

# **Polymers in Tissue Engineering**

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# Organ Donation

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

# Organ Transplant



## Understanding Donation

### Quick Stats & Facts

- 16,900** - 2024 was the 14th consecutive record-breaking year for deceased donations, there were more than 16,900 deceased donors in 2024.
- 8 minutes** - Another person is added to the national transplant waiting list every 8 minutes.
- 100,000 people** - More than 100,000 people are waiting for lifesaving organ transplants.
- 2.5 million** - More than 2.5 million tissue transplants heal lives each year.
- 2 minutes** - It takes less than two minutes to register your decision to be a donor online.
- 1 out of 3** - 1 out of 3 deceased donors is over the age of 50.
- 1 DONOR** - All major religions support donation as a final act of compassion and generosity. More than 45,000 organs from deceased donors were transplanted in 2024.
- 25 million** - More than 170 million people are registered as organ and tissue donors.

Register your decision today at [RegisterMe.org](https://www.registerme.org)

<https://ctmirror.org/2025/08/25/saving-more-lives-through-improving-organ-donation/>

### The U.S. Organ Transplant System

1,000 people waiting for an organ transplant

**100,000+** waiting for an organ transplant

**6,000+** die annually

**One** organization has run the U.S. transplant system since 1984

Modernization Initiative announced! **MARCH 2023**

Source: Health Resources and Services Administration, U.S. Department of Health & Human Services

<https://ldi.upenn.edu/our-work/research-updates/ending-unoss-monopoly-over-the-u-s-organ-transplant-system/>

**1 DONOR** = **8 life-saving organs** tissues & corneas that can improve **75 LIVES**

### EVERY DONATION COUNTS

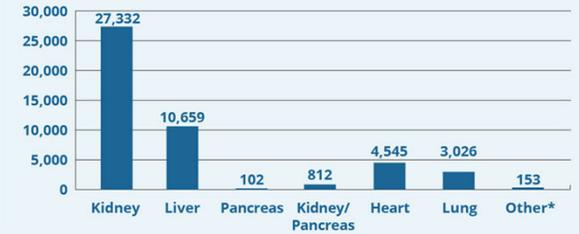
- Lungs** - 3-year survival rate of **68%**
- Liver** - **70%** last 5 years or more
- Pancreas** - improve lives for an average of **10+ YEARS**
- Tissues** - **1 IN 20** Americans will benefit from tissue transplants
- Corneas** - can help improve **20** sight for **YEARS**
- Heart** - 5-year survival rate of **70% OR MORE**
- Kidneys** - improve lives for an average **12-15 YEARS**
- Intestines** - **NEARLY 3000** improved in the U.S. **LIVES** to date

REGISTER TO MAKE A DIFFERENCE TODAY [RegisterMe.org](https://www.registerme.org)

<https://www.baptisthealth.com/blog/family-health/why-you-should-be-an-organ-donor>

## Transplants Performed by Organ

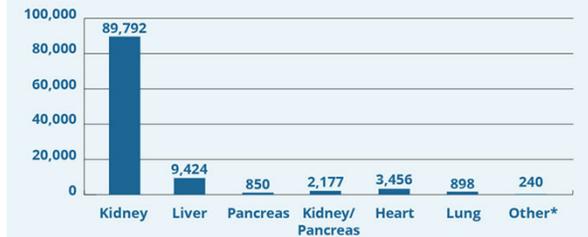
In 2023



\*Other includes allograft transplants like face, hands, and abdominal wall. Based on OPTN data as of September 15, 2024. Data subject to change based on future data submission or correction. Totals may be less than the sums due to patients included in multiple categories.

## Patients on the Waiting List by Organ

As of September 2024



\*Other includes allograft transplants like face, hands, and abdominal wall. Based on OPTN data as of September 15, 2024. Data subject to change based on future data submission or correction. Totals may be less than the sums due to patients included in multiple categories.

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

### 2024 More transplants than ever before

- 3.3%** increase over 2023
- Exceeded **48,000** lifesaving transplants total\*
- 23.3%** increase since 2020\*

Thanks to generous organ donors!

\*Based on OPTN data as of Jan. 10, 2025. Data subject to change based on future data submission or correction.

OPTN Organ Procurement & Transplantation Network

<https://www.hrsa.gov/optn/news-events/news/organ-transplants-exceeded-48000-2024-33-percent-increase-transplants-performed-2023>

# The Benefits of Organ Donation

## How Organ & Tissue Donation Changes Lives

### How is tissue used?

#### Ribs and costal cartilage

Facial reconstruction (jaw, nose, ears) often related to trauma

#### Other bone

Creates pins and screws to promote healing and eliminate the need to remove hardware; also used for spinal fusion spacers, joint replacement and bone regeneration

#### Long bones

Limb salvage in cases of bone cancer (to avoid amputation) and repair of traumatic injuries

#### Tendons

Arthroscopic tendon and ligament repairs, especially in sports injuries

#### Eyes/corneas

Restoration of sight

#### Heart valves

Repairs congenital and acquired heart valve defects

#### Skin

For burn victims; breast reconstruction after mastectomy; abdominal wall repair after hernia surgery; bladder and uterine suspension surgery

#### Veins

For bypass surgery and kidney dialysis shunts

#### Nerves

Can protect a recipient's damaged nerve during healing, repair severed nerves and gap (connect) injured nerves

#### Benefits of tissue transplants (grafts)

- Pliability and flexibility of grafts
- Faster healing times
- Cardiovascular tissue doesn't require anticoagulation therapy and is resistant to infection



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

## Every Donation Counts

One donor can give 8 life-saving organs as well as tissues and corneas that can heal 75 lives with the potential of 125 patients.

### ORGANS

#### Heart



#### Liver



#### Intestines



#### Lungs



#### Pancreas



#### Kidneys



### TISSUES

#### Eyes/corneas

Restoration of sight

#### Heart valves

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

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# **Organs from Animals**

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# 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.).

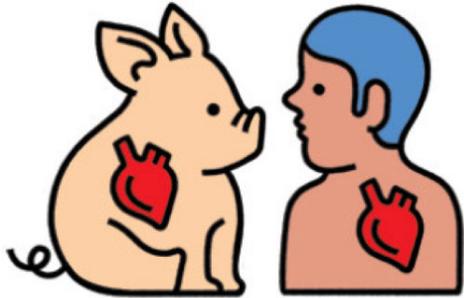
### Xenograft

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

## 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>



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



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

## 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 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.

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=NTY3NzEwMjlyMQS2&spJobID=2231333715&spReportId=MjIzMTMzMzcxNQS2](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=NTY3NzEwMjlyMQS2&spJobID=2231333715&spReportId=MjIzMTMzMzcxNQS2)

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.

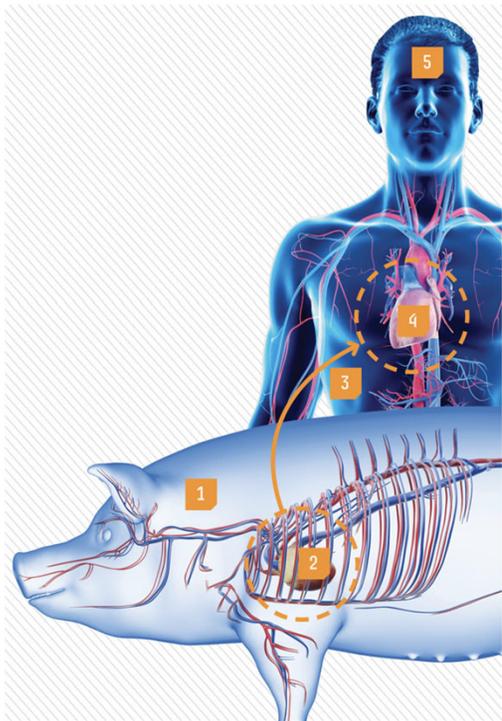
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# From Porcine Pump to Future Breakthroughs

## PORCINE PUMP

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



### 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 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.

## 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.

### 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 (ALS). The results of a preliminary study showed that Relyvrio may slow the rate of decline for ALS sufferers.

### 5 BATTLING CANCER

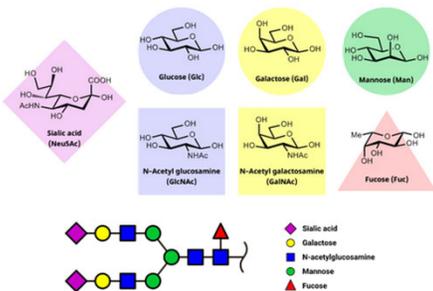
A 13-year-old girl who relapsed with leukaemia became the first patient to receive a genetically engineered immune cells treatment, which sent her into remission.



Issue 174

# From Porcine Pump to Future Breakthroughs

Recent human decedent model studies and compassionate xenograft use have explored the promise of **porcine organs for human transplantation**. To proceed to human studies, a clinically ready porcine donor must be engineered and its xenograft successfully tested in nonhuman primates. Here we describe the design, creation, and long-term life-supporting function of kidney grafts from **a genetically engineered porcine donor** transplanted into a cynomolgus monkey model. The porcine donor was engineered to carry 69 genomic edits, eliminating glycan antigens, overexpressing human transgenes, and inactivating porcine endogenous retroviruses. In vitro functional analyses showed that the edited kidney endothelial cells modulated inflammation to an extent that was indistinguishable from that of human endothelial cells, suggesting that **these edited cells acquired a high level of human immune compatibility**. When transplanted into cynomolgus monkeys, the kidneys with **three glycan antigen knockouts** alone experienced poor graft survival, whereas those with glycan antigen knockouts and human transgene expression demonstrated significantly longer survival time, suggesting **the benefit of human transgene expression in vivo**. These results show that preclinical studies of renal xenotransplantation could be successfully conducted in nonhuman primates and bring us closer to clinical trials of genetically engineered porcine renal grafts.



Glycan structures are conventionally represented using a schematic system in which each type of monosaccharide is represented with a different color

[https://www.glytech-inc.com/hello\\_glycan/what-are-glycans/](https://www.glytech-inc.com/hello_glycan/what-are-glycans/)

Anand 2023, Design and testing of a humanized porcine donor for xenoplantation

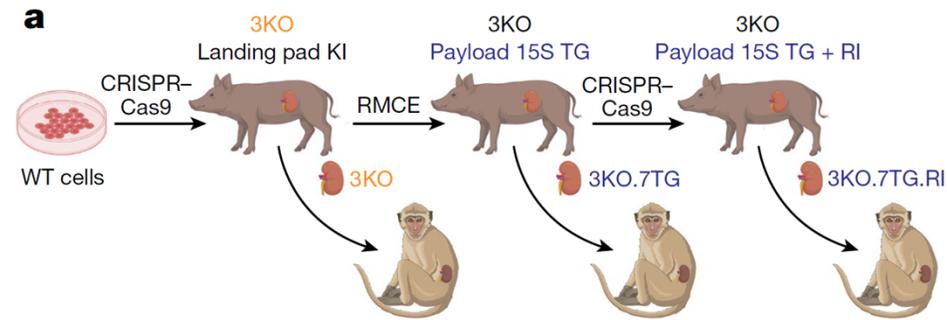
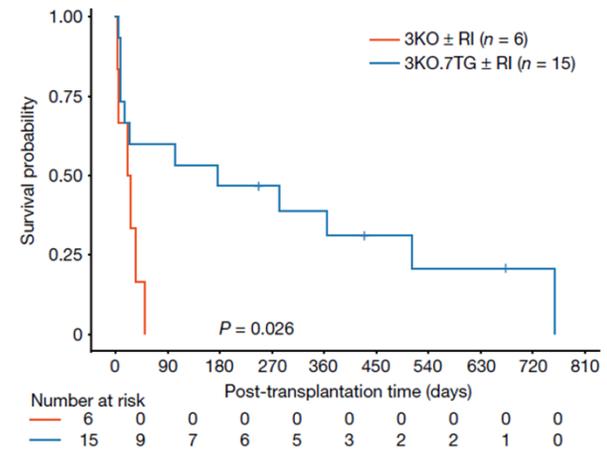


Fig. 2 | Yucatan porcine donor is engineered to carry 69 genomic edits. a, The porcine donor kidney, **3KO.7TG.RI**, was engineered to eliminate three glycan antigens (3KO), overexpress seven human transgenes (PL15S) and inactivate PERV elements (RI) through three rounds of editing and cloning. The donor kidney, 3KO.7TG, carries 3KO and PL15S, without RI. KI, knock in; RMCE, recombinase-mediated cassette exchange.



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# Tissue Engineering

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Since there are not enough donated organs to meet demand, human organs must be obtained through 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 have led to a new research field known as tissue engineering. The tissue engineering approach is still far from perfect and practical. Still, it provides a tool for developing artificial organs in the future through a series of innovations by young scientists.

