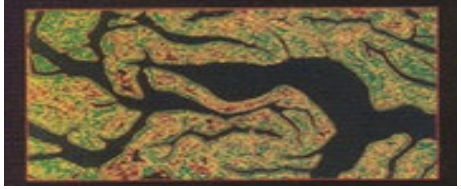
RETINAL DISEASE *
Angiogenesis inhibitors could help clear abnormal blood vessels from the eye



BREAST (AND OTHER) CANCER *
Starving cancers of a blood supply could help eradicate them

ATHEROSCLEROSIS
The plaques that clog vessels may support their own growth by expanding their blood supply

ENDOMETRIOSIS
Agents that block angiogenesis could prevent the growth of uterine tissue outside the uterus

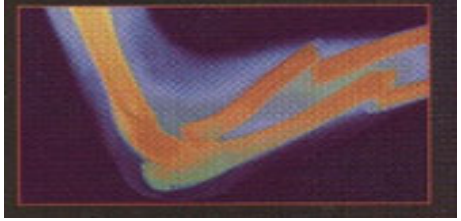


OBESITY
Fat requires miles of blood vessels, which could be trimmed by angiogenesis inhibitors

Proangiogenic agents can stimulate vessel development to treat certain diseases.

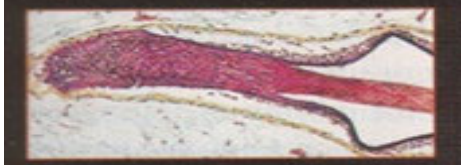
Benefit of Extra Blood Vessel

LIMB FRACTURES
New blood vessels could help repair broken bones

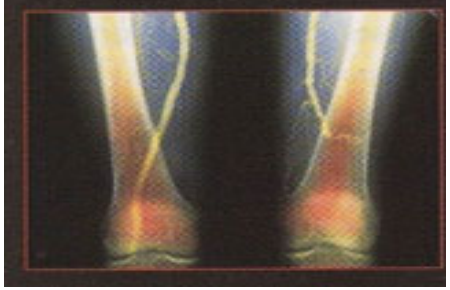


NEURODEGENERATIVE ILLS
An increased blood supply could minimize neuronal damage in the brain

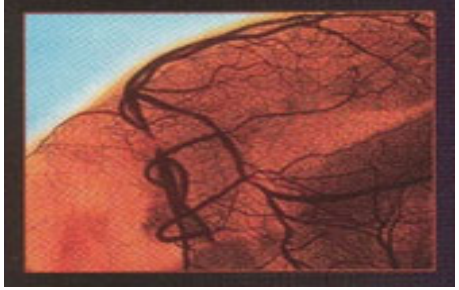
BALDNESS
Hair follicles depend on a good blood supply



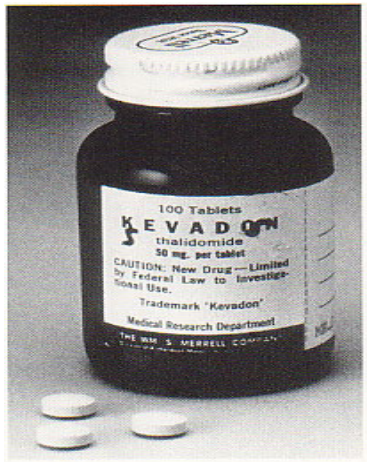
BLOOD CLOTS IN LEGS *
Angiogenesis could bypass clots and improve circulation

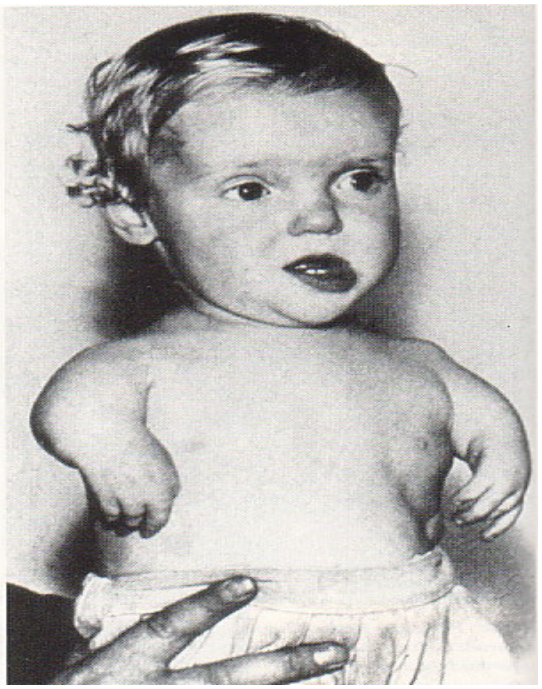


HEART ATTACK *
New coronary vessels could help repair a damaged heart



The Thalidomide Incidence





Above: *Kevadon, also known as thalidomide. It was sold chiefly outside the United States as a sedative despite a lack of testing to determine if it was safe. It caused birth defects when taken in the early months of pregnancy, and led to thousands of cases of premature death and, most famously, a fetal disability in which limbs were stunted. The FDA refused to approve it without better safety data.*

Thalidomide's horrifying effects on newborns became known in 1962.
Distribution of two million tablets by Merrell for investigational use.

Frances Kelsey: Medical officer at FDA Refusal to allow NDA of thalidomide based on insufficient safety data.

Stem Cells

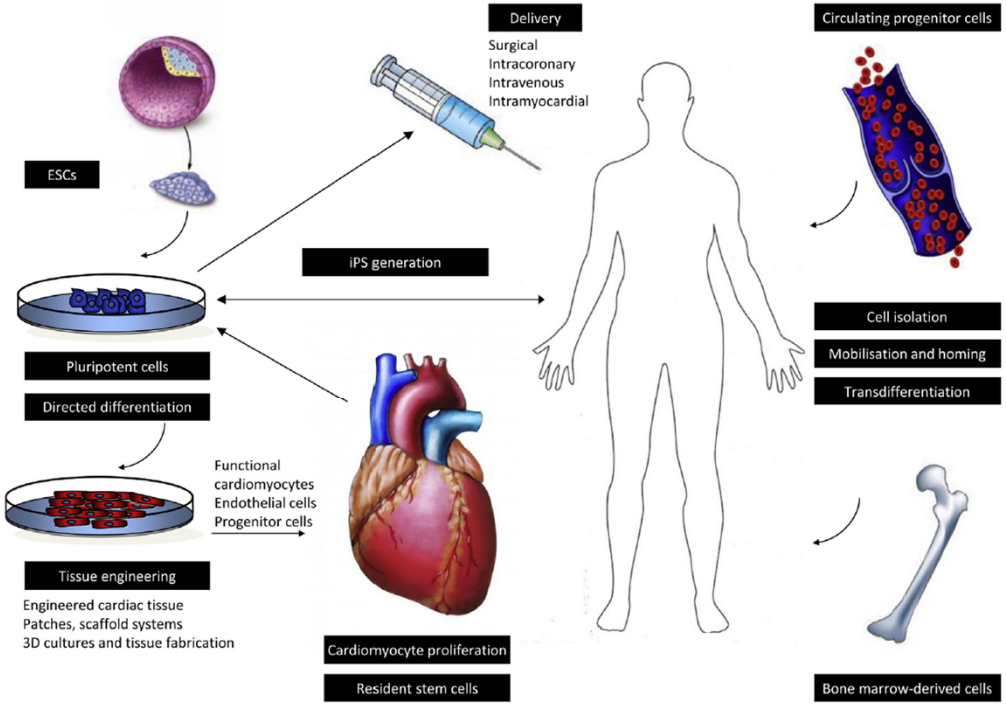


Figure 1 Experimental approaches to cell therapy in heart failure. Clinical trials have been performed with bone marrow-derived cells, circulating progenitor cells and skeletal myoblasts that have been delivered to the heart. Conceptually it would be also possible to achieve by purifying cells of the cardiac lineage from differentiating human embryonic stem cells or induced pluripotent stem cells to the relevant cell type, and thereafter transplanting such cells to the heart of the patient.

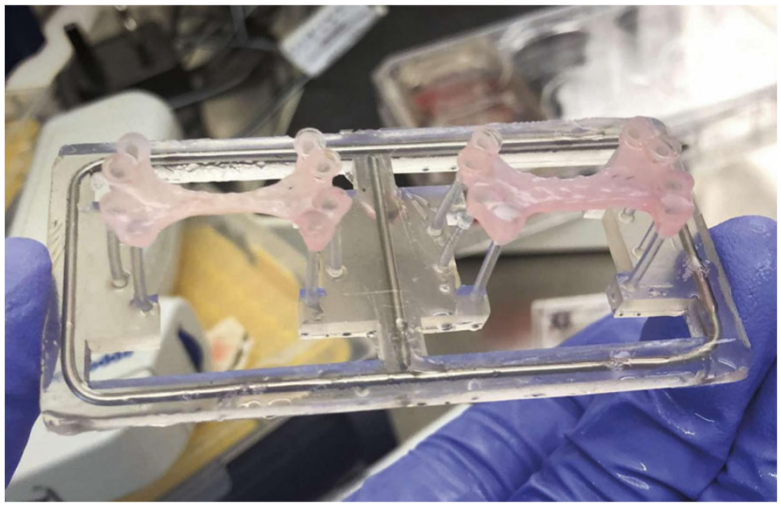
Table 1 Advantages and disadvantages of various cell types isolated from different sources

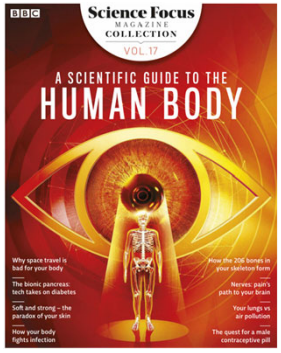
Stem cells	*Cell source*	*Advantages*	*Disadvantages*	*Clinical trials*
Embryonic stem cells, induced pluripotent stem cells	Inner cell mass of the blastocyst, adult fibroblasts Allogeneic cell lines vs autologous somatic cells (iPSC)	Unlimited self-renewal capacity, pluripotency Ability to form functional cardiomyocytes	Ethical and legal considerations; potential teratoma formation from residual cells; immunological problems: graft versus host disease (not with iPSC)	One safety and feasibility transplantation study is recruiting with embryonic stem cell derivatives; none with iPSC
Bone marrow mononuclear cells, haemopoietic stem cells, circulating progenitor cells	Bone marrow, peripheral blood, umbilical cord, placenta	Easy to isolate, proven safety and feasibility to implant Potential for vasculogenesis	Lack of true cardiac differentiation Unknown underlying mechanisms	Medium-scale trials with modest or no benefits; significant reduction in subsequent cardiovascular events
Mesenchymal stem cells	Bone marrow (adherent cells), and other mesenchymal tissues such as adipose tissue	Easy to isolate and expand in culture, abundant supply, low immunogenicity, multipotency	Large heterogeneity; heterotopic differentiation (ventricular ossification) Unknown myocyte regenerative potential	Safety and feasibility studies and multi-centre, randomized clinical trials
Endothelial progenitor cells	Bone marrow, peripheral blood	Important in neovasculogenesis Mobilized from bone marrow or present in the peripheral blood	Heterogeneity; low circulating cell number; reduced cell number in patients with cardiovascular comorbidities	Safety and feasibility studies and multi-centre, randomized clinical trials, no significant benefit
Skeletal myoblasts	Mature skeletal muscle (between sarcolemma and basement membrane) Skeletal muscle biopsy specimen	Extensive scalability; resistance to ischemia; multipotent; no teratoma formation	Controversial data on arrhythmogenesis; lack cardiomyocyte differentiation (dyssynchronous beating)	Large scale clinical trials; no benefit observed
Cardiac resident stem cells and progenitors	Special niches within the myocardium	Resident cells; robust cardiovascular differentiation potential; reduced tumor formation; electrically integrated	Stem cell pool undergo senescence; unknown scalability	Safety/Efficacy studies in progress

Gara 2019, Stem cell therapy to treat heart failure

Pumping Heart Patch

A net of stem cells to graft over the damaged area and support the process of regeneration.





CIRCULATORY SYSTEM

Get advice on what you can do to reduce the risk of getting heart disease bbc.in/2GEnCKe

HEART OF THE MATTER

Keeping blood circulating around the network of vessels and organs in your body is a 24/7 job. And there's one four-chambered bundle of muscle and nerves at the heart of the entire operation…

words by SIMON CROMPTON

Heart specialists like the term 'perfuse'. It's a word that sums up the role of the circulatory system – the heart and its approximately 100,000km network of veins, arteries and capillaries that carry blood to virtually every cell in your body. The system perfuses each organ in blood, supplying just the right amount of oxygen, nutrients and regulatory hormones to keep it healthy.

At the same time, the circulatory system takes away carbon dioxide and waste products that would otherwise harm cells. It also regulates your body's temperature and transports your white blood cells around your body so they're where they are needed to fight infection.

If you imagine the circulatory system as a simple pump with tubes, think again. "The circulatory system is a fantastically sophisticated machine," says Barbara Casadei, Professor of Cardiovascular Medicine at the British Heart Foundation's Centre of Research Excellence, University of Oxford. "After nearly 30 years in cardiology, I still find it amazing that it can selectively perfuse each organ according to its needs at the time, yet still maintain blood pressure in the whole system."

A SELF-AWARE SYSTEM

Your circulatory system (also known as the cardiovascular system) consists of: your heart, the arteries that take oxygenated blood from your heart, the veins that bring deoxygenated blood back to your heart, and the tiny blood vessels called capillaries that branch off arteries and veins – feeding (and taking waste products from) every cell in your body.

But it's not a simple circular system. Your heart is, in effect, two pumps joined together, each powering separate but

Your heart started beating 22 days after you were conceived and will continue to do so for your entire life

linked circuits. One circuit feeds deoxygenated blood to your lungs to get rid of waste carbon dioxide and pick up oxygen. The other pumps this oxygenated blood all around your body.

On the way around your body, blood passes through the gut where it picks up nutrients to feed organs, through the kidneys and liver where waste products are filtered out, and is then carried on to the brain, which demands large amounts of oxygen and nutrients.

What's remarkable is the way the flow is regulated. Your heart needs to pump hard and maintain a good blood pressure, so that plenty of blood reaches your brain even when you're standing and gravity is pushing it to your toes. But too hard a flow would cause damage to your blood vessels and delicate tissues, particularly the brain.

So your veins have valves to prevent backward flow and your capillaries dilate and contract to divert flow to areas where blood is most needed. If you're running, for example, blood will be diverted away from your digestive organs and towards your leg muscles. Receptors throughout the system act as sensors, detecting blood pressure and chemical changes in the blood, which are translated into nerve and hormone signals that, in turn, control how wide your blood vessels open and how fast your heart beats.

"It's a wonderful complex system, so it's not surprising that if something goes wrong, we are in serious trouble," says Prof Casadei.

UNDER PRESSURE

As your blood moves around your body it pushes against the sides of blood vessels. This pressure is a product of both how narrow your blood vessels are and how hard your heart is working. You can run into problems if your blood pressure is too high or too low so it good to get it tested occasionally. A blood pressure test provides two readings. One is the pressure when your heart is pushing blood into the arteries (systolic pressure) and one is in the instant between

TOP LEFT: Your blood pressure provides an indication of the condition of your circulatory system

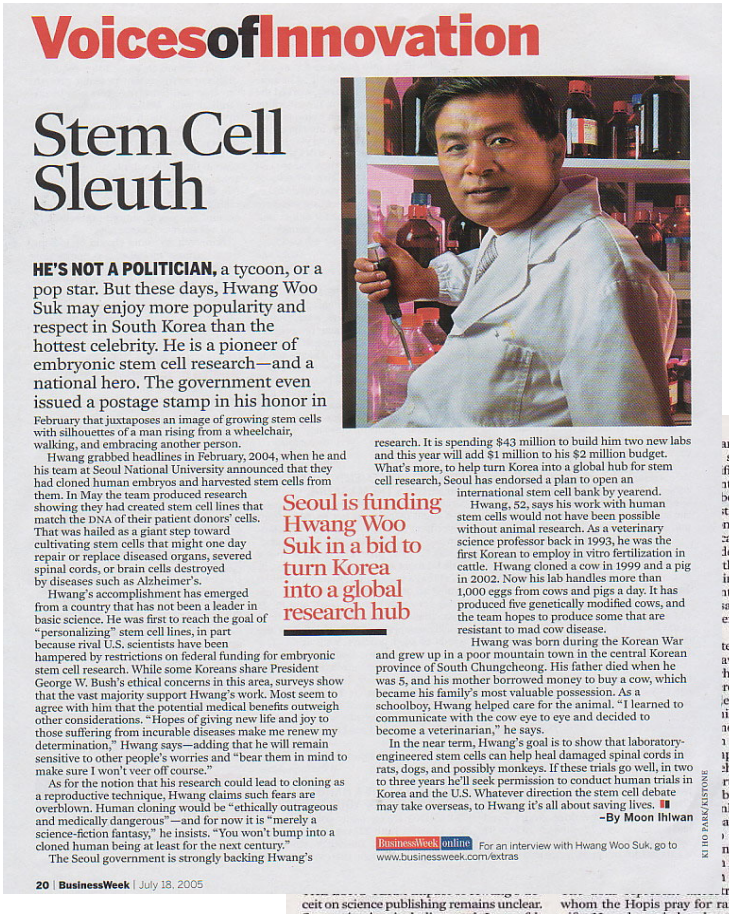
TOP: Ultrasound scans are used to check on the heart beat of babies as they develop in the womb

ABOVE: Atherosclerosis, the accumulation of fatty plaque in your arteries, is a common cause of heart attacks and strokes

Stem Cells

Research on tem cells was a sensational topic for a while, as stem cells showed a potential to revolutionize the entire medicine. Then, the dream was busted by a scientist who fabricated the data on his stem cell research. This made a full stop to the stem cell research throughout the world.

BusinessWeek, Jan 23, 2006



Voices of Innovation

Stem Cell Sleuth

HE'S NOT A POLITICIAN, a tycoon, or a pop star. But these days, Hwang Woo Suk may enjoy more popularity and respect in South Korea than the hottest celebrity. He is a pioneer of embryonic stem cell research—and a national hero. The government even issued a postage stamp in his honor in February that juxtaposes an image of growing stem cells with silhouettes of a man rising from a wheelchair, walking, and embracing another person.

Hwang grabbed headlines in February, 2004, when he and his team at Seoul National University announced that they had cloned human embryos and harvested stem cells from them. In May the team produced research showing they had created stem cell lines that match the DNA of their patient donors' cells. That was hailed as a giant step toward cultivating stem cells that might one day repair or replace diseased organs, severed spinal cords, or brain cells destroyed by diseases such as Alzheimer's.

Hwang's accomplishment has emerged from a country that has not been a leader in basic science. He was first to reach the goal of "personalizing" stem cell lines, in part because rival U.S. scientists have been hampered by restrictions on federal funding for embryonic stem cell research. While some Koreans share President George W. Bush's ethical concerns in this area, surveys show that the vast majority support Hwang's work. Most seem to agree with him that the potential medical benefits outweigh other considerations. "Hopes of giving new life and joy to those suffering from incurable diseases make me renew my determination," Hwang says—adding that he will remain sensitive to other people's worries and "bear them in mind to make sure I won't veer off course."

As for the notion that his research could lead to cloning as a reproductive technique, Hwang claims such fears are overblown. Human cloning would be "ethically outrageous and medically dangerous"—and for now it is "merely a science-fiction fantasy," he insists. "You won't bump into a cloned human being at least for the next century."

The Seoul government is strongly backing Hwang's research. It is spending $43 million to build him two new labs and this year will add $1 million to his $2 million budget. What's more, to help turn Korea into a global hub for stem cell research, Seoul has endorsed a plan to open an international stem cell bank by yearend.

Seoul is funding Hwang Woo Suk in a bid to turn Korea into a global research hub

Hwang, 52, says his work with human stem cells would not have been possible without animal research. As a veterinary science professor back in 1993, he was the first Korean to employ in vitro fertilization in cattle. Hwang cloned a cow in 1999 and a pig in 2002. Now his lab handles more than 1,000 eggs from cows and pigs a day. It has produced five genetically modified cows, and the team hopes to produce some that are resistant to mad cow disease.

Hwang was born during the Korean War and grew up in a poor mountain town in the central Korean province of South Chungcheong. His father died when he was 5, and his mother borrowed money to buy a cow, which became his family's most valuable possession. As a schoolboy, Hwang helped care for the animal. "I learned to communicate with the cow eye to eye and decided to become a veterinarian," he says.

In the near term, Hwang's goal is to show that laboratory-engineered stem cells can help heal damaged spinal cords in rats, dogs, and possibly monkeys. If these trials go well, in two to three years he'll seek permission to conduct human trials in Korea and the U.S. Whatever direction the stem cell debate may take overseas, to Hwang it's all about saving lives.

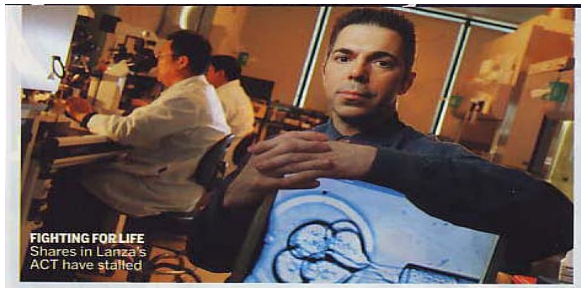
–By Moon Ihlwan

BusinessWeek online For an interview with Hwang Woo Suk, go to www.businessweek.com/extras

KI HO PARK/XISTONE

20 | BusinessWeek | July 18, 2005

BusinessWeek, Jul. 18, 2005



BIOTECH

A 'BODY BLOW' TO STEM CELL RESEARCH

Funding in the U.S. wasn't so hot even before the Korean scandal broke. Now...

BY ARLENE WEINTRAUB

ON A RAINY DAY IN Worcester, Mass., just before New Year's, Dr. Robert Lanza threw his arms up in despair. He had just heard initial reports that South Korean scientist Hwang Woo Suk faked 11 lines of embryonic stem cells he claimed to have created through cloning last May, with the results published in the prestigious journal *Science*. Lanza is vice-president of medical and scientific development for Advanced Cell Technology Inc., one of the few publicly held U.S. companies that are researching embryonic stem cells. He fears that Hwang's fabrication, which was confirmed on Jan. 10, will set the already embattled field back indefinitely. "The reputation of the science has suffered," Lanza laments.

The Korean debacle is a black eye that America's stem cell pioneers can ill afford. Federal funding is still limited to research on just a few batches, or "lines," of the embryonic cells. And the promised $3 billion in funding from California's Proposition 71 is tied up by lawsuits challenging its constitutionality. The political overhang has kept most venture capitalists away from anyone with the phrase "stem cell" in their business plan. When the Korean scandal started erupting in December, shares of ACT fell 16% and rival Geron Corp. tumbled 4%, despite the fact that interest in biotech was strong and the Amex Biotech Index was up 6% in the same period. Korea "is just another body blow," says ACT investor William Woodward of Santa Monica (Calif.)-based Anthem Venture Partners.

As the custodians of academic research, journals such as *Science* bear some responsibility for the tightening purse strings. *Science* previously said it would withdraw Hwang's May paper and on Jan. 10, the magazine said it would withdraw a 2004 paper by Hwang as well. Both papers, like many in well-known journals, were "peer reviewed," meaning they were critiqued by two or more independent scientists before they were published. But the vetting clearly was inadequate, and *Science* Editor-in-Chief Donald Kennedy has said in a statement that the journal will consider "additional procedural safeguards."