# 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 because they are prone to **mechanical failure over the long term.** 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 involves moving organs or tissues from their original location to replace damaged tissue, e.g., the saphenous vein as a bypass graft or the patellar tendon for anterior cruciate ligament (ACL) repair. However, often this strategy fails to replace all the functions of the original tissue. Additionally, the development of malignant tumors, surgical complications, and morbidity at the donor sites are major problems in the 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 normal tissue development, tissue engineering is a strategy to restore, maintain, and improve tissue function, ultimately aiming toward complete organ replacement.

# 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

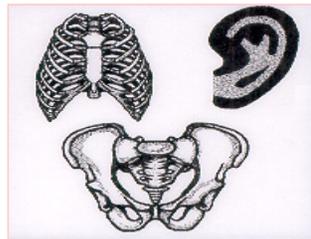
Solution casting  
Spray casting  
Melt molding: compressing molding  
Electrospinning

### Porous structure

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



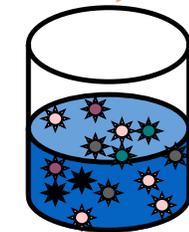
## Natural Organs



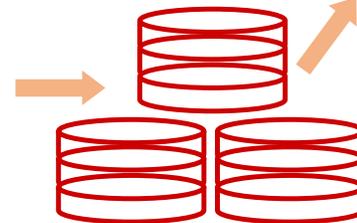
## Scaffolds in Organ Shape and Size



## Cell Seeding



Isolation of Cell *in vitro*



Large Scale Cell Culture *in vitro*

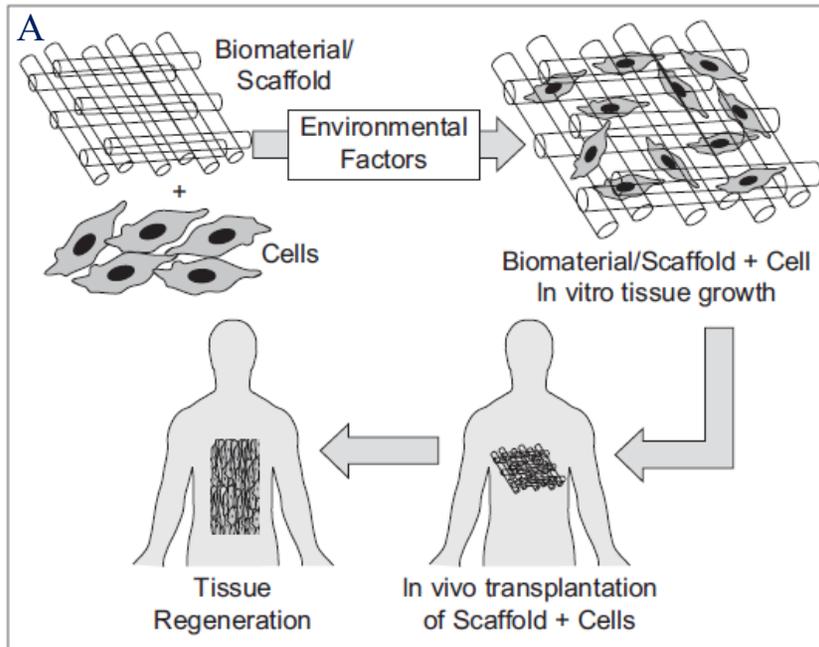
## New Tissue Engineered Organs

## Preparation of Artificial Organs

# Traditional Tissue Engineering Approaches: Two Main Strategies

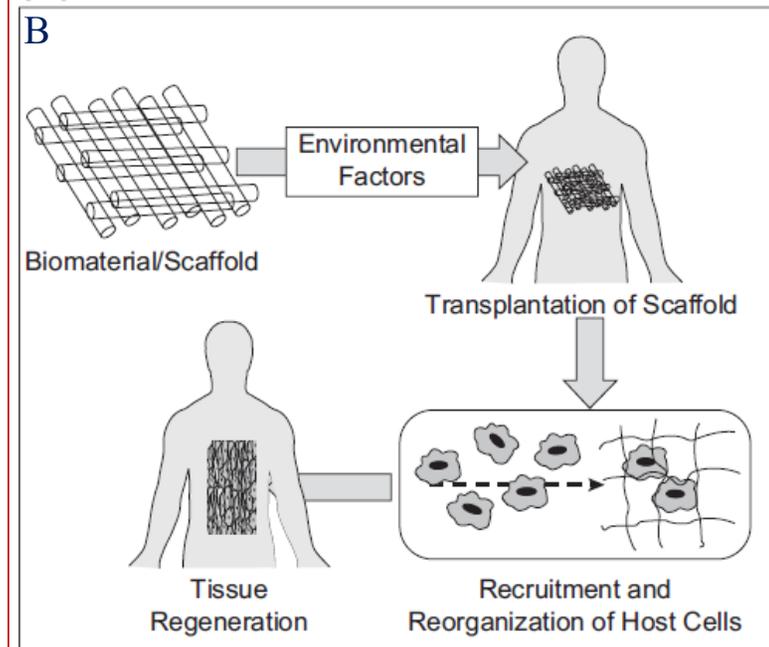
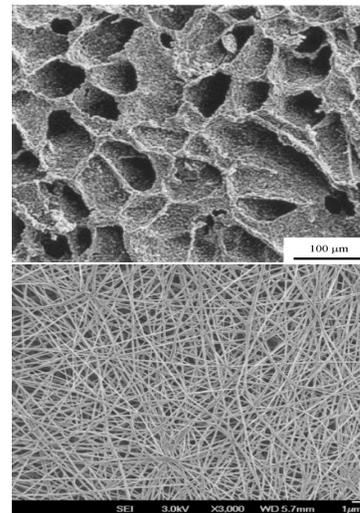
(1) Transplantation of a tissue grown *in vitro* consisting of an artificial matrix with cells and growth factors

(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** must be porous for cells to grow and form their own extracellular matrix. Example structures are shown below.



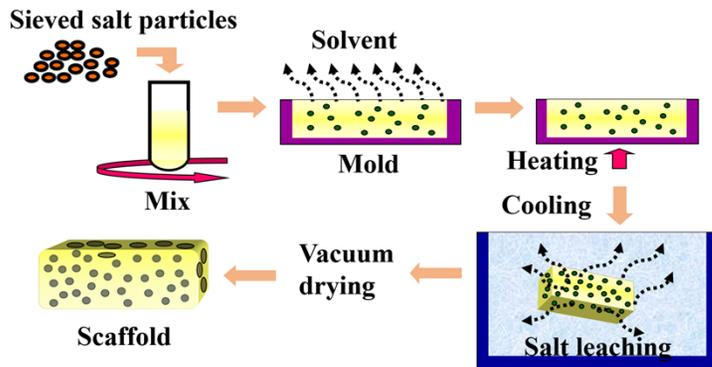
In vivo tissue engineering by transplantation of scaffold and recruitment and reorganization of host cells

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 (PLGAs)**, are integrated with microenvironmental factors (such as cytokines, growth factors, mechanical forces, physico-chemical factors, spatial and temporal signals, and extracellular matrix molecules).

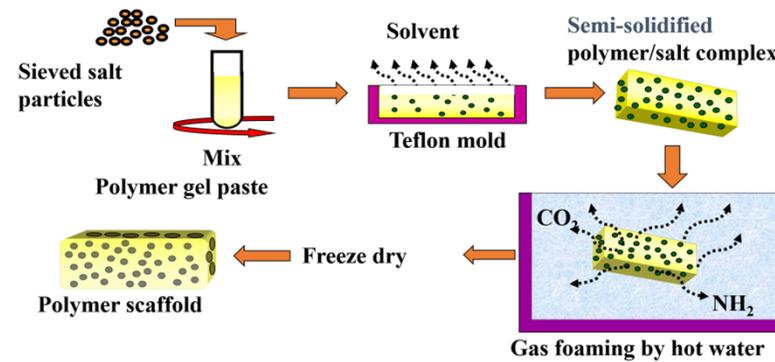
# Porous Scaffold Preparation

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

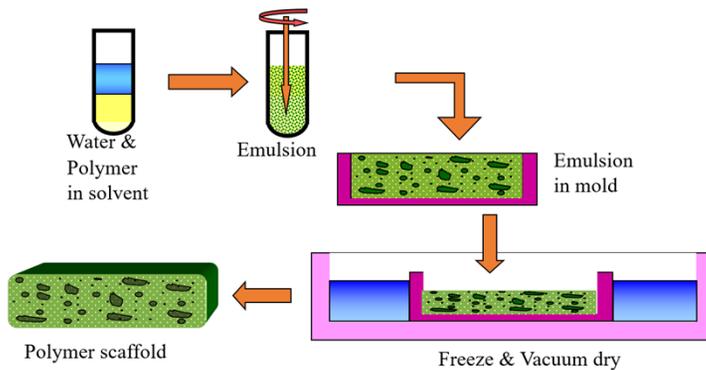
## Particulate Leaching



## Gas Generation

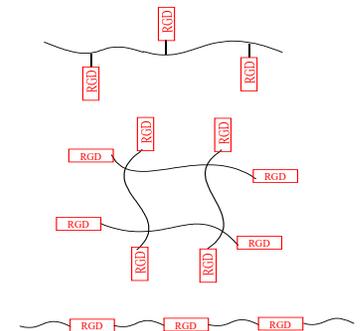


## Emulsion Freeze-drying



## Signaling Factors

<b>RGD</b>	Fibronectin, Collagen, Fibrinogen, Laminin, Vitronectin
<b>YIGSR</b>	Laminin
<b>IKVAG</b>	Laminin
<b>LRE</b>	Laminin
<b>REDV</b>	Fibronectin
<b>DGEA</b>	Collagen
<b>VTXG</b>	Thrombospondin
<b>VGAPG</b>	Elastin



# Tissue Engineering Materials

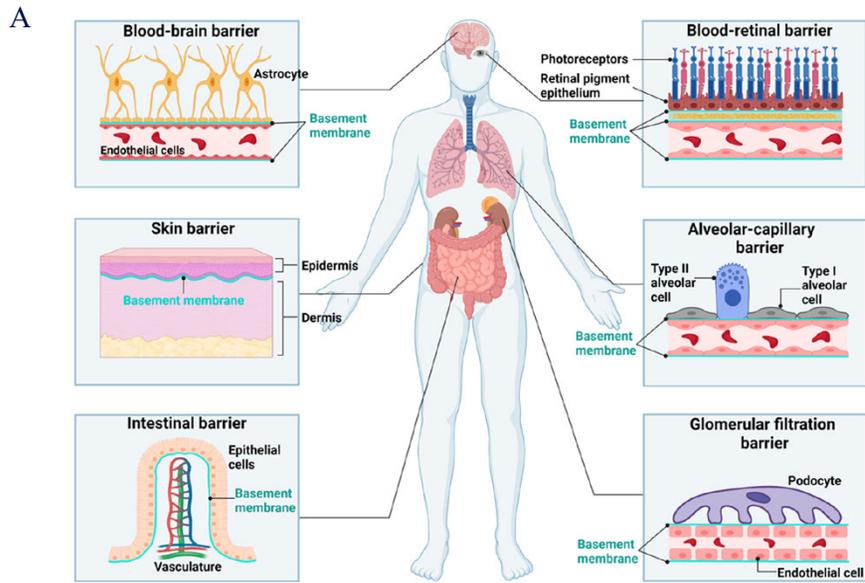


Figure 1. **Basement membrane** location: Basement membrane (BM) is ubiquitous in the human body and is located adjacent to the epithelium, endothelium, and parenchymal cells including muscle, adipose as well as nerve cells. It is involved in many vital physiological processes and is found in many organ 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.

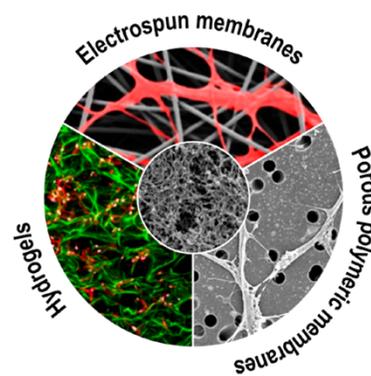


Figure 3. Structural resemblance of native basement membrane with synthetic mimics: Schematic represents the structural resemblances

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

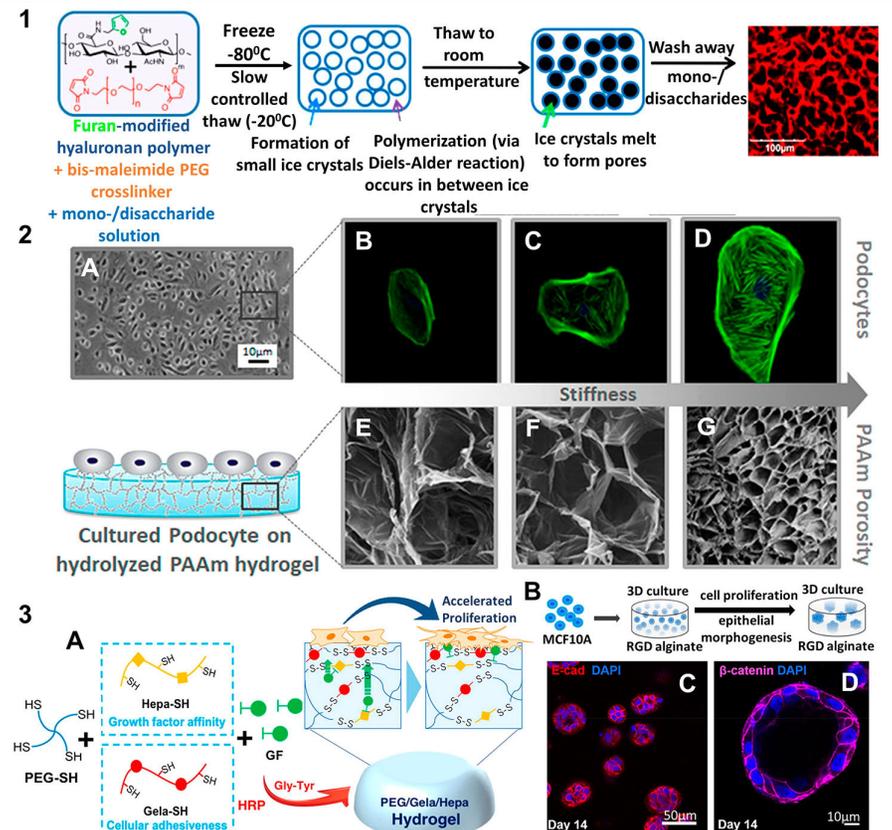


Figure 5. **Optimization of hydrogel properties to mimic basement membrane structure and function:** Modification of physical and chemical properties of hydrogels have been addressed in varying methods that allow their use as scaffolds to mimic BM: (1) Limitations of pore formation have been overcome 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.

# Tissue Engineered Organs

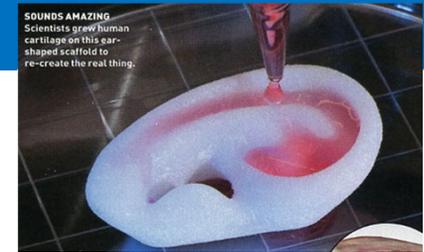
When tissue engineering first began in the mid-1980s, scientists created many tissue-like objects. An ear or a nose grown in mice attracted a lot of attention. Since then, numerous tissue-looking objects have been made.

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 in tissue engineering, the progress in developing artificial organs has been painfully slow. So, remember that **a good idea is just an idea, unless it is translated into a practical solution.** There are many other promising ideas, such as gene therapy and nanomedicine, but they 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.



## Children receive new ears grown from their own cells in world first

"They've shown that it is possible to get close to restoring the ear structure"

Harry Cockburn

Wednesday 31 January 2018 18:55 GMT

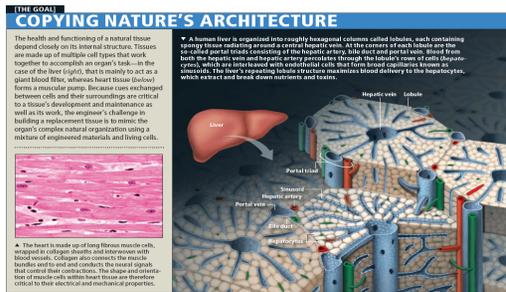


Cartilage cells taken from the recipient's unaffected ear are grown on an artificial mould before it is grafted into place (Cao et al/EBioMedicine)

# The Extracellular Matrix (ECM)

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

## Natural Tissues



Professor Luis Solorio

The TMET Lab engineers physiologically-relevant in vitro platforms, scaffolding, and other devices to investigate key cellular events, improve regeneration, or perform precision medicine tests clinically.

Some of our models include:

- A micro-actuating lung model
- A pre-metastatic niche and wound healing mimetic
- Mandible scaffolding for bone regeneration
- Tissue property and physiology characterization
- A tumbling robot for precise movement of payload in patients

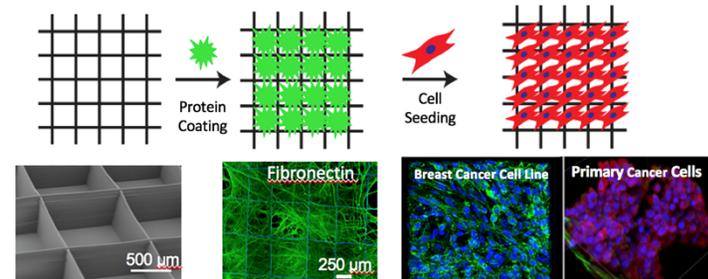
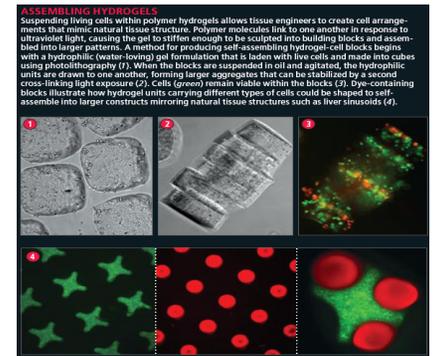
These platforms use a variety of matrices, cell types, and tissues to study or improve patient outcomes related to regenerative medicine, drug delivery and cancer progression.

*Tissue  
MicroEnvironment  
& Therapeutics  
Lab (TMET)*

## Synthetic Materials

## Advanced Building Materials

Engineers want to reproduce the internal structure of a natural tissue as closely as possible because cells depend on environmental cues to maintain themselves and to do their jobs. New techniques and materials are giving tissue engineers finer control and faster methods of creating cell constructs designed to grow into a functioning implant.



# Next Generation Tissue Engineers

The field of tissue engineering has evolved from its early focus on engineering tissue substitutes to current efforts to build 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.

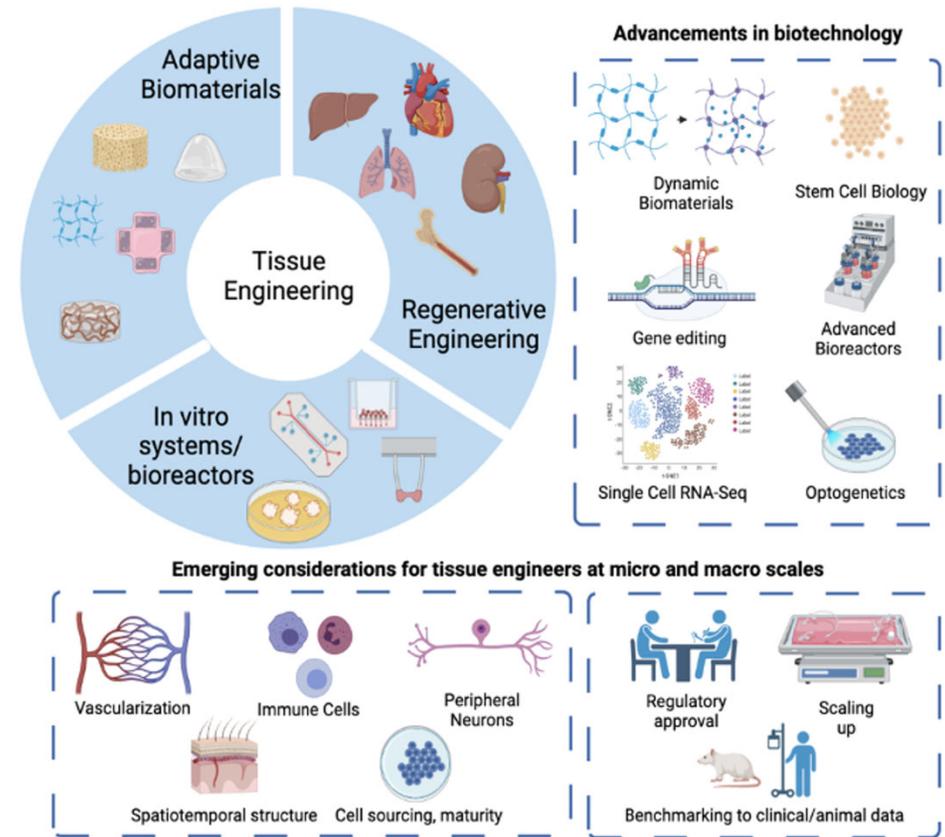
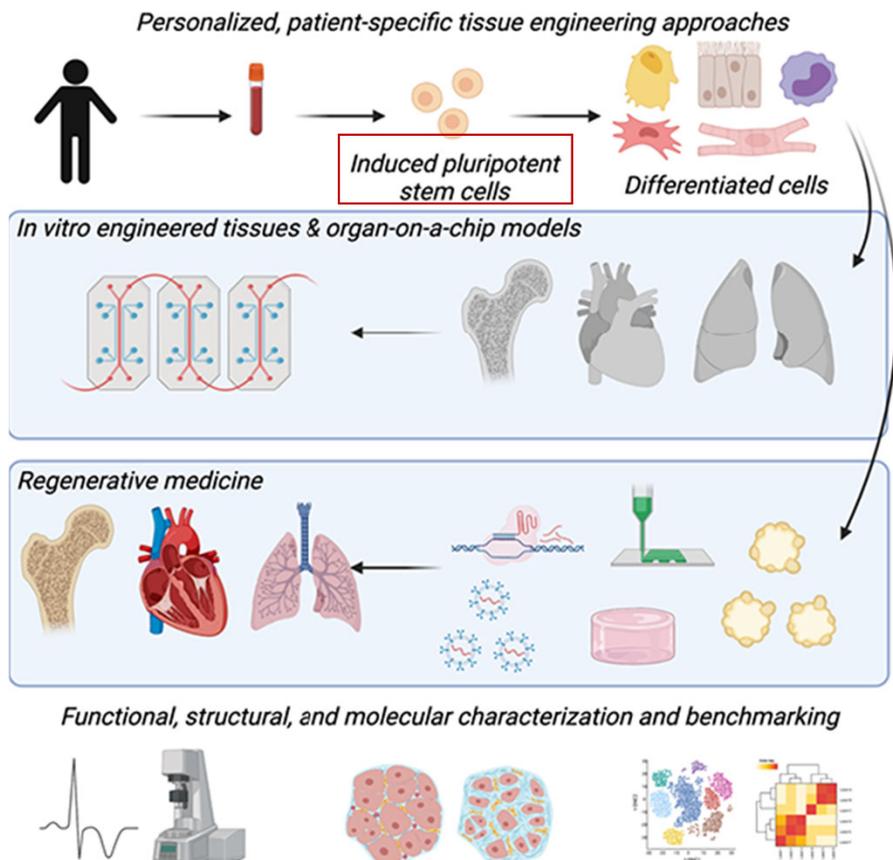


Figure 2. State of the field of tissue engineering. Innovations in biomaterials, regenerative engineering strategies, in vitro systems, and biotechnology have propelled the advancement of tissue engineering over the last 20 years. Emerging considerations for continued acceleration focus on building complexity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

# Materials of Human Origin for Tissue Engineering

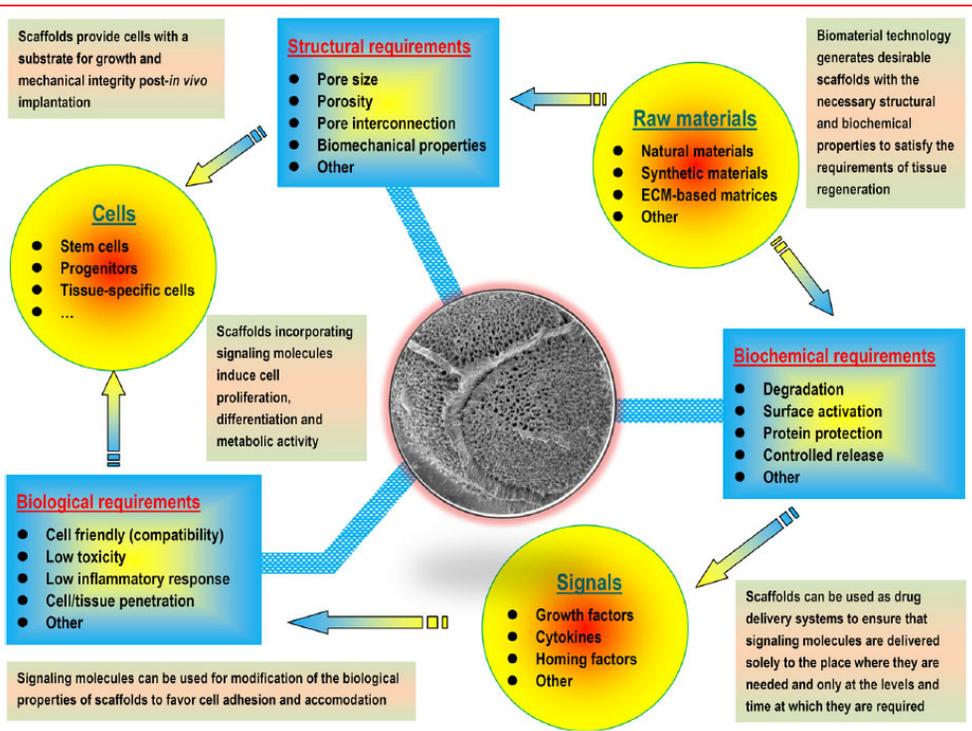


Fig. 2. Schematic representation of pivotal factors (structural, mechanical, biochemical and biological) involved in the design of biomaterials (templates) for tissue engineering that coax cells to behave in the same or a similar manner as their natural in vivo counterparts