In the tiny world of stem cell research, that's a scary message. Because commer-

FALLOUT Hwang's deceit could shake up peer review

SHAWN G. HENRY

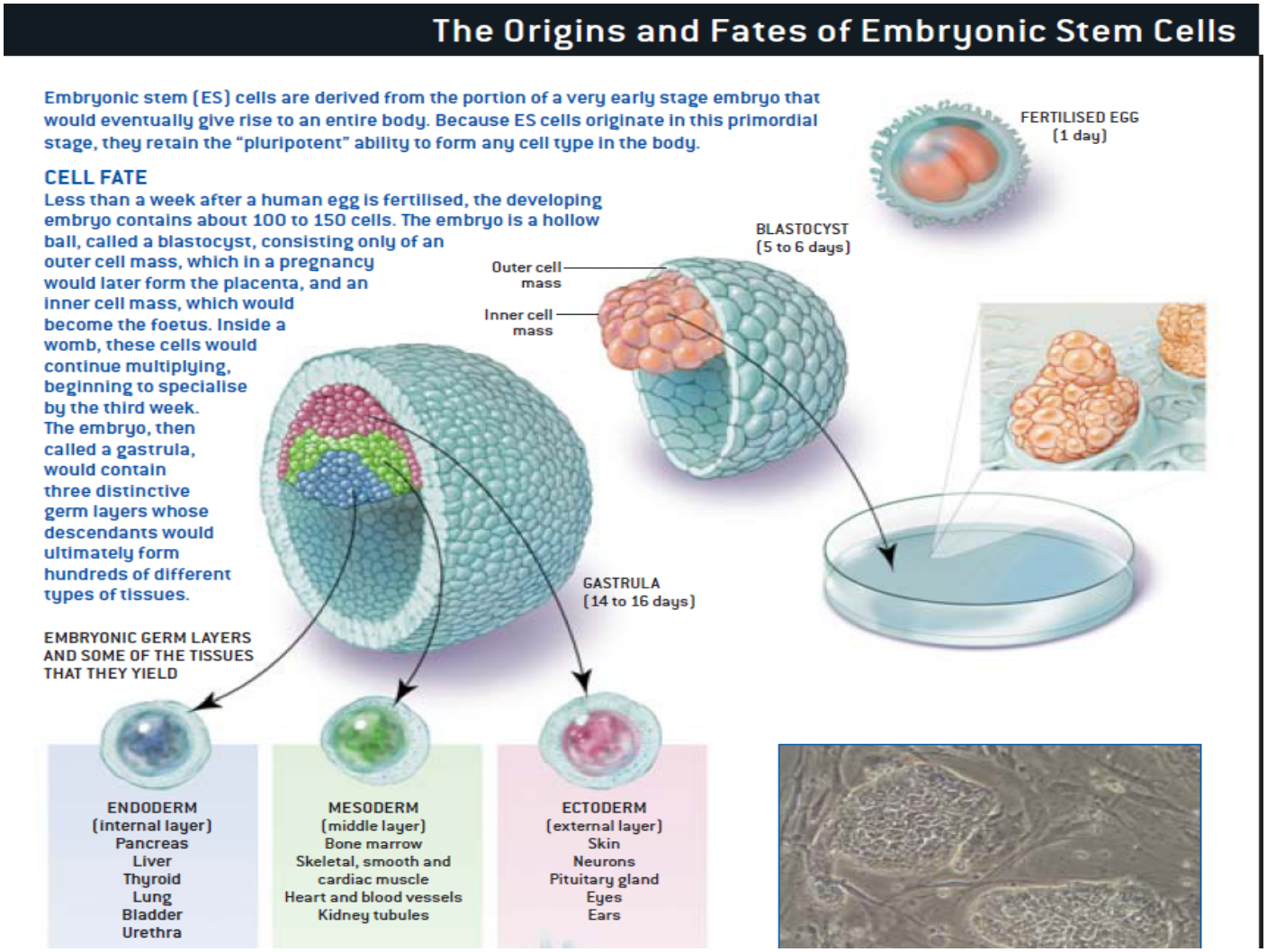
ang's original should have fied by an in- lab. Yet boss, Michael t, concedes nly so much an do if a sci- determined to them. "They into labs and ntific experi- ays West, entific officer

ted stem cell ave left ACT ho joined the ron in 1998, ence of deep- ile, across the nent was fun- as Hwang's, pression that hind. rtions. When by going pub- nkers were so any opted in- a shell com- ndian dolls. It initial public was perfect: tral spirits to

ceit on science publishing remains unclear. Some scientists, including ACT's Lanza, felt whom the Hopis pray for rain and other gifts. New shares in hand, ACT raised $18 million from hedge funds and other risk-taking investors—enough to carry the company into 2007. But the spirits didn't smile on this stock, which fell from a high of $7 to a recent $1.90 a share.

Lanza says the stem cell flap hurts patients the most. He often thinks about an incident in 2004 when a police officer followed him to work. Lanza thought he was about to get snared for speeding. But the cop just wanted an update on ACT's research. One promising project was using stem cells to make retinal pigment epithelial (RPE) cells, which the eye needs in order to see. The policeman's teenage son was suffering from a degenerative eye disease and was about to go blind. "I was in tears," Lanza says. "We didn't have the money to keep the cells going." Thanks to fresh capital from the Hopi doll company merger, that research is back on track. A Red Tailed Hawk doll sits in Lanza's office, reminding him of the strange reversal of fortune that reinvigorated ACT. Now Hwang's lab has crumbled, notes Lanza, and "we're still alive."

Embryonic Stem Cells

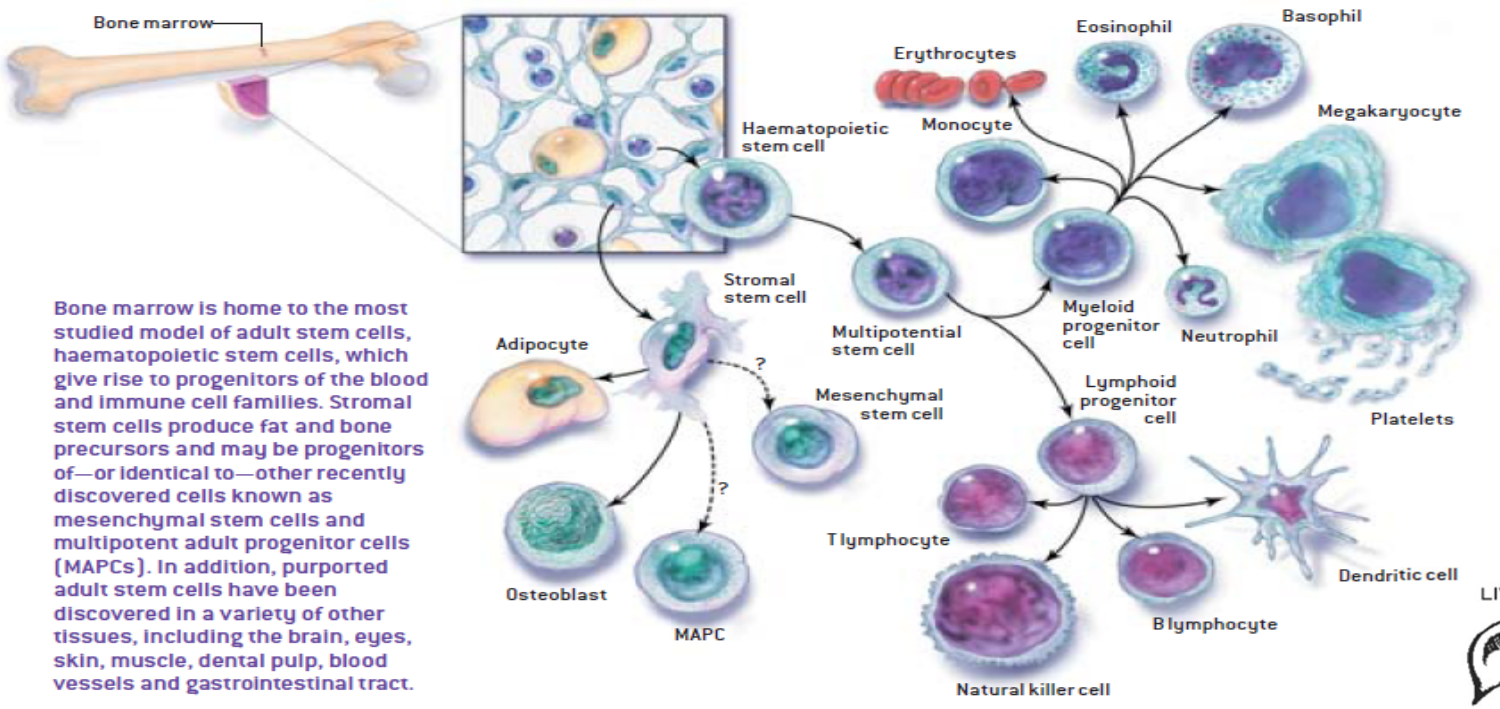


Grow your own eye: biologists have coaxed cells to form a retina, a step toward growing replacement organs outside the body.

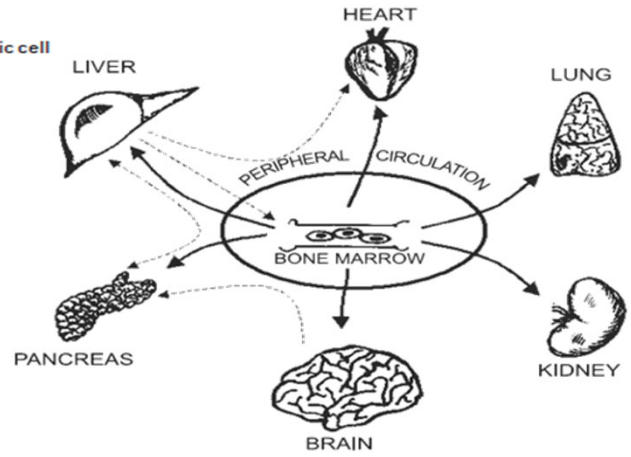
Sasai, Y. Scientific American 307(5): 44-49. November 2012.

Haematopoietic Stem Cells

Stem Cell Storehouse



Bone marrow is home to the most studied model of adult stem cells, haematopoietic stem cells, which give rise to progenitors of the blood and immune cell families. Stromal stem cells produce fat and bone precursors and may be progenitors of—or identical to—other recently discovered cells known as mesenchymal stem cells and multipotent adult progenitor cells (MAPCs). In addition, purported adult stem cells have been discovered in a variety of other tissues, including the brain, eyes, skin, muscle, dental pulp, blood vessels and gastrointestinal tract.



The interchangeable stem cell hypothesis: Primitive stem cells may exist within the bone marrow that are capable of homing to tissues that need repair or regeneration (solid lines). Likewise, progenitor cells from an adult organ, such as liver, may act as a source of regeneration-competent cells for another tissue, including the bone marrow (dotted lines).

Keeping Cells Alive

Cells are an essential component in tissue engineering. Cells may not be available when necessary. For this reason, cells may have to be frozen until use. Frequently cells die during freezing and thawing processes.

Stem cells are an ideal choice for any tissue engineering, although we still have to understand more how to make them grow into specific cell types for certain tissues and organs.

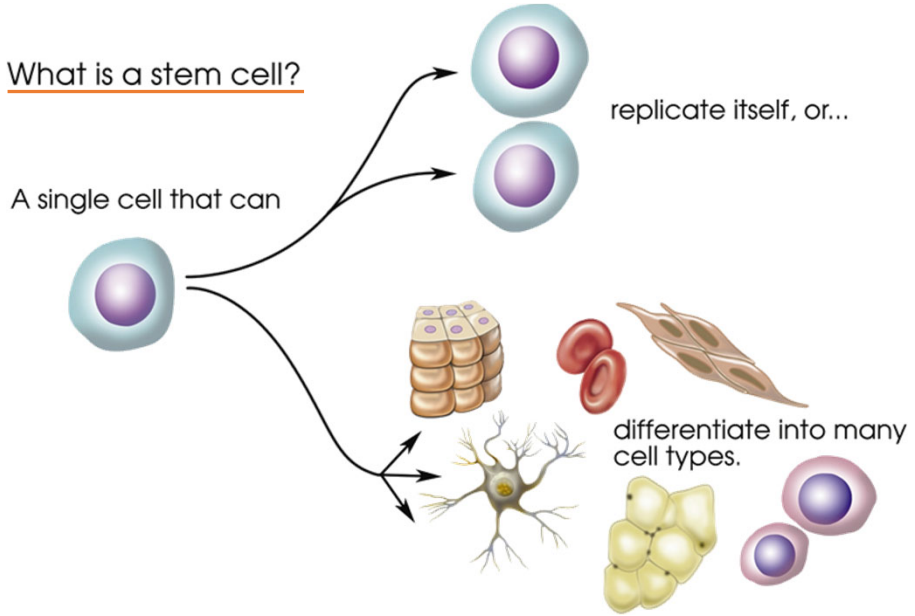
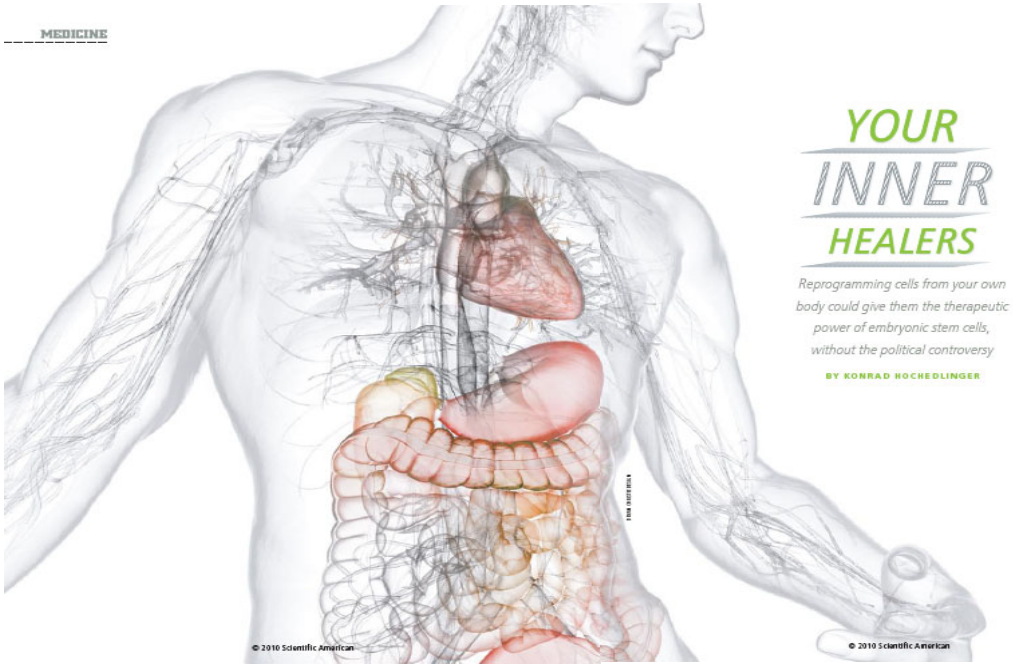


Image prepared by Catherine Twomey for the National Academies, Understanding Stem Cells: An Overview of the Science and Issues from the National Academies, http://www.nationalacademies.org/stemcells.