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

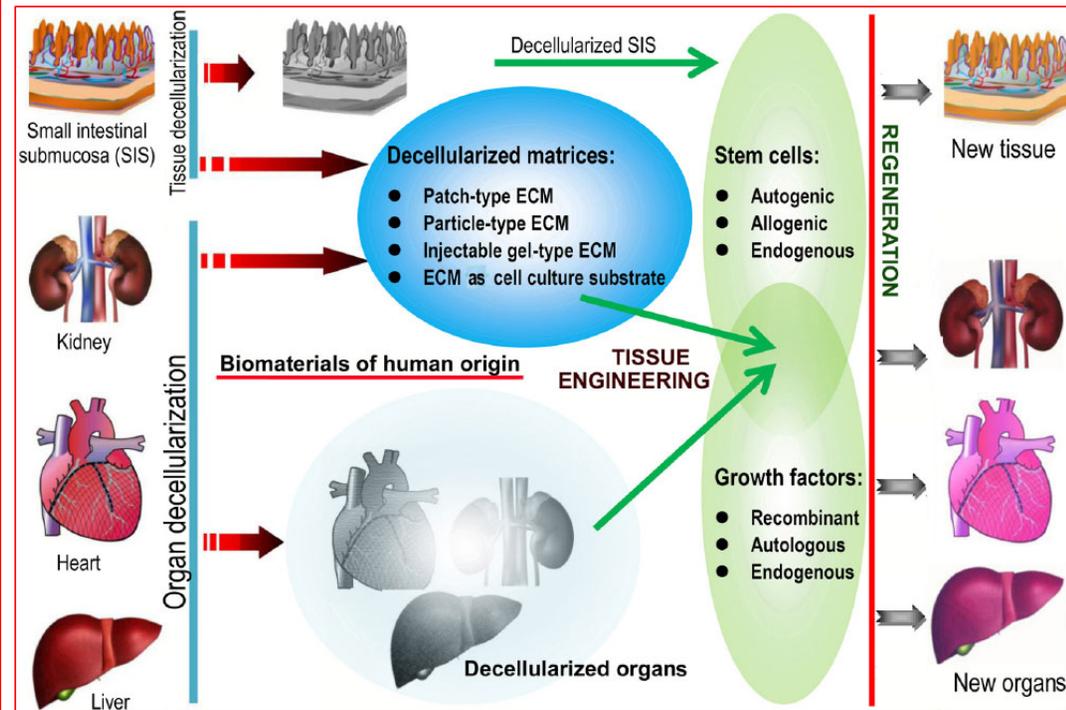
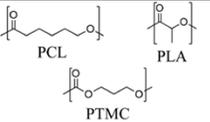
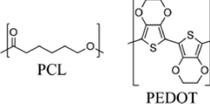
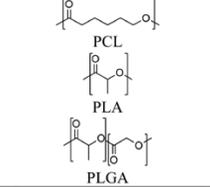
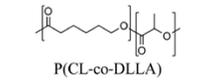
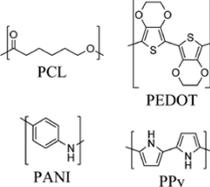


Fig. 12. Decellularized matrices from tissues (e.g., small intestinal submucosa (SIS)) or organs (e.g., kidney, heart and liver) that have native-like extra cellular matrix (ECM) microstructures, compositions and biomechanical properties (schematic is not to scale). These decellularized ECMs may maintain 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].

# Synthetic Polymers for Tissue Engineering

Table 1. Structure–property guide and suggestions on the use of synthetic polymers as TE scaffolds

Target tissue	Chemical structure	Derived properties	Resulting desired scaffold characteristics	Polymer suggestions	Repeating units
Adipose	Long aliphatic moieties, asymmetric monomers	Low glass transition temperature and crystallinity	Soft and pliable	P(CL-co-LA) P(LA-co-TMC)	
Muscle	Non-crosslinked, aliphatic moieties	Soluble, electrospun and printable	Fibrillar, myoblast attachment and proliferation	PCL-co-conductive polymer (e.g. PEDOT, PPy, PANI, polyaniline)	
	Long aliphatic moieties	Elasticity	Myotube differentiation		
	Alternating double and single bonds	Conductivity	Restoration of contractile function		
Vessels	Long aliphatic moieties	Elasticity	Mimicking of native vessels	PCL-PGS, P(CL-co-LA), PLGA	
	Non-crosslinked, aliphatic moieties	Soluble	Fibrillar (electrospinning)		
	Asymmetric monomers	Low T <sub>g</sub> and low crystallinity, fast degradation	Cell infiltration and prevention of calcification		
	Citric acid moiety	Calcium chelating anticoagulant	Anti-thrombogenic		POC inner layer
Nerves	Long aliphatic moieties	Soft	Attachment and growth of neurons	P(CL-co-DLLA)	
	Long methylene moieties	Low wettability	3D neuronal growth and differentiation		
	Non-crosslinked, aliphatic moieties	Processable	Porous, permeable		
	Alternating double and single bonds	Conductive	Neuron excitation	PCL-co-conductive polymer (e.g. PEDOT, PPy, PANI, polyaniline)	

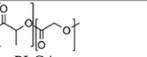
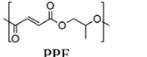
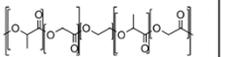
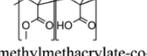
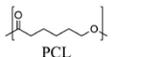
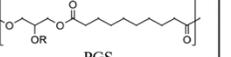
Skin	Polar groups	Hydrophilicity, water content	Cell adhesion, proliferation, and differentiation	PVA, PVP, PLGA	
	Long aliphatic moieties	Elasticity	Mimicking of native tissue		
	Non-crosslinked, aliphatic moieties	Processable	Porous, permeable, vascularization		
Bone	Non-crosslinked, aliphatic moieties	Processable, 3D printable	Interconnected macroporosity, facilitates bone growth, vascularization	PCL, PLGA, PLA, PLGA-PEG-PLGA, PPF based composites with bioactive additives	
	Stereoregularity	Semicrystalline, rigid	Provide mechanical support		
Cartilage	Polar groups	Hydrophilicity, water content, crosslinkable	Hydrogel, mimicking native cartilage	PEG/PEO, PVA, Polyacrylates Modified with amine groups	
	Long aliphatic sequences	Low T <sub>g</sub> and crystallinity	Deformability		
	Surface amine groups	Cell-scaffold interactions	Chondrogenesis		
Cornea	Long aliphatic sequences, asymmetric monomers	Amorphous, elastic	Transparency, softness	PCL-natural polymers PCL-PGS blends	
	Polar groups	Hydrophilicity	High water content		
	Non-crosslinked, aliphatic moieties	Soluble	Fibrillar (via electrospinning and bioprinting)		

Table 1 includes the influence of the chemical structure on the properties that control their suitability for the different tissue engineering applications. All of these have been reported to efficiently regenerate the target tissues, but besides that, the commercial availability and pre-existing regulatory approval were considered, which may be crucial factors in accelerating clinical applications.

# Synthetic & Natural Materials for Tissue Engineering

## Overview of the Origin and Applications of Injectable Cryogels

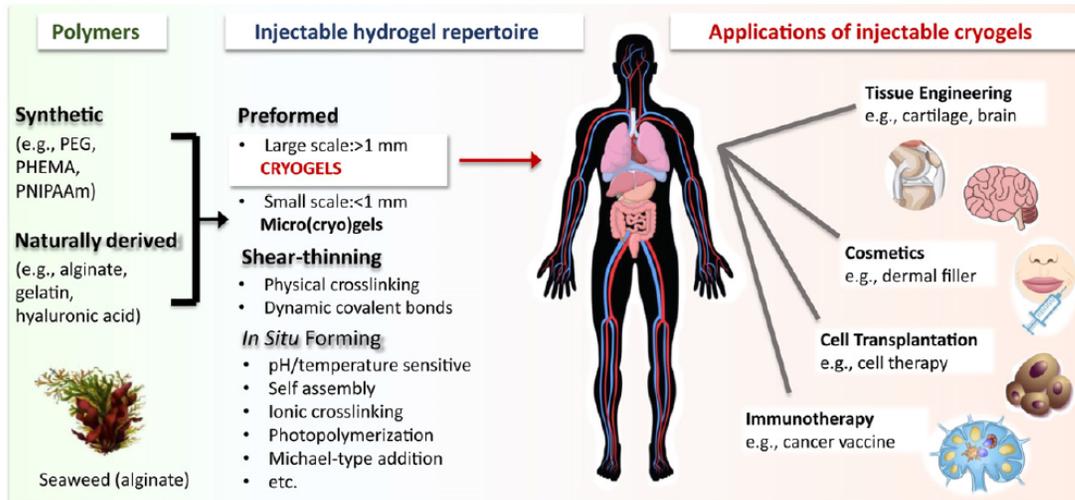


Fig. 1. Hydrogels can be fabricated using synthetic or naturally derived polymers. Within the injectable hydrogel repertoire, many in situ forming gels such as shear-thinning and small-scale gels have been developed. Cryogels are the only readily available large-scale, preformed, and injectable macroporous 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

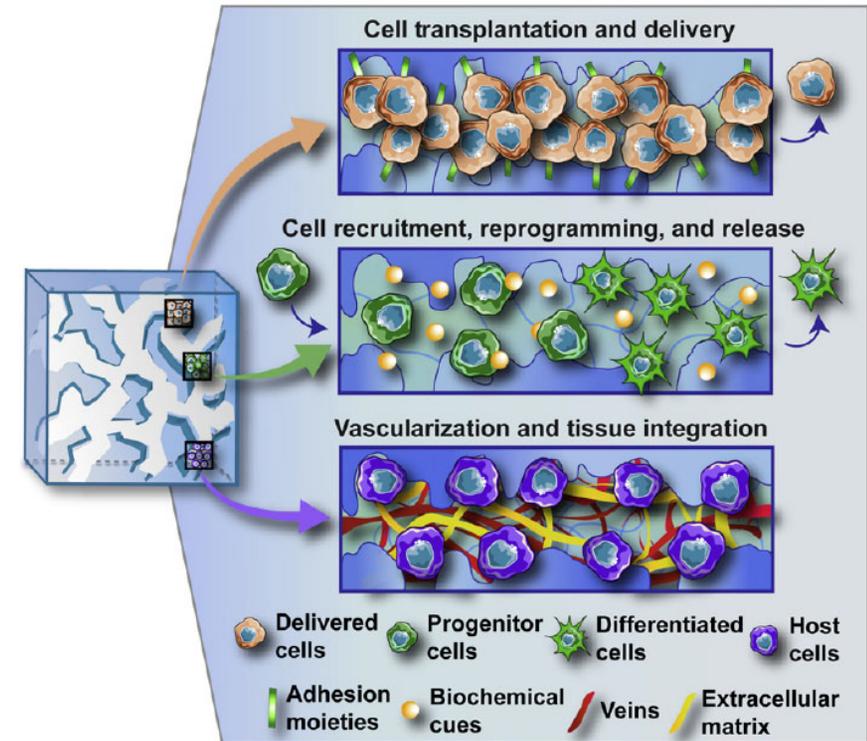


Figure 3. Cryogels are suitable delivery systems and create a confined niche for several biomedical applications. The interconnected macropores are an ideal environment that allows cell attachment and protection during transplantation. Furthermore, the open macroporous structure facilitates recruitment 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

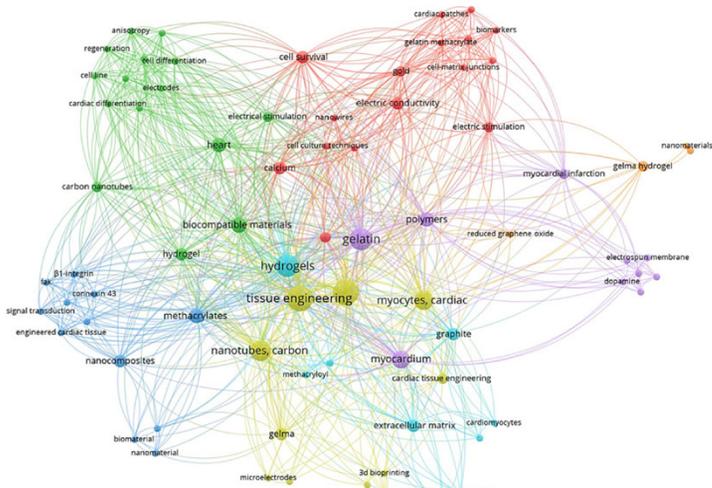


Fig. 1. Bibliometric map using PubMed data published between 2010 and 2023, searching for the keywords “GelMA” and “cardiac tissue” and “nanomaterial”, created with VOSviewer software version 1.6.16 [20].