Reprogramming Cells



YOUR INNER HEALERS

Reprogramming cells from your own body could give them the therapeutic power of embryonic stem cells, without the political controversy

BY KONRAD HOCHEDLINGER

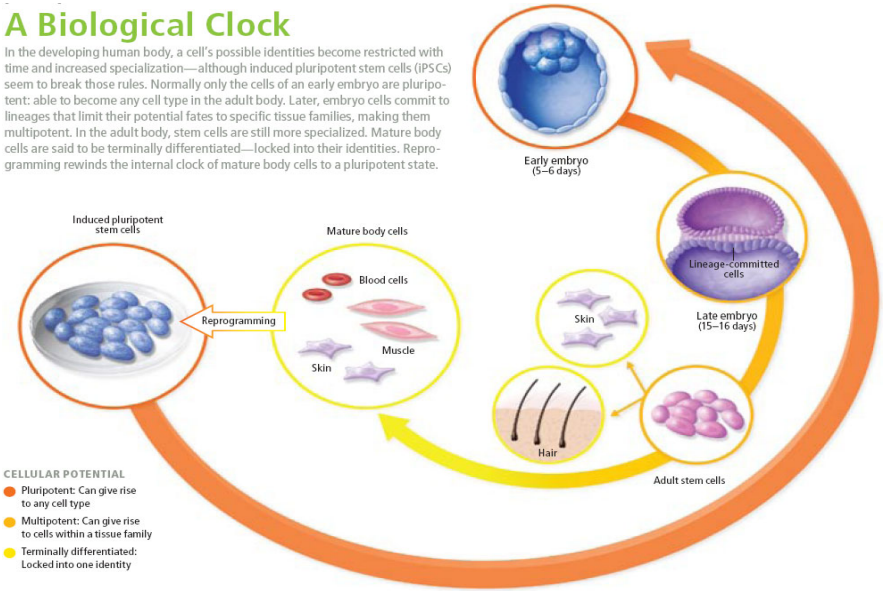
KEY CONCEPTS

- Induced pluripotent stem cells are mature body cells that have been made to change their identities and revert to an embryonic state—without the help of eggs or embryos.
- Rejuvenating the normal body cells of any individual—than converting them to any of the 220 human cell types—could yield new disease treatments and custom replacement tissues.
- Scientists are now working to understand how these cells are able to reverse their biological clocks and whether the newest kind of stem cell will prove as powerful as embryonic cells.

—*The Editors*

A Biological Clock

In the developing human body, a cell's possible identities become restricted with time and increased specialization—although induced pluripotent stem cells (iPSCs) seem to break those rules. Normally only the cells of an early embryo are pluripotent: able to become any cell type in the adult body. Later, embryo cells commit to lineages that limit their potential fates to specific tissue families, making them multipotent. In the adult body, stem cells are still more specialized. Mature body cells are said to be terminally differentiated—locked into their identities. Reprogramming rewinds the internal clock of mature body cells to a pluripotent state.

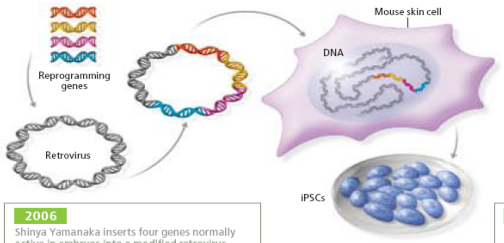


CELLULAR POTENTIAL

- Pluripotent: Can give rise to any cell type
- Multipotent: Can give rise to cells within a tissue family
- Terminally differentiated: Locked into one identity

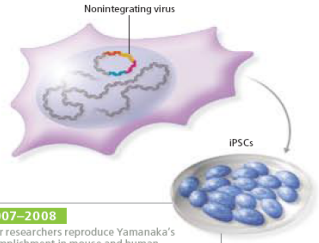
Rapid Progress toward Safe Cell Rejuvenation

Just four years ago scientists in Japan first showed that a set of genes ferried by a retrovirus could transform the skin cells of adult mice into pluripotent stem cells. Many researchers have since been working to achieve the same end in simpler, safer and more efficient ways—key steps to making therapy a reality.



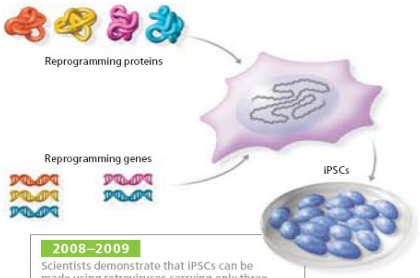
2006

Shinya Yamanaka inserts four genes normally active in embryos into a modified retrovirus, which he then injects into mouse skin cells. The virus inserts the genes into the mouse DNA, and the genes then begin reprogramming the skin cells into induced pluripotent stem cells (iPSCs).



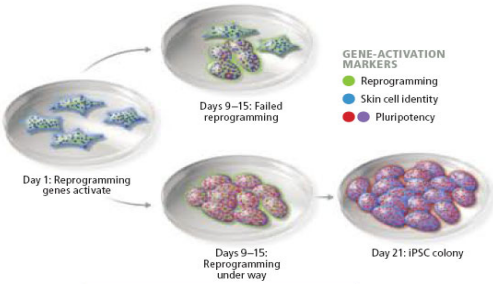
2007–2008

Other researchers reproduce Yamanaka's accomplishment in mouse and human cells. Experiments also show that delivery of the four reprogramming genes by viruses that do not permanently integrate into cellular DNA still succeeds in producing iPSCs.



2008–2009

Scientists demonstrate that iPSCs can be made using retroviruses carrying only three of the original Yamanaka reprogramming genes, then only two, or just by introducing the proteins encoded by the four reprogramming genes directly into cells.



2009–2010

Scientists focus on raising efficiency by identifying distinct patterns of gene activation (revealed by fluorescent markers) characterizing cells that will successfully convert to iPSCs. Skin cell identity and reprogramming-gene markers give way to pluripotency markers.

Tissue Regeneration: FDA

Regenerative Medicine Cellular Products

Advanced analytical methods for assessing the efficacy of regenerative medicine cellular products

Cell therapy related devices ReCell® Autologous Cell Harvesting

- An autograft-sparing technology indicated for use at the patient's point-of-care for preparation of an autologous epithelial cell suspension to be applied to a prepared wound bed.
- The suspension is used to achieve epithelial regeneration for definitive closure of burn injuries, particularly in patients having limited availability of donor skin for autografting.
- Pre-Market Approval (PMA) approved, September 2018

Slide by **Kyung Sung, Ph.D.**
FDA Branch Chief
Cellular and Tissue Therapies Branch (CTTB)
Office of Cellular Therapy and Human Tissue (OCTHT)
Center for Biologics Evaluation and Research (CBER)
https://www.fda.gov/vaccines-blood-biologics/biologics-research-projects/investigating-effects-cell-materials-interactions-safety-and-effectiveness-cell-based-products

Licensed Cellular Products

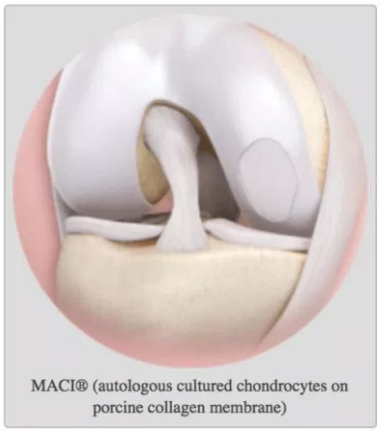
- **PROVENGE (sipuleucel-T):** Autologous T-cell immunotherapy for treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer
- **HPC (hematopoietic progenitor cells), Cord Blood:** For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
- **LAVIV (Azficel-T):** Autologous fibroblasts for improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults
- **GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in bovine collagen):** For topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults
- **MACI (Autologous Cultured Chondrocytes on porcine collagen membrane):** For repair of single or multiple symptomatic, full-thickness cartilage defects of the knee with or without bone involvement in adults
- **STRATAGRAFT (Allogeneic Cultured Keratinocytes and Dermal Fibroblasts in murine collagen-dsat):** Treatment of adults with thermal burns containing intact dermal elements for which surgical intervention is clinically indicated (deep partial-thickness burns)
- **RETHYMIC allogeneic processed thymus tissue–agdc:** For immune reconstitution in pediatric patients with congenital athymia

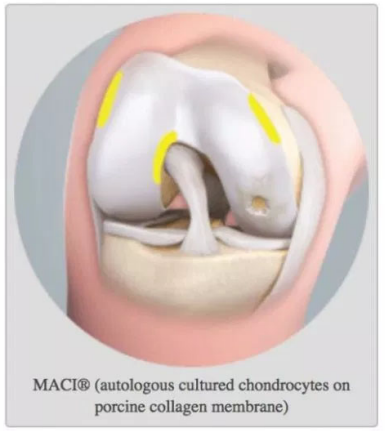
Slide by Kyung Sung, Ph.D., FDA

Cell-Device Combination Product

Tissue engineered product

- Expanded autologous chondrocytes (biologic constituent) + porcine-derived Type I/III collagen scaffold (device constituent)
- Biologics License Application (BLA) approved in December 2016
- Indicated for the repair of single or multiple symptomatic, full-thickness cartilage defects of the adult knee, with or without bone involvement.

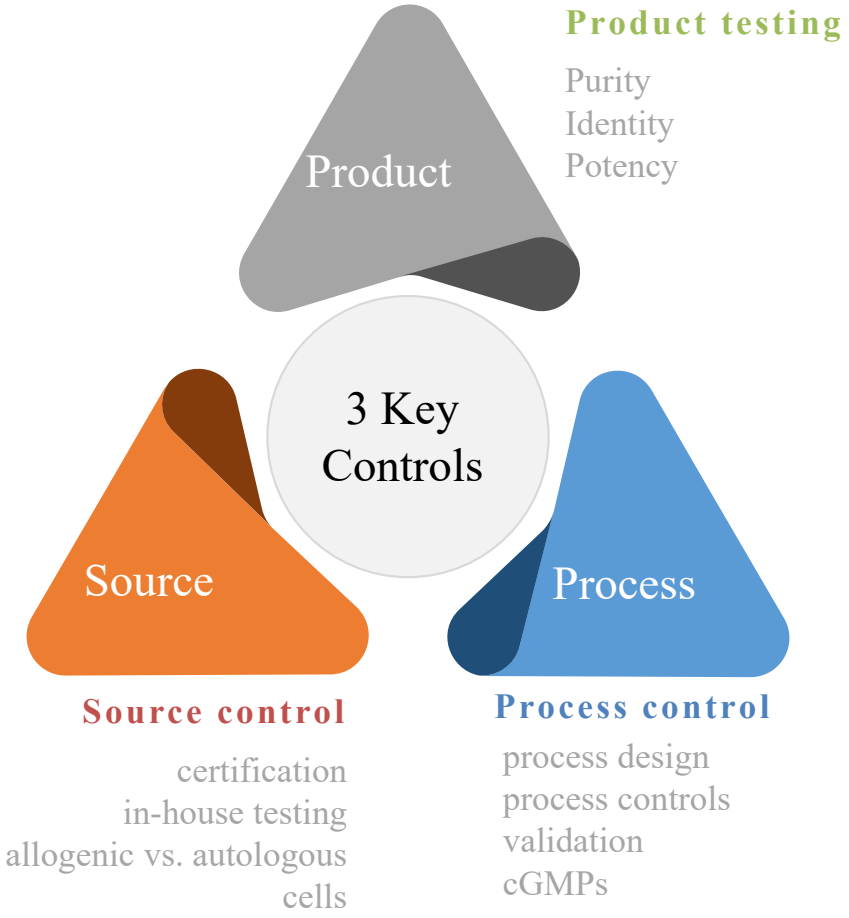

MACI® (autologous cultured chondrocytes on porcine collagen membrane)


MACI® (autologous cultured chondrocytes on porcine collagen membrane)

Slide by Kyung Sung, Ph.D., FDA

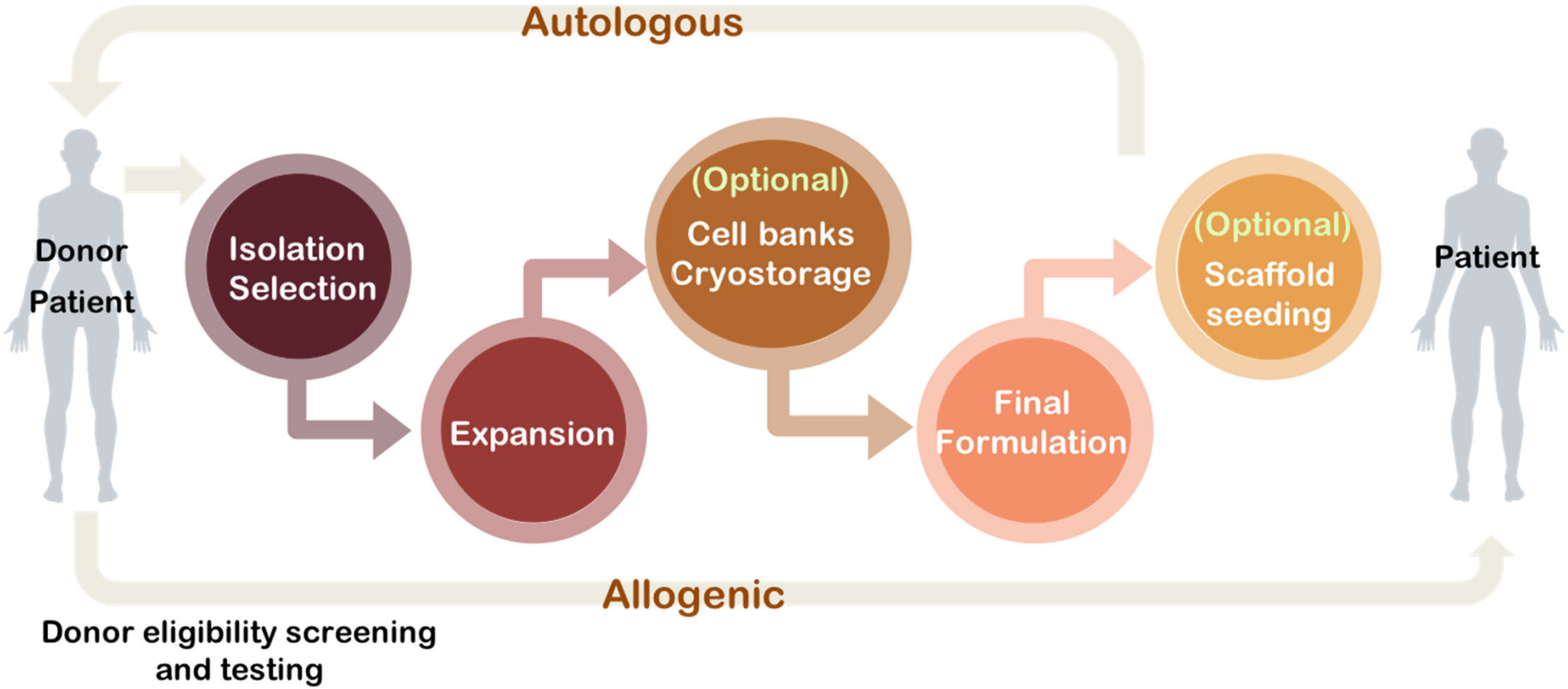
Chemistry, Manufacturing, and Controls (CMC)

- **CMC** = Product manufacturing and testing
- How do you make the product?
- What do you use to make the product?
- Product safety and quality testing
- Product stability
- Other controls – product container, labels, tracking



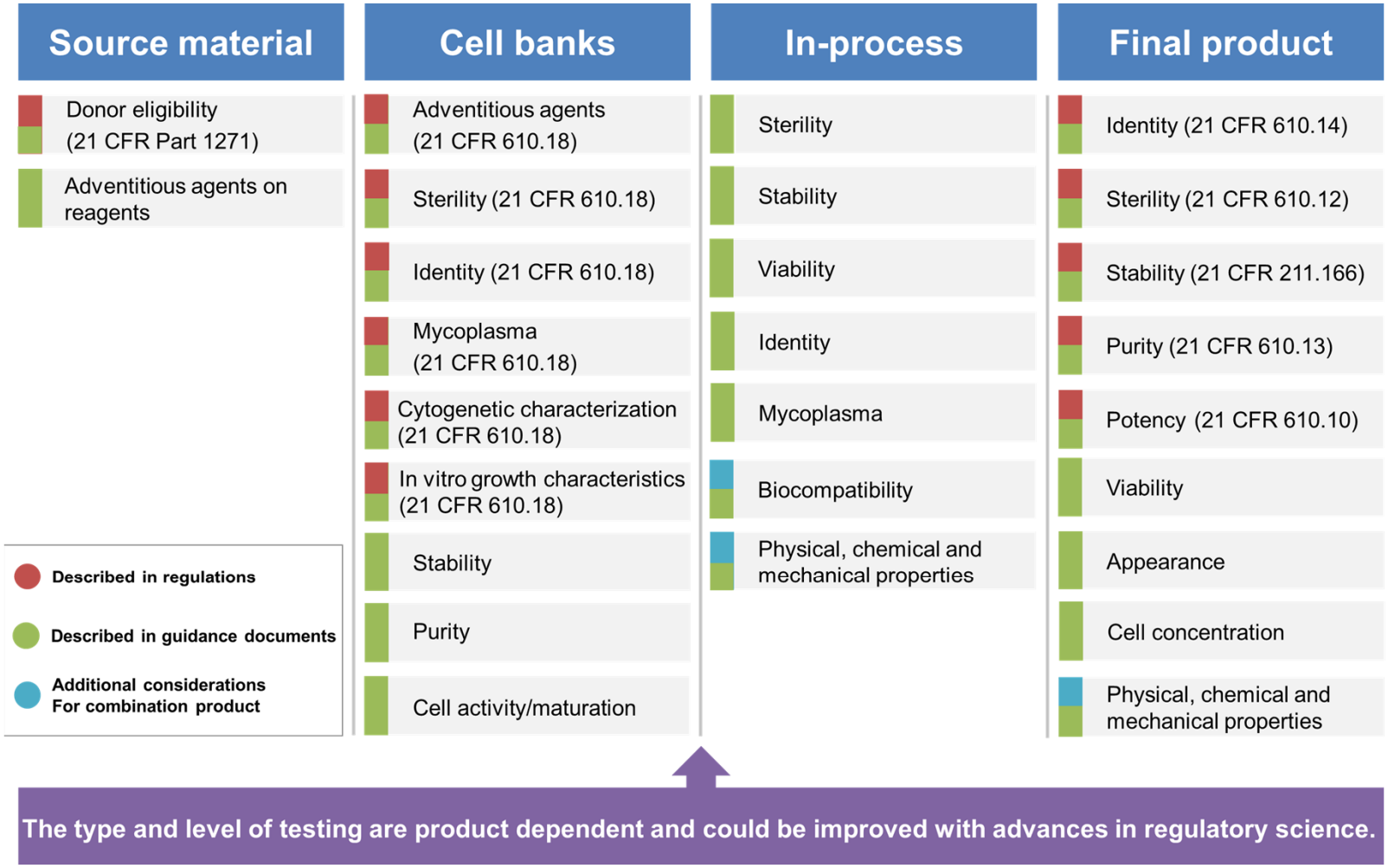
Slide by Kyung Sung, Ph.D., FDA

Cellular product manufacturing process



Slide by Kyung Sung, Ph.D., FDA

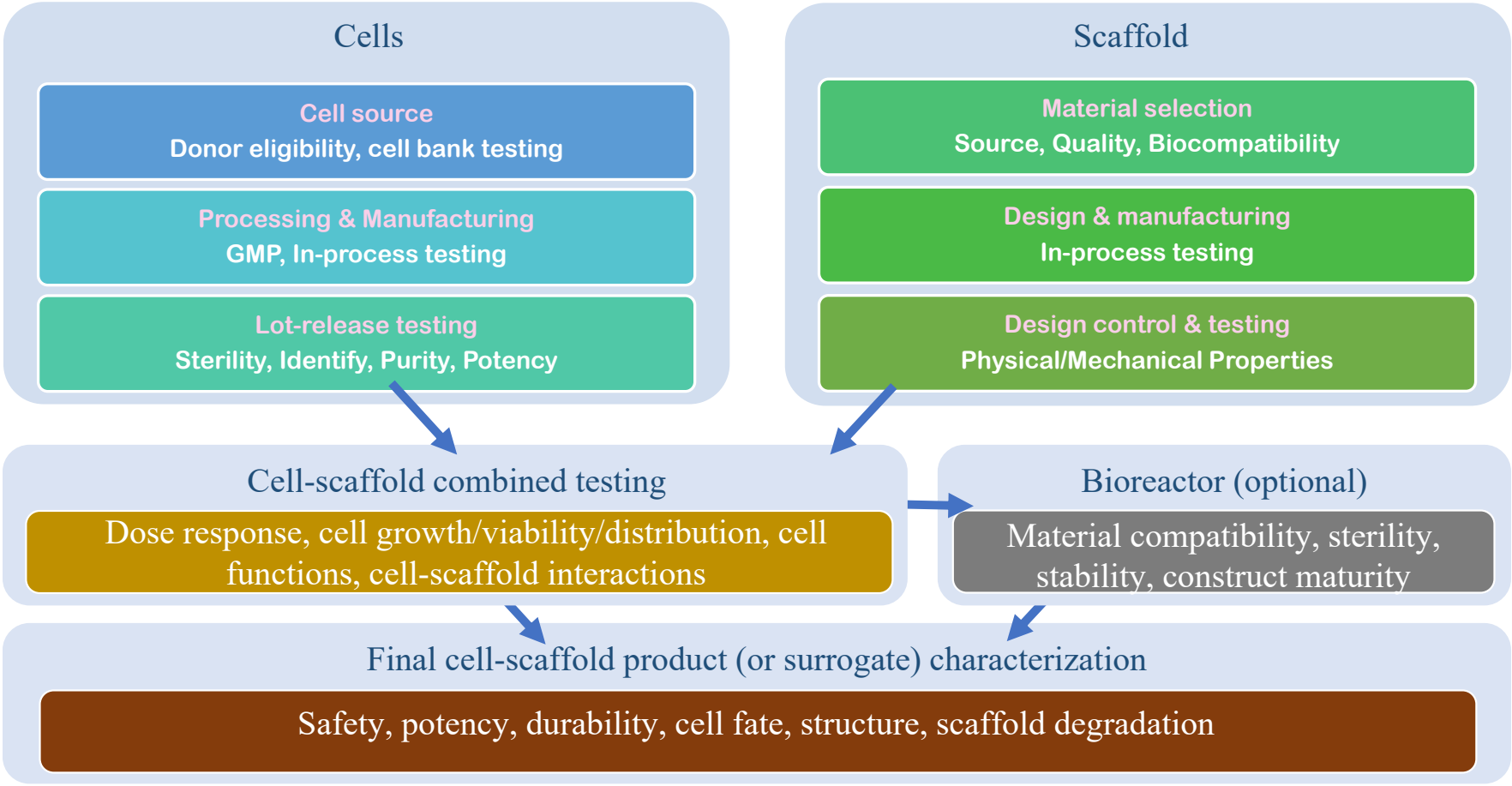
An Example of Testing for Cellular Products including Cell-Scaffold Combination Products



Lam et al., 2021, Curr Opin Biomed Eng

Slide by Kyung Sung, Ph.D., FDA

Safety and Effectiveness Consideration for a Ccell-Scaffold Product



Sung et al., 2019, Principles of Tissue Engineering

Slide by Kyung Sung, Ph.D., FDA

Cell Therapy Challenges and Regulatory Science Goals

Challenges

- Inadequate markers predictive of cell state and cell fate
- Poor understanding of how cells interact with their microenvironment
- Poor understanding regarding the impact of manufacturing parameters on product quality