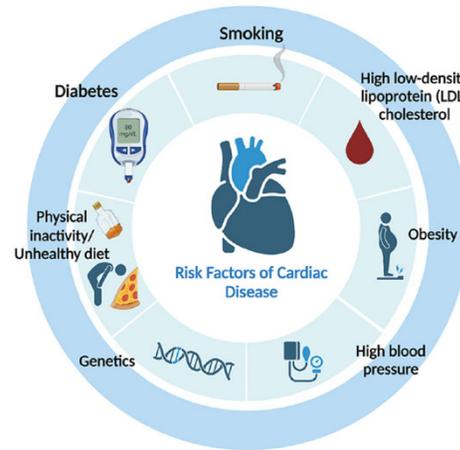


Fig. 2. Main cardiovascular disease risk factors: behavioral risk factors are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol and underlying determinants of CVDs as age, poverty, stress and hereditary factors.

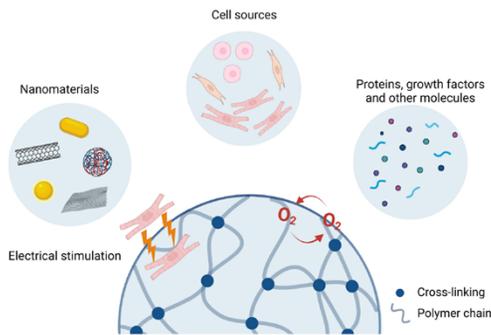


Fig. 3. Illustrative scheme identifying potential applications of hydrogels in tissue engineering. It is also possible to add growth factors and other molecules that will help the material’s communication and cell adhesion. Nanomaterials may also stimulate the electrical communication between cells,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

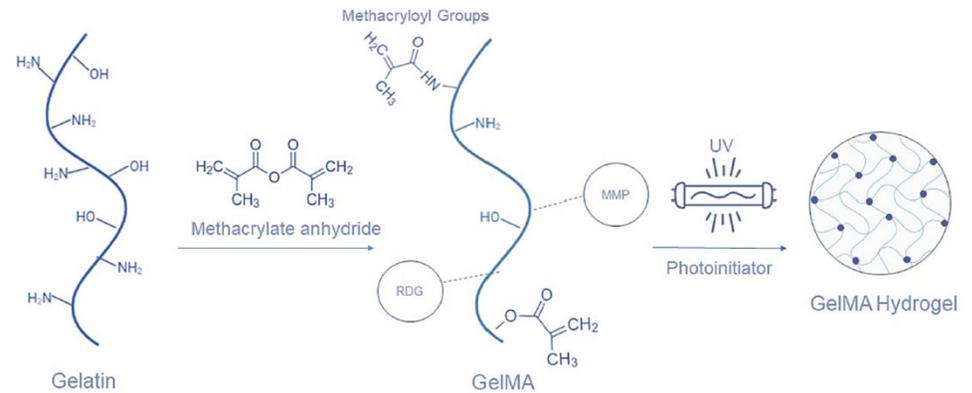


Fig. 4. Synthesis of methacrylated gelatin (GelMA). The primary amine groups of gelatin react with methacrylic anhydride (MA) to add methacryloyl pendant groups. Bioactives from gelatin, such as arginine-glycine-aspartic (RGD) and matrix metalloproteinases (MMP), are still kept in the GelMA chain (first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

Lisboa 2024, Nanomaterials-combined methacrylated gelatin hydrogels (GelMA) for cardiac tissue constructs

# Advanced Smart Biomaterials and Constructs

REVIEW ARTICLE OPEN

## Advanced smart biomaterials and constructs for hard tissue engineering and regeneration

Ke Zhang<sup>1,2</sup>, Suping Wang<sup>2,3</sup>, Chenchen Zhou<sup>3</sup>, Lei Cheng<sup>2,3</sup>, Xianling Gao<sup>2,4</sup>, Xianju Xie<sup>1,2</sup>, Jirun Sun<sup>5</sup>, Haohao Wang<sup>2,3</sup>, Michael D. Weir<sup>2</sup>, Mark A. Reynolds<sup>2</sup>, Ning Zhang<sup>1,2</sup>, Yuxing Bai<sup>1</sup> and Hockin H. K. Xu<sup>2,6,7</sup>

Hard tissue repair and regeneration cost hundreds of billions of dollars annually worldwide, and the need has substantially increased as the population has aged. Hard tissues include bone and tooth structures that contain calcium phosphate minerals. Smart biomaterial-based tissue engineering and regenerative medicine methods have the exciting potential to meet this urgent need. Smart biomaterials and constructs refer to biomaterials and constructs that possess instructive/inductive or triggering/stimulating effects on cells and tissues by engineering the material's responsiveness to internal or external stimuli or have intelligently tailored properties and functions that can promote tissue repair and regeneration. The smart material-based approaches include smart scaffolds and stem cell constructs for bone tissue engineering; smart drug delivery systems to enhance bone regeneration; smart dental resins that respond to pH to protect tooth structures; smart pH-sensitive dental materials to selectively inhibit acid-producing bacteria; smart polymers to modulate biofilm species away from a pathogenic composition and shift towards a healthy composition; and smart materials to suppress biofilms and avoid drug resistance. These smart biomaterials can not only deliver and guide stem cells to improve tissue regeneration and deliver drugs and bioactive agents with spatially and temporarily controlled releases but can also modulate/suppress biofilms and combat infections in wound sites. The new generation of smart biomaterials provides exciting potential and is a promising opportunity to substantially enhance hard tissue engineering and regenerative medicine efficacy.

*Bone Research* (2018)6:31; <https://doi.org/10.1038/s41413-018-0032-9>

Zhang 2018, Advanced smart biomaterials and constructs for hard tissue engineering and regeneration

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# Scaffolds

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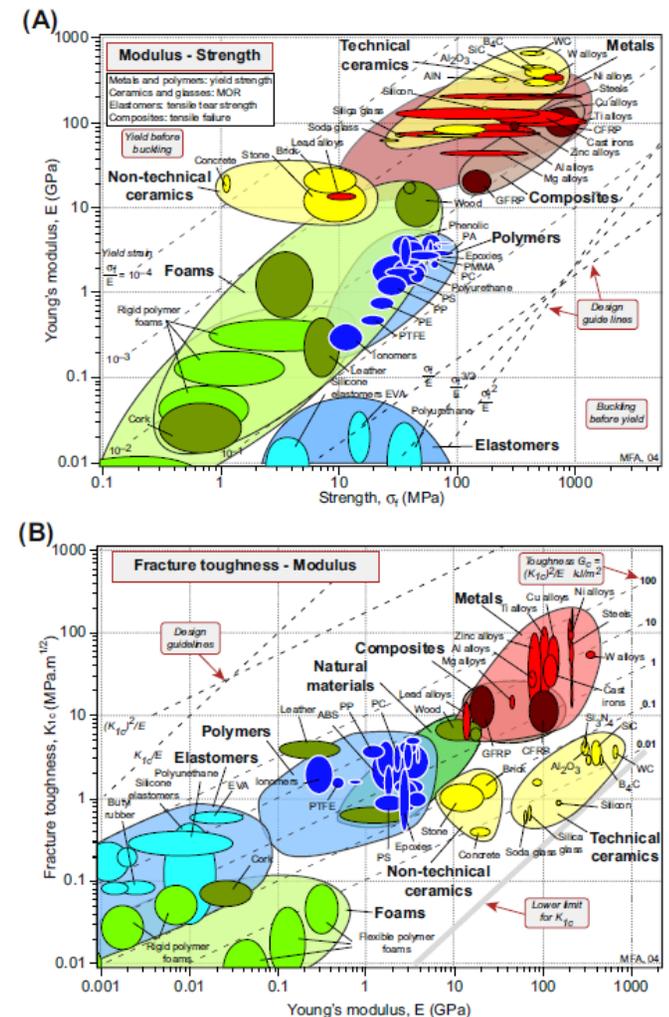
# Design Principles in Scaffolds

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

Given the mechanical support functions defined for a specific clinical application of a scaffold, and the increasingly recognized **influence of mechanical parameters on host response and cell behavior, selecting materials with suitable mechanical properties 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 being prone to 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.



# 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.

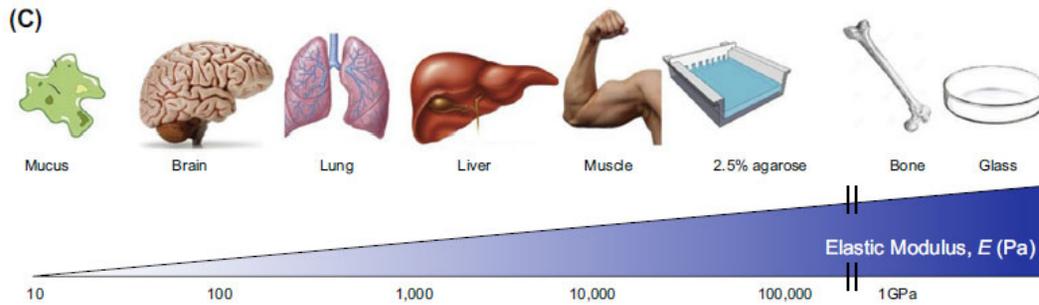
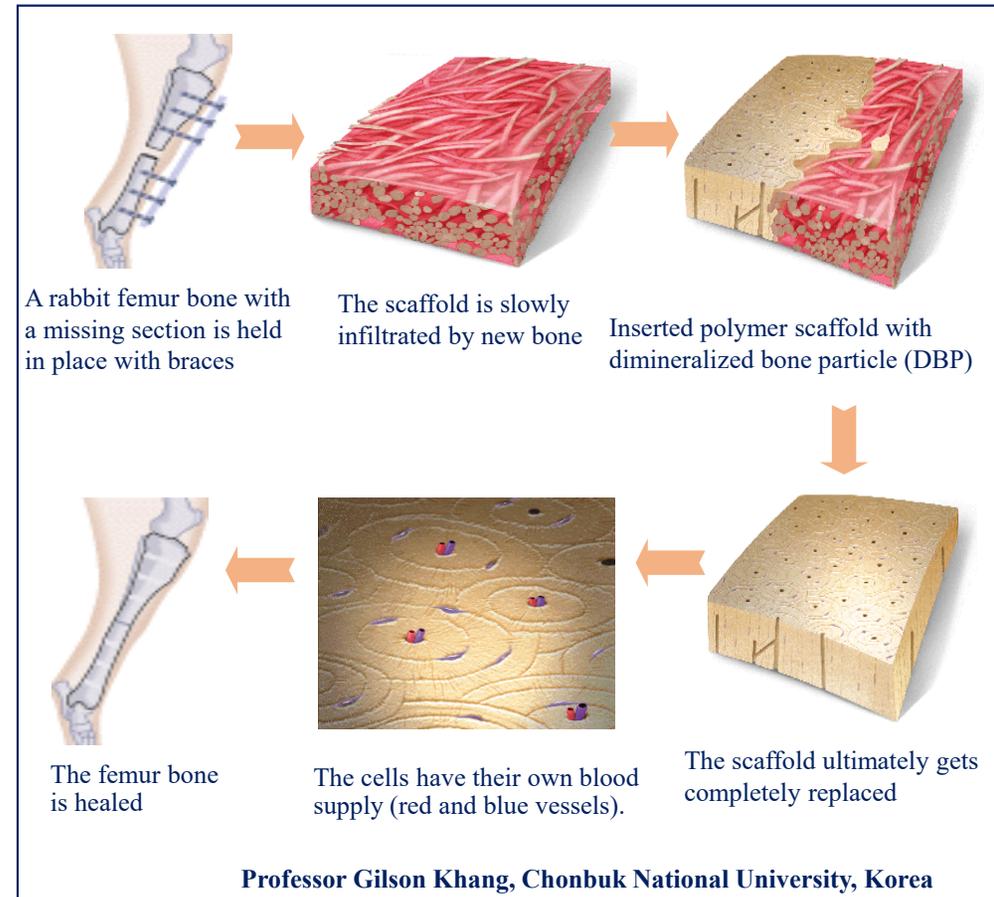


FIGURE 30.2 (C) Young's, or elastic, modulus of tissues [21].