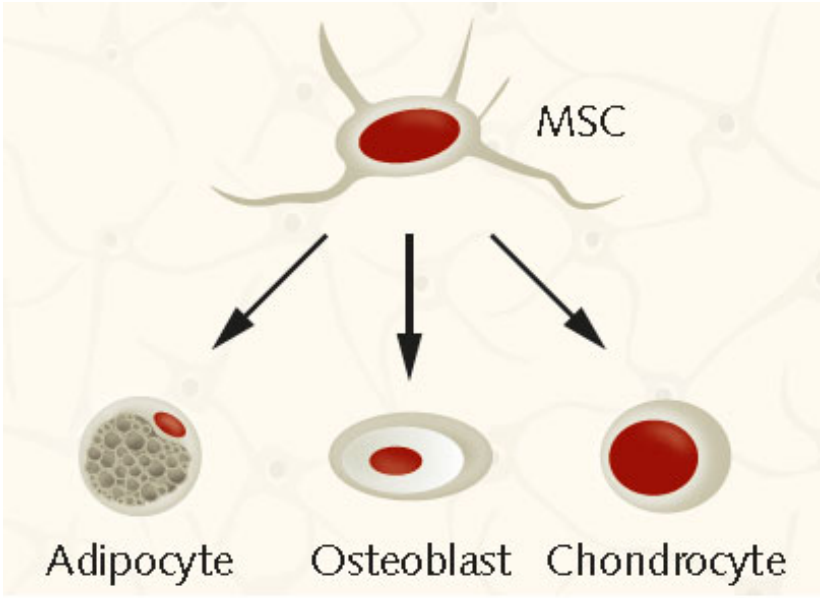
Regulatory science goals

- Develop and improve test methods for cell product characterization
- Identify product attributes that are quantifiable and predictive of safety and effectiveness
- Identify product quality attributes that are predictive of safety and effectiveness

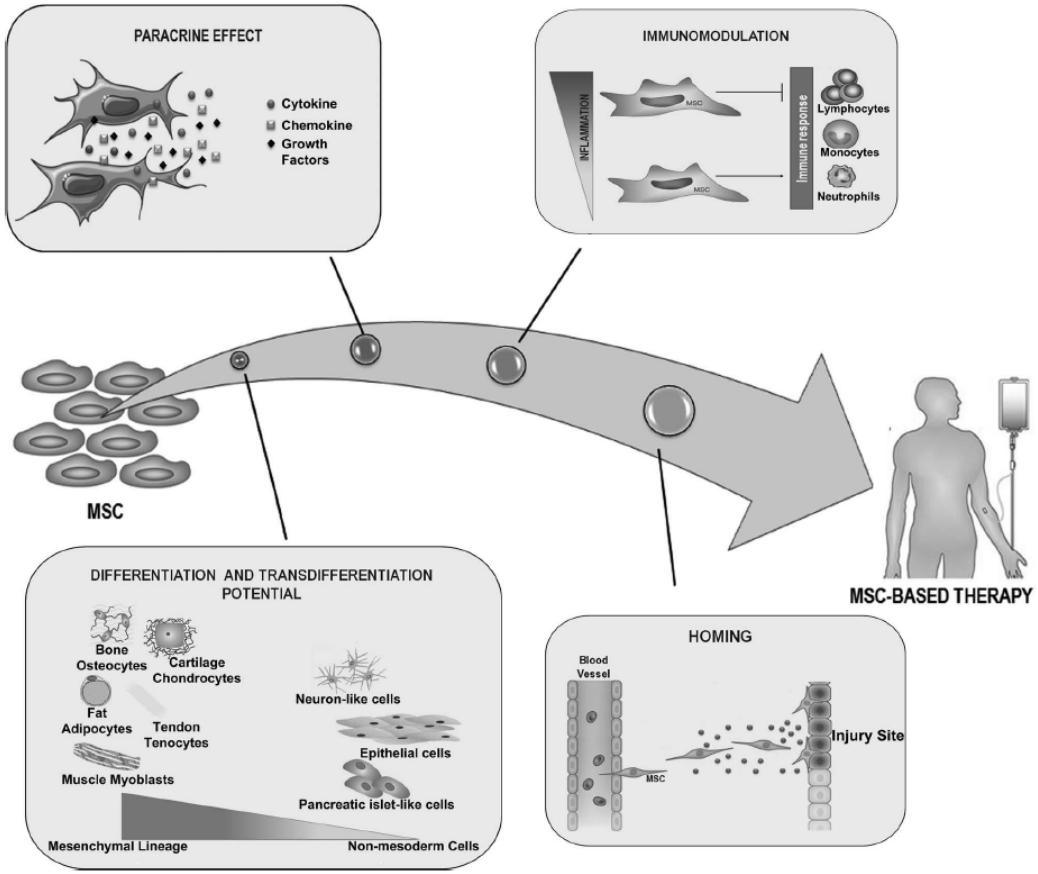
Slide by Kyung Sung, Ph.D., FDA

Mesenchymal Stromal (Stem) Cells (MSCs)

Adult, multipotent stromal cell derived from various tissue sources



Sigma-Aldrich



Adapted from Squillaro et al, *Cell Transplantation*, 2016

Slide by Kyung Sung, Ph.D., FDA

Use of MSCs in Clinical Trials

Received: 4 July 2019 | Accepted: 26 August 2019
DOI: 10.1002/sctm.19-0202

HUMAN CLINICAL ARTICLE

STEM CELLS TRANSLATIONAL MEDICINE

Trends in mesenchymal stem cell clinical trials 2004-2018: Is efficacy optimal in a narrow dose range?

Maciej Kabat[1] | Ivan Bobkov[1] | Suneel Kumar[2] | Martin Grumet[1]

ELSEVIER

Archives of Medical Research

Archives of Medical Research 52 (2021) 93–101

EPIDEMIOLOGICAL

Mesenchymal Stem Cells Current Clinical Applications: A Systematic Review

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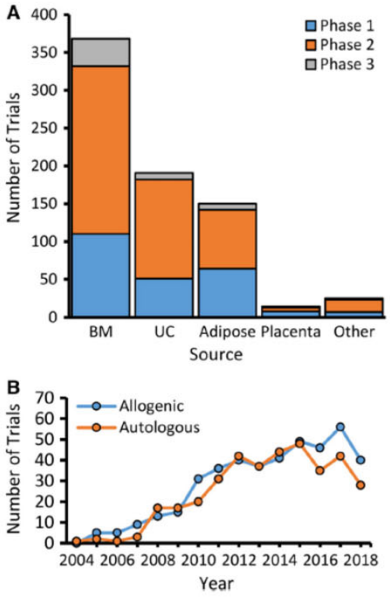


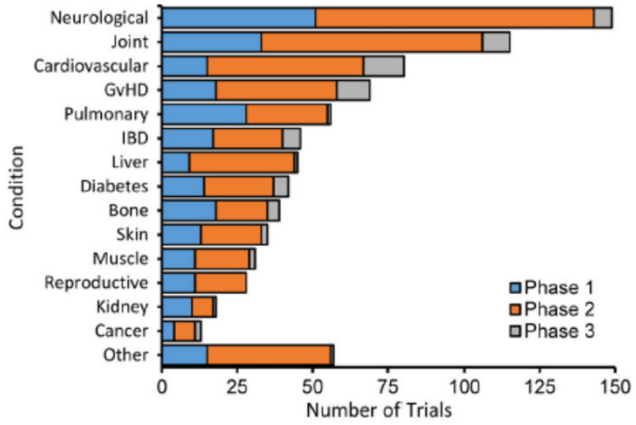
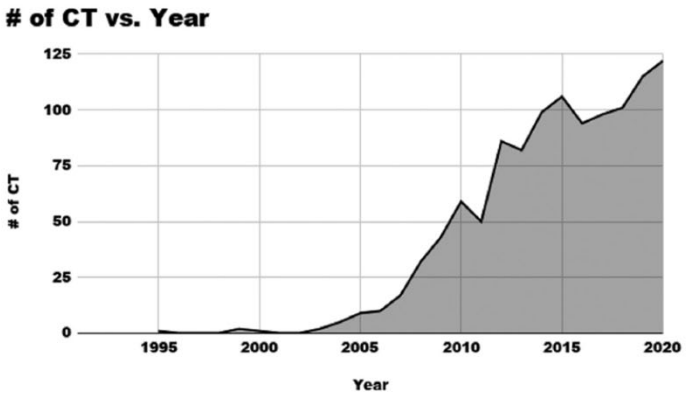


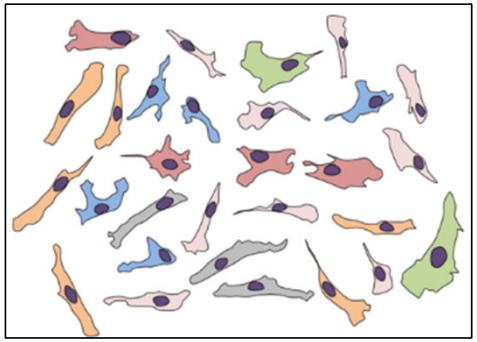
Table 2. Listed MSC Clinical Trials around the world

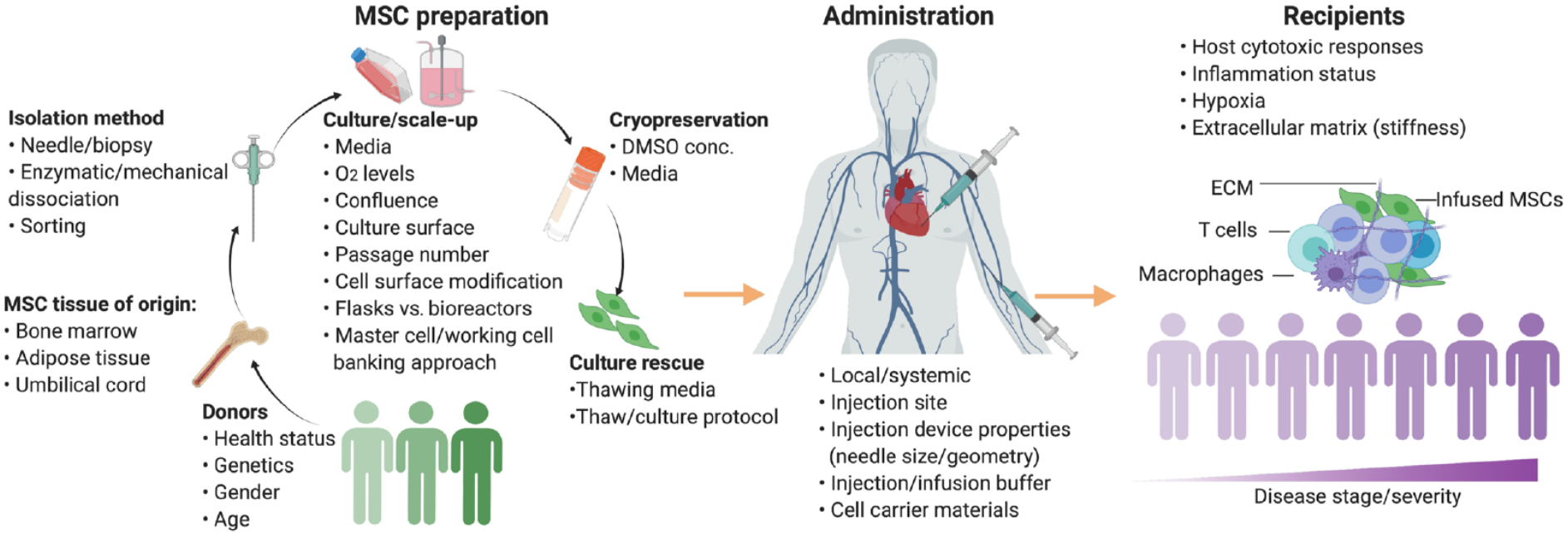
Country	Total	Percentage	Phase1	Phase2	Phase3
China	228	25.25	60	154	13
United States	186	20.60	83	88	13
Spain	69	7.64	9	59	1
South Korea	62	6.87	20	32	10
Iran	44	4.87	25	15	4
Brazil	21	2.33	6	12	3
France	20	2.21	2	16	2
Jordan	20	2.21	8	12	0
India	19	2.10	4	13	1



Slide by Kyung Sung, Ph.D., FDA

MSC Functional Heterogeneity





Levy et al, *Science Advances*, 2020

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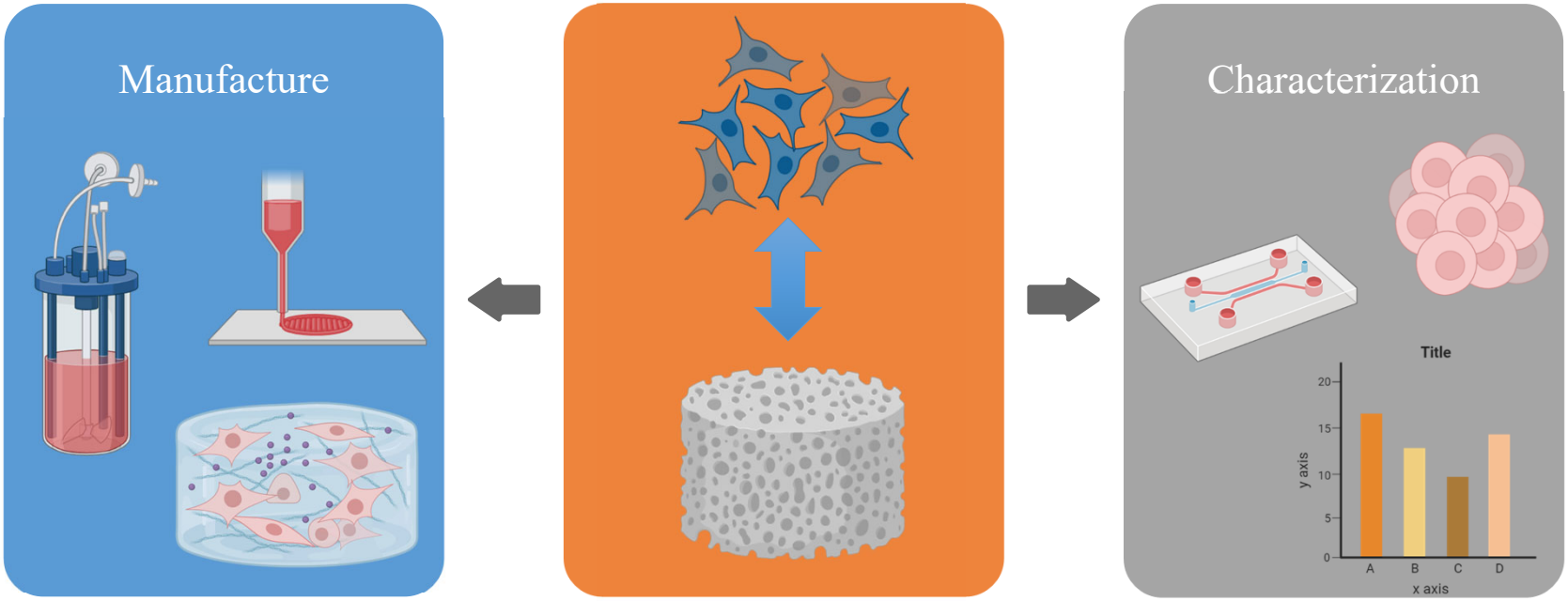
Cell-Materials Interactions

Investigating the effect of cell-materials interactions on the safety and effectiveness of cell-based products



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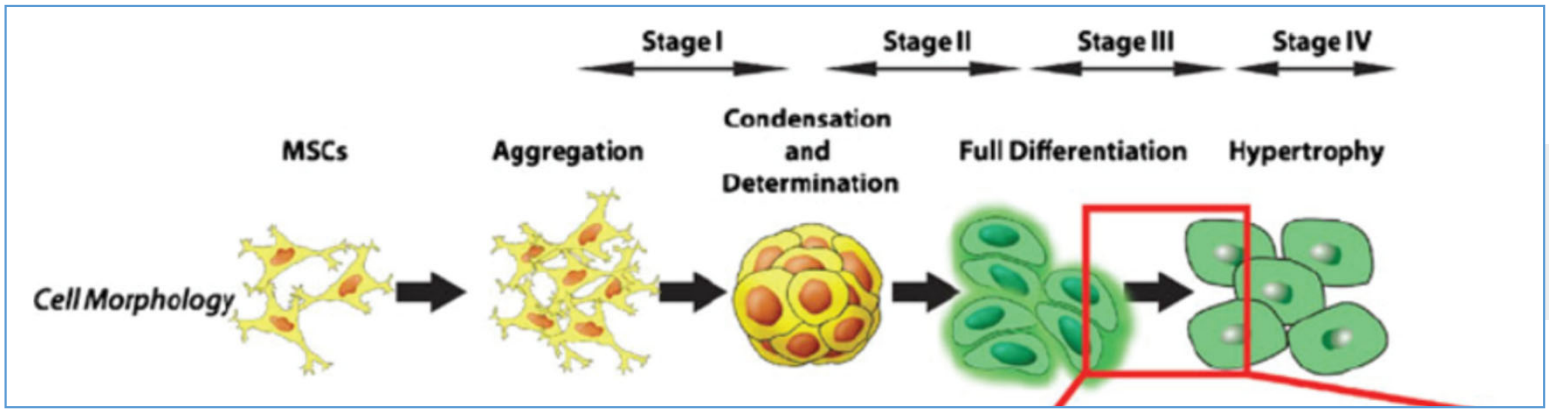
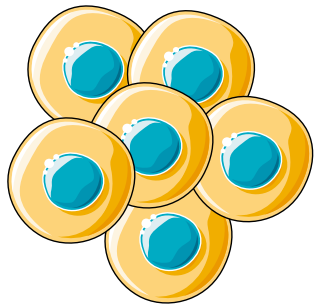
3D In Vitro System for MSC Chondrogenesis

MSCs are alternative cell source for treating cartilage defect due to their capacity for easy isolation and chondrogenic differentiation.



Gadjanski et al. Stem Cell Rev and Rep (2011)

3D aggregates



Recapitulate cell-cell contacts during condensation

Lam et al. Stem Cell Transl Med (2018)

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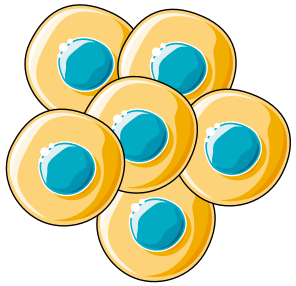
Experimental Scheme

8 MSC lines

Early passage: P2/P3

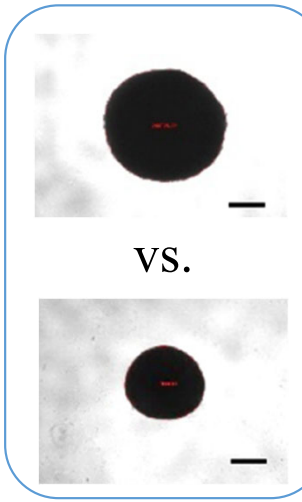
Late passage: P5

MSC aggregates



- **21 days** of culture
- 10ng/mL TGF-β3

- Image acquisition at days **1, 4, 7, 11, 14, 18, and 21**



- **Spheroid morphology quantification:** 14 shape and size features
- **Matrix deposition:** Sulfated glycosaminoglycan (GAG) normalized to the total DNA amount
- **Chondrogenic gene expression:** type IIα1 collagen, aggrecan, and type IIα1/type I collagen fold ratio
- **Histology:** GAG and collagen

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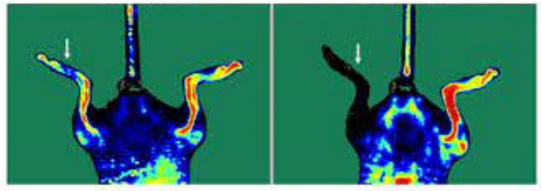
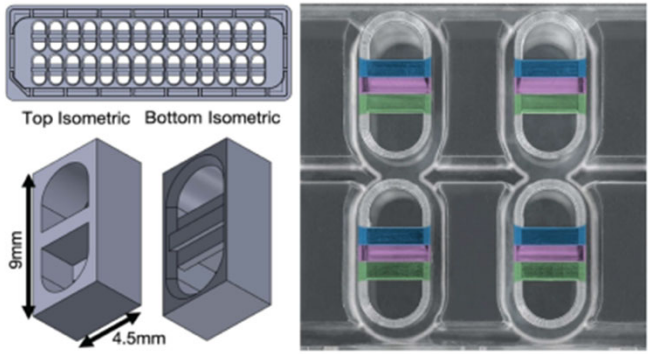
Human Blood Vessel Chips to Predict Vasculogenic Potential of MSCs

- CBER scientists use human blood vessel chips to identify multipotent stromal cells (MSCs) that are more likely to stimulate blood vessel regeneration via paracrine interactions as an alternative to the traditional murine hindlimb ischemia model.
- Scientists and physicians are very interested in using donated human MSCs to treat patients with various vascular diseases. However, the ability of MSCs to stimulate vessel regeneration varies depending on the donor and the manufacturing conditions under which the cells are prepared for therapeutic use.
- A quantitative method for predicting which MSCs will effectively stimulate vessel regeneration would improve manufacturers' ability to prepare safe and effective MSC products.

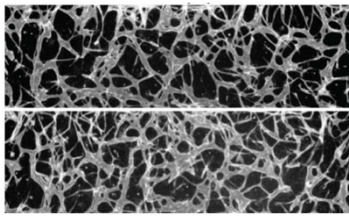
Traditional murine hindlimb ischemia model



High throughput human blood vessel chips enabling compartmentalized co-culture



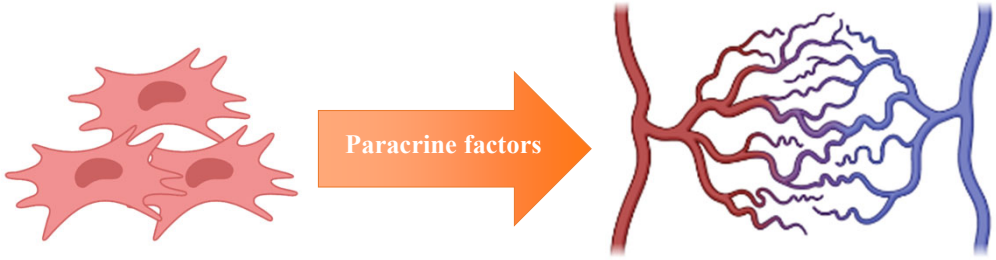
Vasculatures formed within the chips



QUREATOR
CREATE TO CURE

Slide by Kyung Sung, Ph.D., FDA

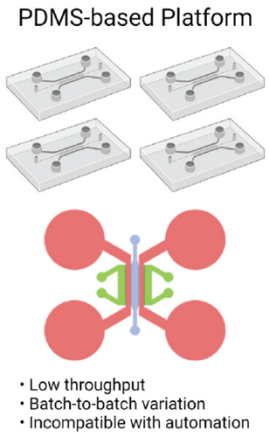
MPS for Evaluating MSC-Vasculogenic Bioactivity

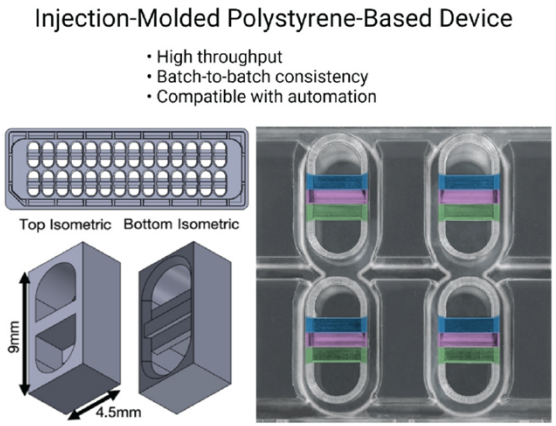


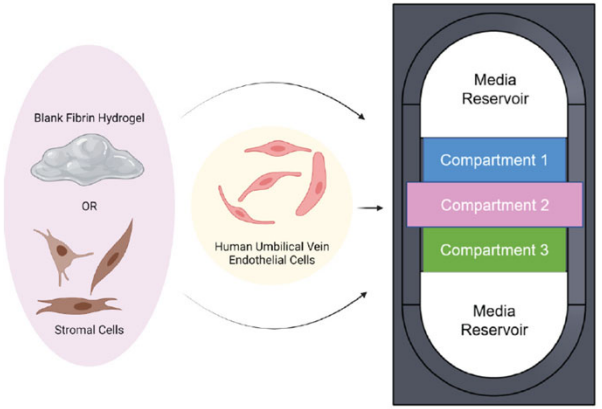
Objective: to develop and qualify a functionally relevant potency assay for measuring vasculogenic bioactivity of MSC products

Experimental Overview

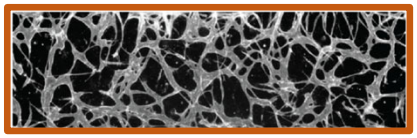
MPS Selection → MPS Platform Optimization → Assay Design → Assay Qualification