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.

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>



# Injectable Hydrogels

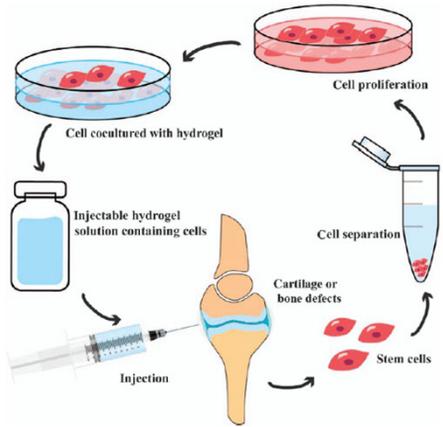


Figure 1. Schematic illustration of approaches to make injectable hydrogels for cartilage- and bone tissue-engineering applications.

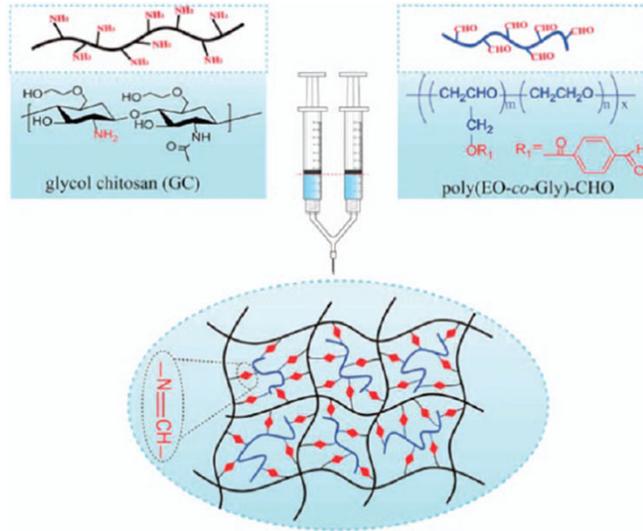


Figure 5. Schematic illustration of injectable hydrogels prepared by Schiff base cross-linking between aqueous solutions of GC and poly(EO-co-Gly)-CHO.230

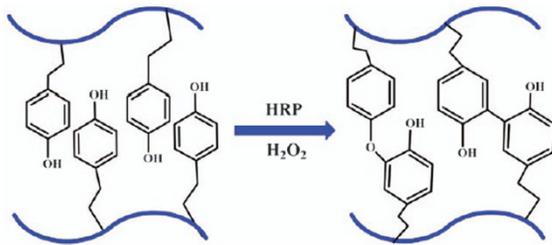


Figure 4. Schematic illustration of injectable hydrogels prepared by the enzymatic cross-linking method with horseradish peroxidase (HRP) and  $H_2O_2$ .



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

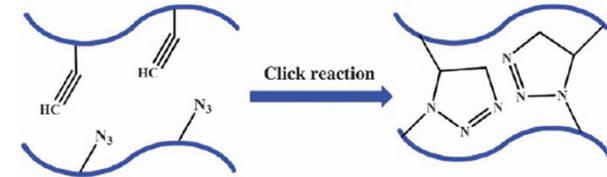


Figure 7. Schematic illustration of injectable hydrogels prepared by click chemistry. (Azide and alkyne groups).

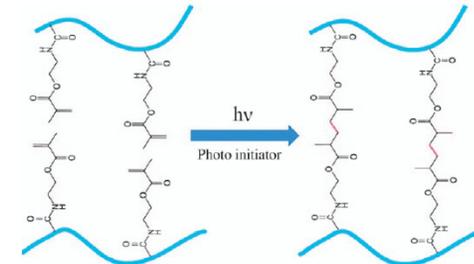


Figure 8. Schematic illustration of injectable hydrogels prepared by the photo-cross-linking method.

# Functional Semi-Interpenetrating Polymeric Materials

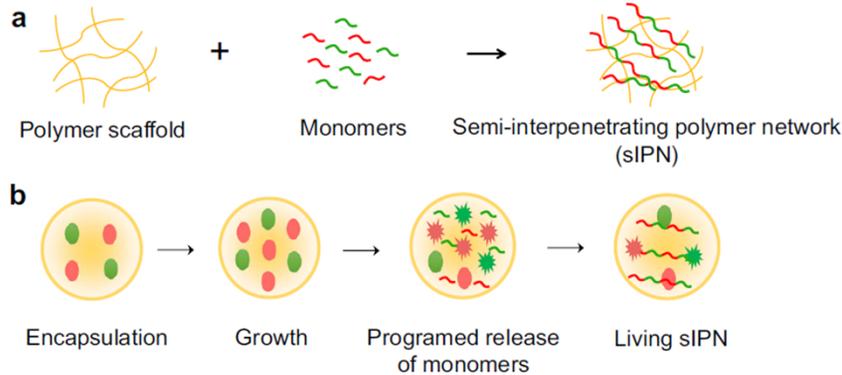


Fig. 1 Conventional and living fabrication of semi-IPN. a) A semi-interpenetrating polymer network (sIPN) is defined as a linear or branched polymer in the presence of another cross-linked polymer. sIPN can be assembled by dissolving monomers (for the 2nd component) in a crosslinked scaffold (the 1st 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

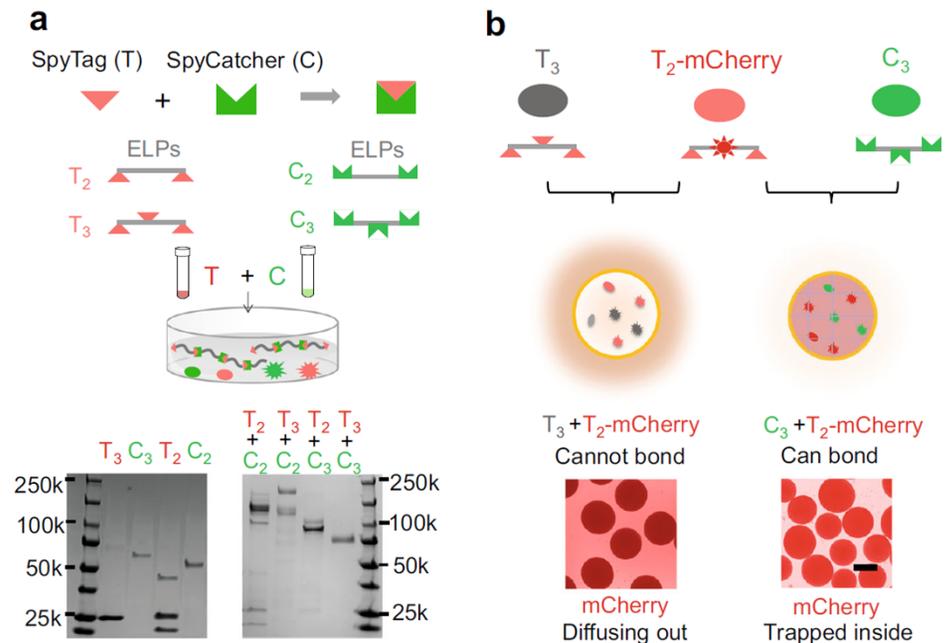


Fig. 2 Formation of living sIPN.

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<sub>2</sub>-mCherry) or MC(T<sub>3</sub>)) and bacteria expressing proteins with multiple SpyCatchers (C, MC(C<sub>2</sub>) or MC(C<sub>3</sub>)) 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<sub>2</sub>-mCherry) and MC(C<sub>3</sub>) were mixed, pelleted and encapsulated with chitosan. As a control, MC(C<sub>3</sub>) was replaced with MC(T<sub>3</sub>). 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

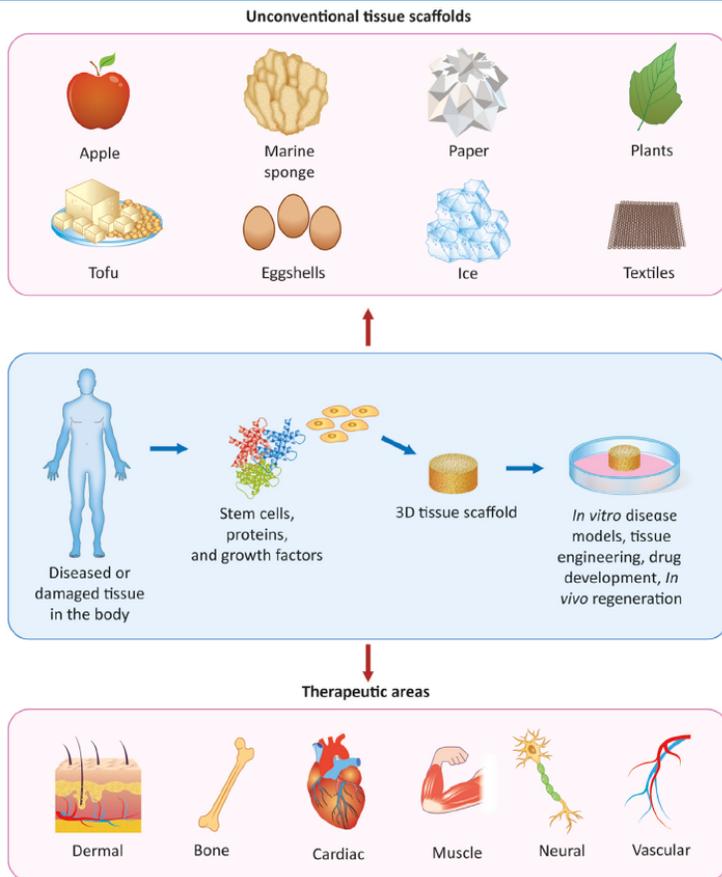


Figure 1. These approaches can cover a plethora of therapeutic and research areas including dermal, bone, cardiac, muscle, neural, and vascular tissues.

Nguyen 2019, Unconventional tissue engineering materials in disguise

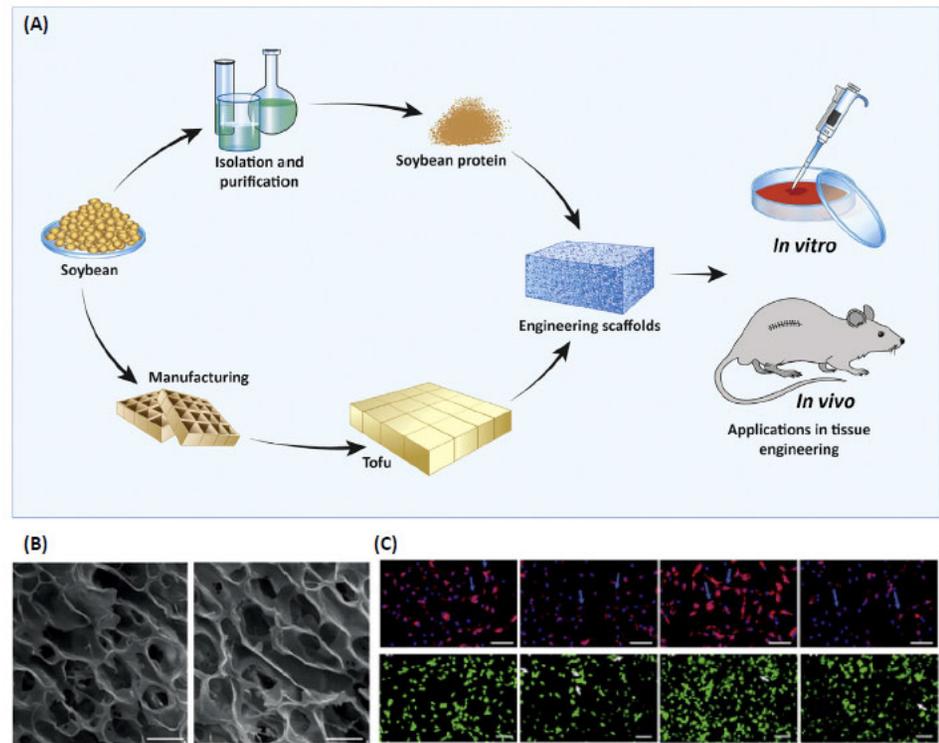


Figure 6. Biomimetic Protein Scaffolds from Tofu. (A) A schematic diagram illustrating the fabrication process from soybean to tofu-based engineered scaffold. The scaffold was seeded with 3T3 cells and cultured in vitro. The 3D construct was also implanted into mice for in vivo testing, which did not 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  $\mu\text{m}$ . (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  $\mu\text{m}$ . (A–C) Adapted, with permission, from [54].