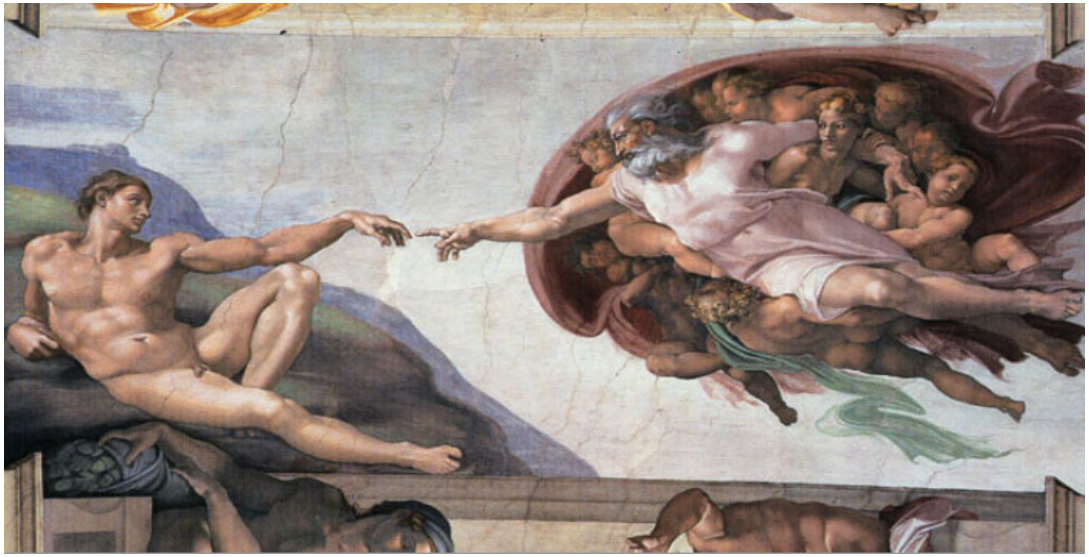
Vasculature formed within MPS



Slide by Kyung Sung, Ph.D., FDA

Cloning

Creation of Adam

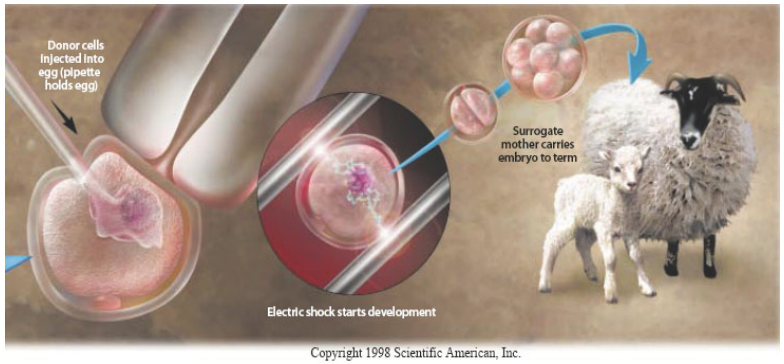


The Creation of Adam by Michelangelo Buonarroti

Creation of Life
Synthetic biology remakes organisms, but can it bring inanimate matter to life?
Biello 2010, Creation of life (David Biello, Scientific American, June 2010, p. 42)

Cloning History

- February 1997—the first cloning of a mammal, Dolly the sheep, from an adult body cell is announced.
- March 1997—President Clinton bans federal funding of human-cloning research.
- 1998–2000—Researchers clone mice, calves, goats, and pigs. A bull is "recloned" from a cloned bull.
- April 2000—Scientists find that cloning can restore body cells to a youthful state.
- October 2001—The first cloned human embryos are created at Advanced Cell Technology Lab in Worcester, MA



Cloning for Medicine by Ian Wilmut

MEGAN AND MORAG (*above*) were the first mammals cloned from cultured cells. That basic technique has allowed the creation of cloned sheep carrying human genes. Such animals produce milk that can be collected and processed (*left*) to yield therapeutic human proteins.

Sheep
Ian Wilmut and Keith Campbell

All previous cloning experiments used donor nuclei from cells in early embryos. In this experiment, the donor nuclei came from a slightly different source: cultured sheep cells, which were kept alive in the laboratory.

Wilmut and Campbell transferred the nuclei from cultured cells into enucleated sheep egg cells. The lambs born from this procedure were named Megan and Morag.

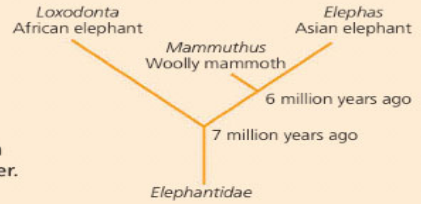
This experiment showed that cultured cells can supply donor nuclei for cloning by nuclear transfer. Because scientists had already learned how to transfer genes into cultured cells, this experiment showed that it might be possible to use such modified cells to create transgenic animals—such as cows that could make insulin for diabetics in their milk.

Jurassic Ark

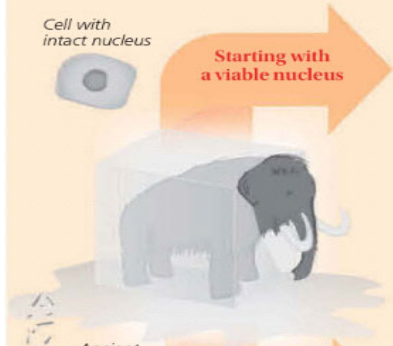
Jurassic Ark

MAKING A WOOLLY MAMMOTH

Reviving a woolly mammoth will be no easy feat, but below are two plausible methods. The first requires remarkable luck to find a mammoth cell with a viable nucleus. The second requires painstaking efforts — which are underway — to modify the Asian elephant's genome to resemble the mammoth's. Eventually, scientists might use mammoth genome sequences to synthesize chromosomes, which could be placed in a nucleus that then is placed in a cell and grown into an embryo, which could be implanted in a surrogate mother.



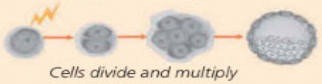
The Asian elephant is the woolly mammoth's closest living relative.



Cloning With Somatic Cell Nuclear Transfer

1 Extract egg from Asian elephant donor, then swap in nucleus from mammoth cell.

2 Electrically shock the egg to stimulate cell division, creating an embryo.

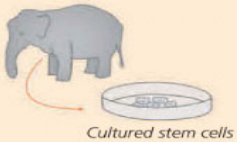


3 Implant embryo into the uterus of a surrogate mother elephant.

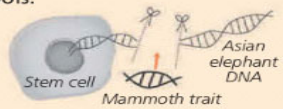
4 After gestation, Asian elephant mother gives birth to a cloned baby mammoth.

Building a Hybrid With Genome Engineering

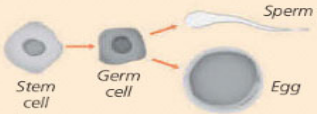
1 Cultivate Asian elephant stem cells.



2 Modify their genomes to substitute mammoth genes that confer cold resistance using genome-engineering tools.



3 Convert modified stem cells into germ cells, which form modified elephant sperm and egg.



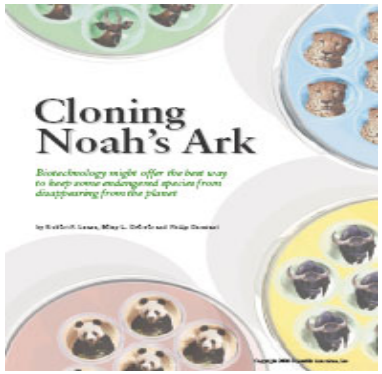
4 Fertilize the modified elephant egg with modified elephant sperm, creating embryo of mammoth-elephant hybrid.

5 Implant hybrid embryo into the uterus of surrogate mother elephant; mother gives birth to a mammoth-elephant hybrid.

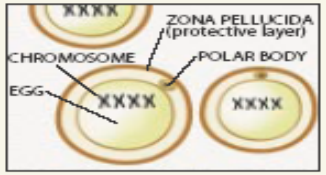
6 See if the baby has mammoth cold-resistance traits. Make additional changes, if needed, creating a herd of mammoth-elephant hybrids that can flourish on the tundra.

The elephant genome is modified with mammoth genes for cold resistance, such as long hair, extra subcutaneous fat and an altered blood protein.

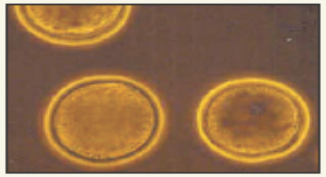
Cloning

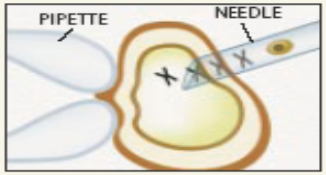


THE NUCLEAR TRANSFER (CLONING) PROCESS

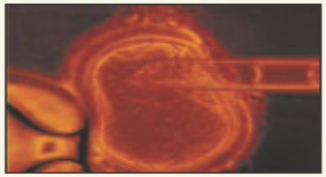


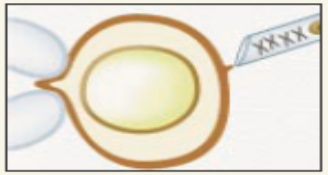
Recipient eggs are coaxed to mature in a culture dish. Each has a remnant egg cell called the polar body.



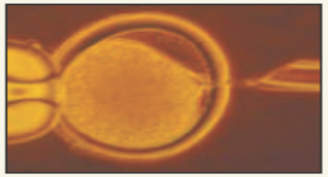


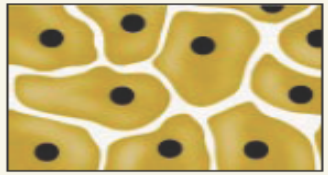
The polar bodies and chromosomes of each egg are drawn into a needle. A pipette holds the egg still.



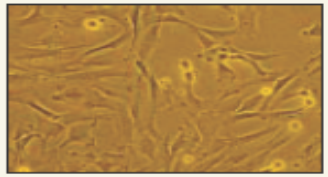


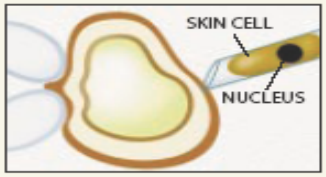
Once the chromosomes and polar body are removed, all that remains inside the zona pellucida is cytoplasm.



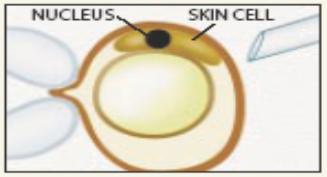


Skin cells called fibroblasts are isolated from the animal to be cloned and grown in culture dishes.

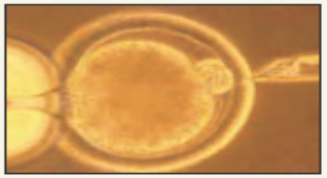




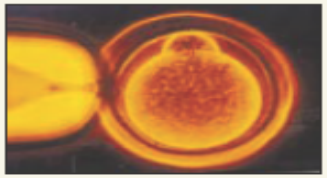
An entire skin cell is taken up into the needle, which is again punched through the zona pellucida.

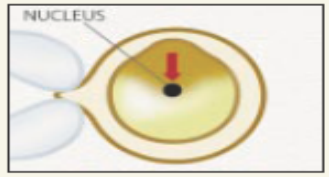


The skin cell is injected underneath the zona pellucida, where it remains separate from the egg cytoplasm.

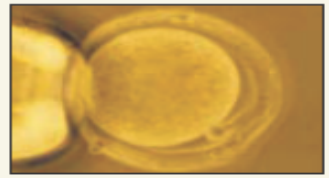


Each injected egg is exposed to an electric shock that fuses the skin cell with the egg cytoplasm.



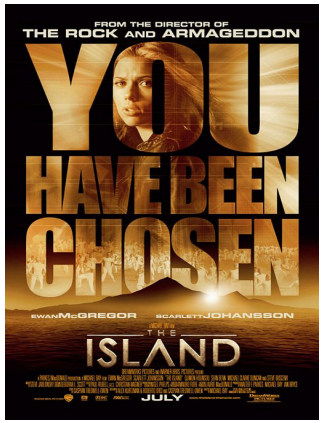


The skin cell's nucleus, with its genes, enters the egg cytoplasm. Within a few hours, the fused cell begins to divide.



Make Your Own Bank of Organs through Cloning

If you clone yourself, you will have another you. When you need a fresh organ, you can have it from your clone. This is the main story of the movie, The Island.



There is a fiction book about cloning a human. Klone in the book title is the name of the clone of the main character of the book. The cloned you may do the job for you when you are so busy. Your productivity will increase and you can be at different places at the same time, because your clone is you. But the truth is that your clone is not you. Your clone is another human being.