# Conductive Biomaterials

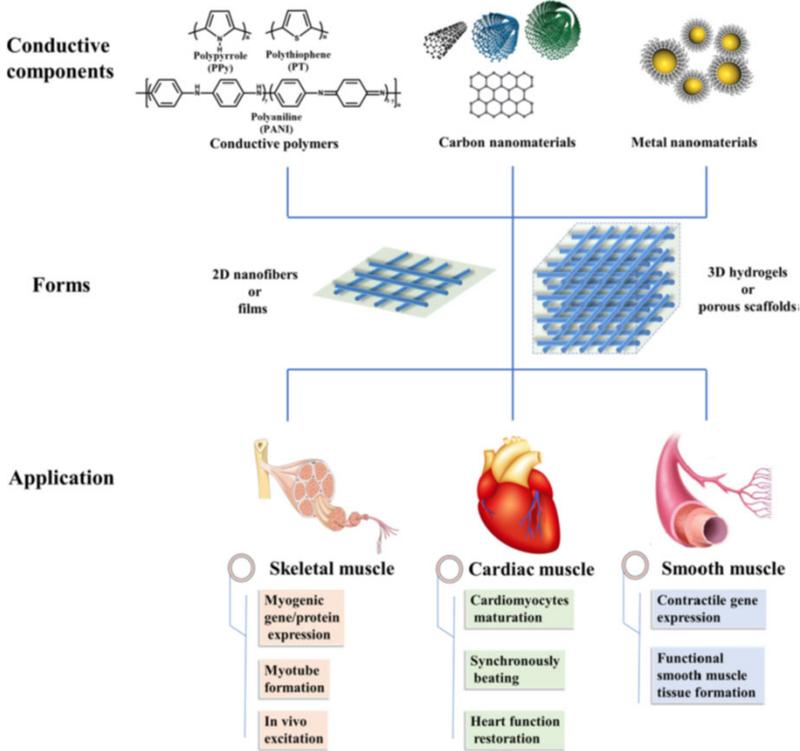


Fig. 1. Schematic illustration of conductive biomaterials' fabrication, forms and their application in muscle tissue repair.

Dong 2020, Conductive biomaterials for muscle tissue engineering

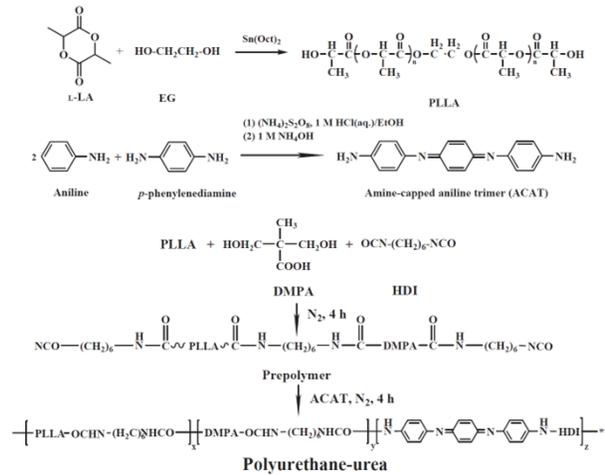


Fig. 3. Synthetic methods of PLLA, ACAT, and PUU copolymer.

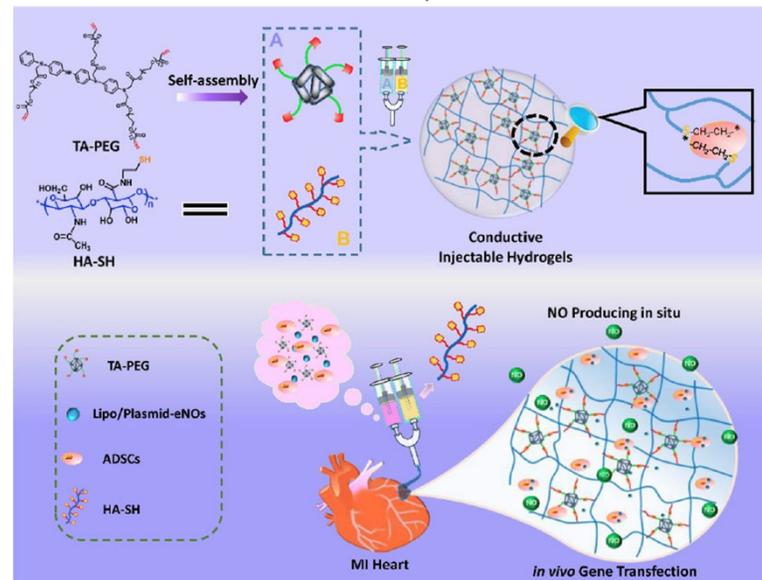


Fig. 4. Schematic sketch showing how to construct the conductive injectable hydrogel. TA-PEG is soluble in water and the hydrogel can form in aqueous solution. DNA-eNOs nanoparticles and ADSCs are encapsulated inside this hydrogel to treat myocardial infarction.

# 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.  $\beta$ -O-4' and carbon-carbon linkages such as  $\beta$ -5',  $\beta$ - $\beta'$ , and 5-5'.

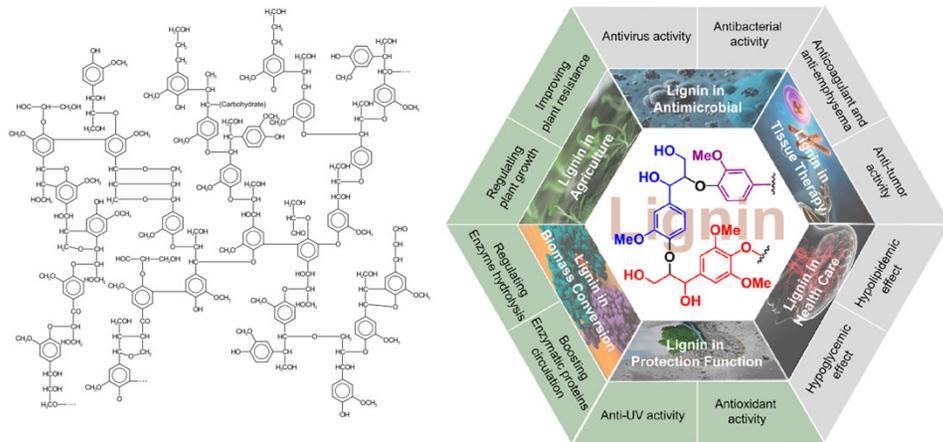


Figure 1. Biological activities and effective utilizations of lignin.

## Lignin

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

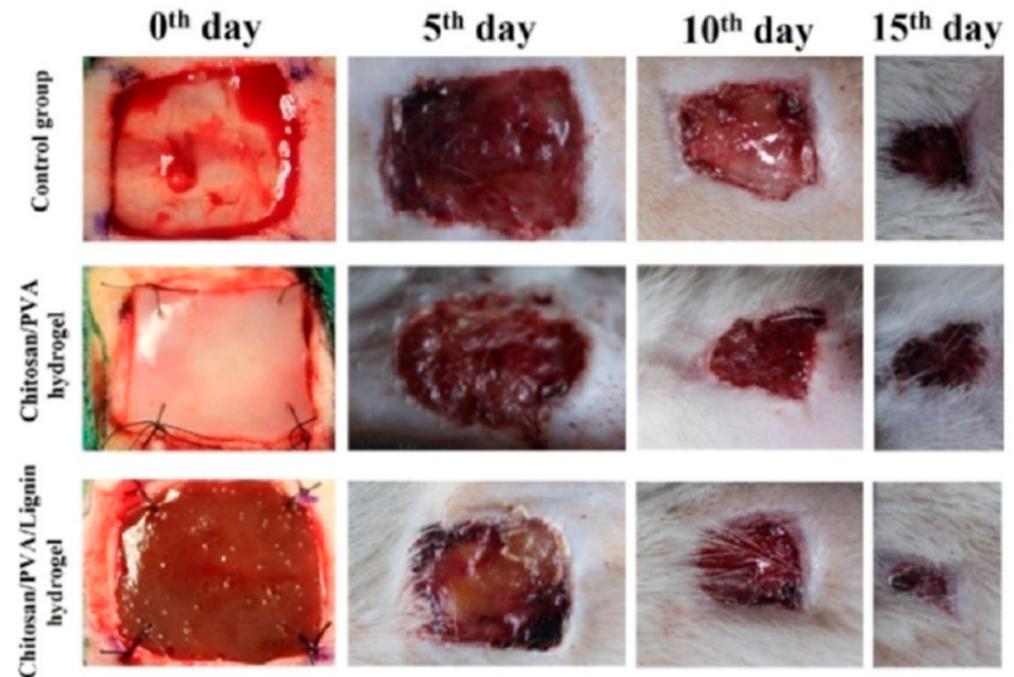
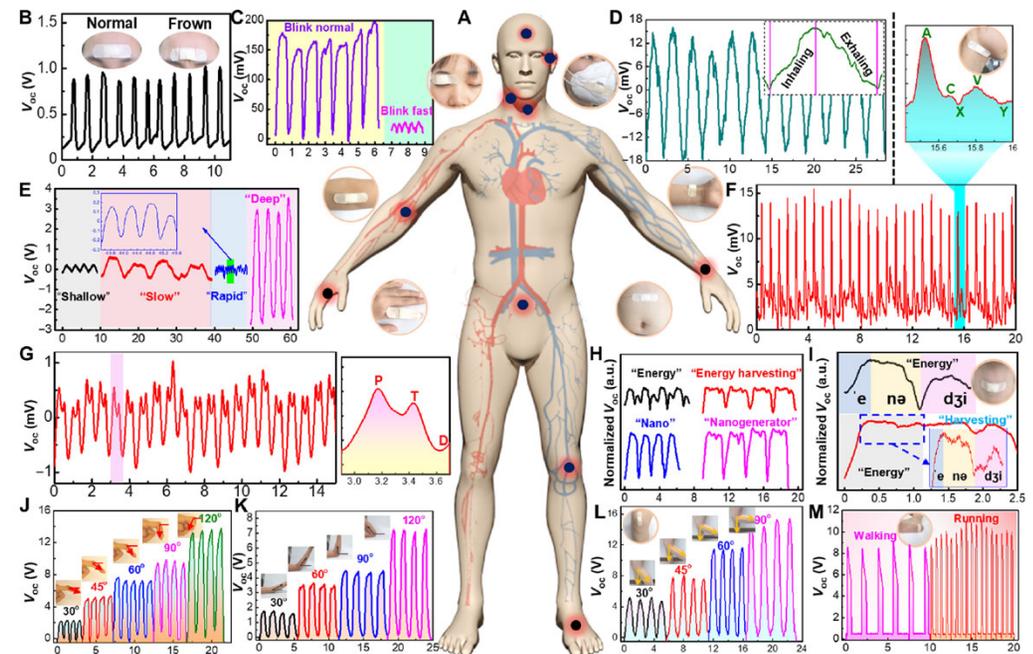
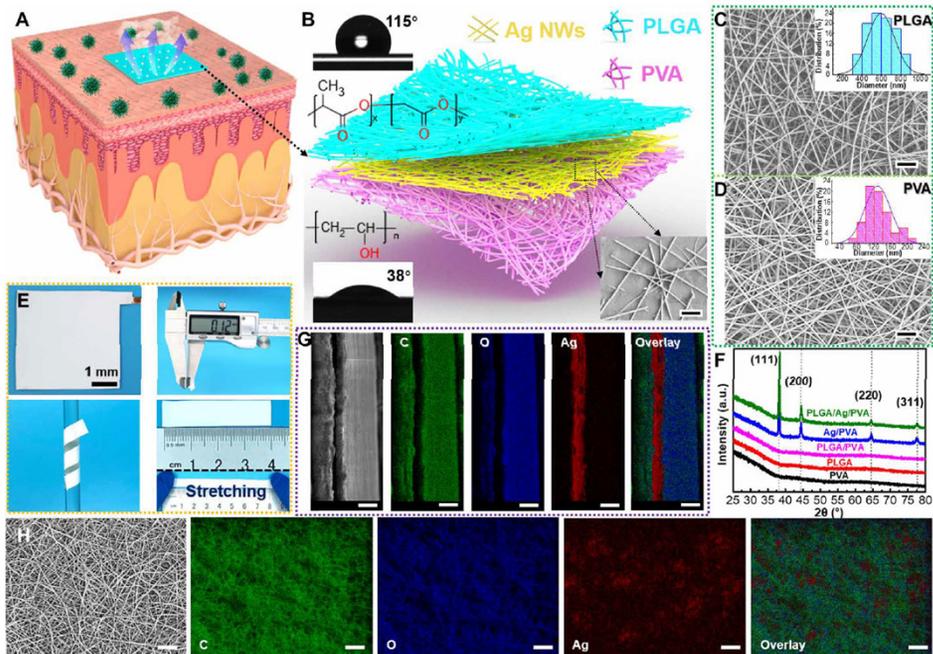


Figure 3. (a) Wound healing status of a blank control group, chitosan-PVA hydrogel, and chitosan-PVA-lignin hydrogel (lignin 10 wt %) at days 0, 5, 10, and 15.

# 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 poly(lactic-co-glycolic acid) (PLGA) and poly(vinyl 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

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# **Organ-on-a-Chip**

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# Organ-on-a-Chip

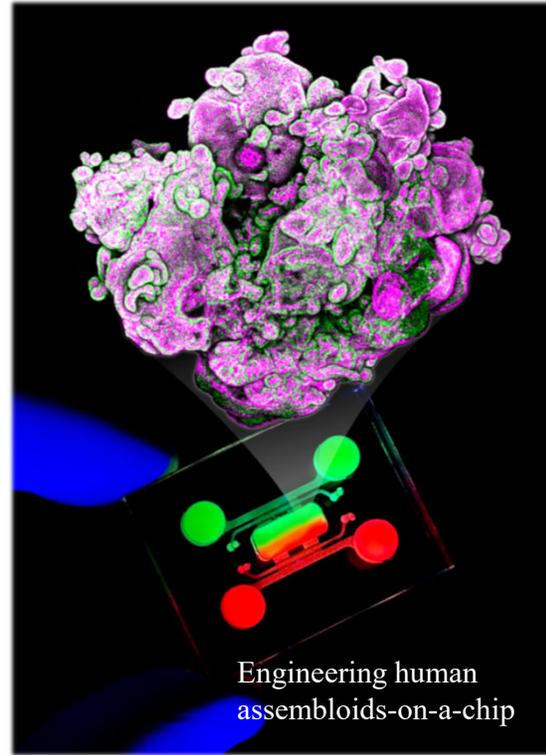
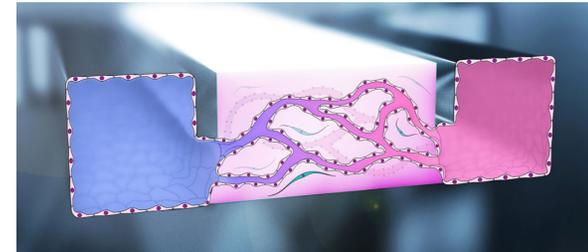
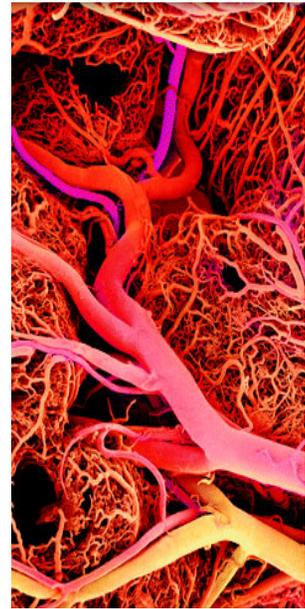
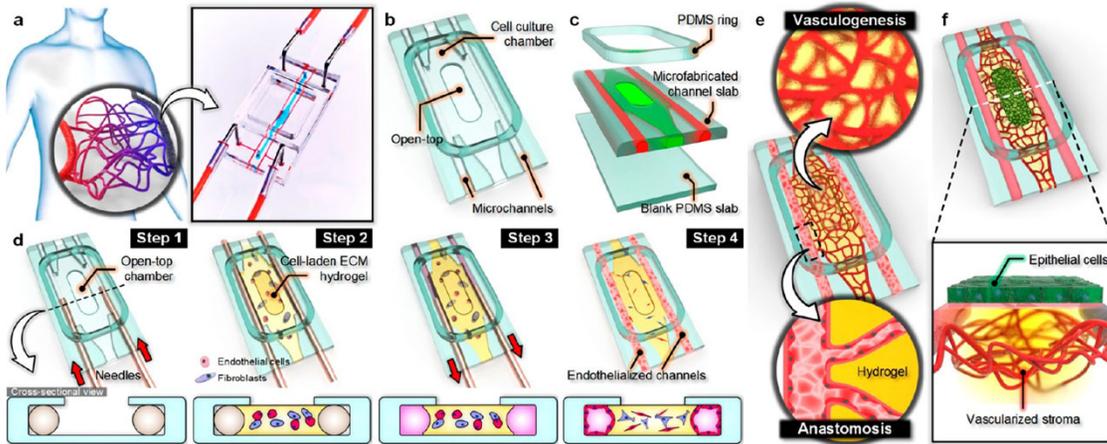
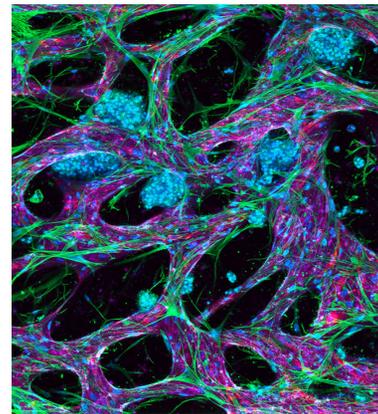


Figure 1. A microengineered in vitro 3D culture platform to produce self-assembled and perfusable microvascular beds. a. Our microengineered cell culture device is used to engineer in vivo-like 3D networks of blood vessels. b. The device consists of a cell culture chamber with a top opening and two parallel microchannels used for controlled vascular perfusion. c. This multilayered device is fabricated by sealing microfabricated channels against a blank PDMS slab and bonding a PDMS ring used as a medium reservoir. d. To form the vasculature, template needles are inserted into the microchannels (Step 1), and this step is followed by the injection of endothelial cells and fibroblasts suspended in an ECM hydrogel solution into the cell culture chamber (Step 2). Once gelation is complete, the needles are pulled out to generate hollow microchannels (Step 3), which are then seeded with vascular endothelial cells to create endothelialized and externally accessible compartments on both sides of the cell-laden scaffold (Step 4). e. During cell culture, the endothelial cells in the hydrogel undergo vasculogenesis and self-assemble into interconnected endothelial tubes that together form a 3D multicellular structure reminiscent of microvascular plexus in vivo. The vessels in the hydrogel also anastomose with the endothelium in the side channels to form a perfusable network. f. The open top of the cell culture chamber can be used for culturing another cell type, such as epithelial cells, directly on the surface of the vessel-containing ECM hydrogel scaffold to mimic the structural organization of vascularized 3D tissues in a more realistic manner.

Paek 2019, Microphysiological engineering of self- assembled and perfusable microvascular beds for the production of vascularized three-dimensional human microtissues

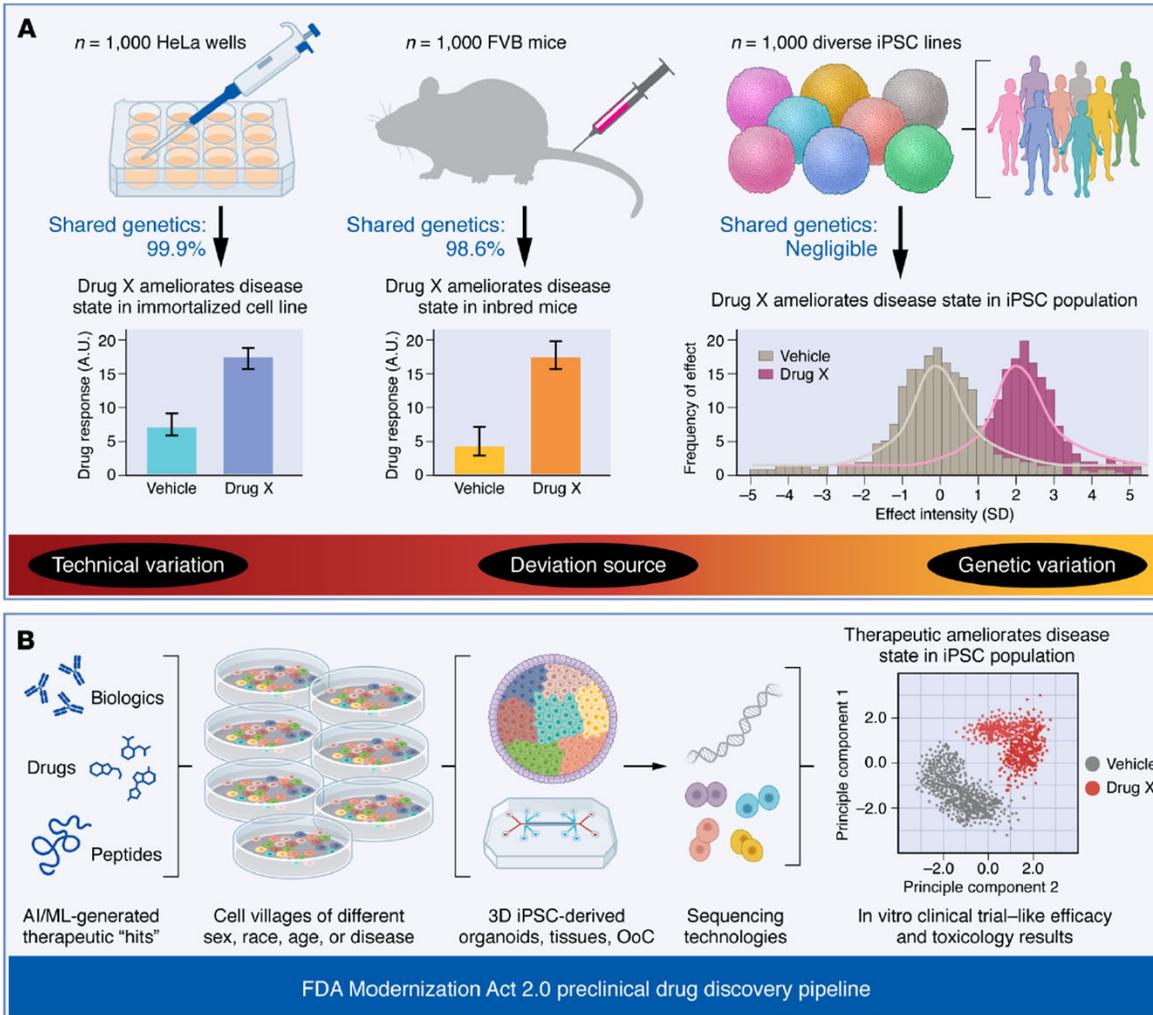


Prof. Estelle Park



Engineering human  
assembloids-on-a-chip

# Human Organoids: Beyond Animal Models



The discovery and development of effective therapeutics to combat diseases is a cornerstone of biomedical research. Preclinical studies serve as a crucial gateway, bridging scientific discovery with tangible patient outcomes, yet still often fail to provide positive results. The FDA Modernization Act 2.0 has paved the way for alternative methods to bolster the preclinical data pipeline, aiming to reduce the dependence on animal models that have frequently resulted in therapeutic dead ends. Among these new alternatives, iPSCs in particular have gained prominence for their potential to offer more physiologically relevant insights into disease mechanisms and drug responses. When paired with emerging bioengineered 3D techniques and sequencing technologies, iPSCs currently represent the best hope for improving our preclinical-to-clinical trial pipeline for new therapeutics.

Figure 1. Lack of genetic diversity and pharmacogenomic differences between model animals and humans lead to the high termination rate of phase I and II clinical trials. (A) Immortalized cell lines such as HeLa cells and inbred rodent models are commonly used as the gold standard of preclinical validation due to their ease of use. However, tissue cultures use immortalized cell lines with 99.9% shared genetics on average and rodent experiments use inbred strains with 98.6% shared genetics on average. Data from technical replicates of therapeutic agents can have positive results with low standard deviations that may be misleading. In contrast, testing therapeutic agents on patient-specific iPSCs more accurately represents the full genetic and pharmacogenomic diversity of human populations. Here, experimental results would reflect the effects of therapeutic agents in responders and nonresponders across a large cohort of individuals (rather than in a single immortalized cell line or a chosen rodent strain), hence providing more reliable safety and efficacy data prior to proceeding to clinical trials. (B) Innovative techniques for cultivating multiple iPSC lines together and segregating their transcriptomic and genetic signals have given rise to the concept of "cell villages." These pooled populations are then differentiated into various cell types and incorporated into 3D models such as organoids or organs-on-chips, closely mimicking the human in vivo environment. These cell village tissues can then be subjected to therapeutic agents identified from AI/ML models, and the application of single-cell technologies allows for elucidation of each cell line's distinct gene expression response to the therapeutic intervention. This transformative approach of clinical-trial-in-a-dish holds immense potential for enhancing our understanding of drug safety and efficacy, ultimately increasing the likelihood of therapeutic success in early clinical trials.

# Microfluidic Organs-on-a-Chip

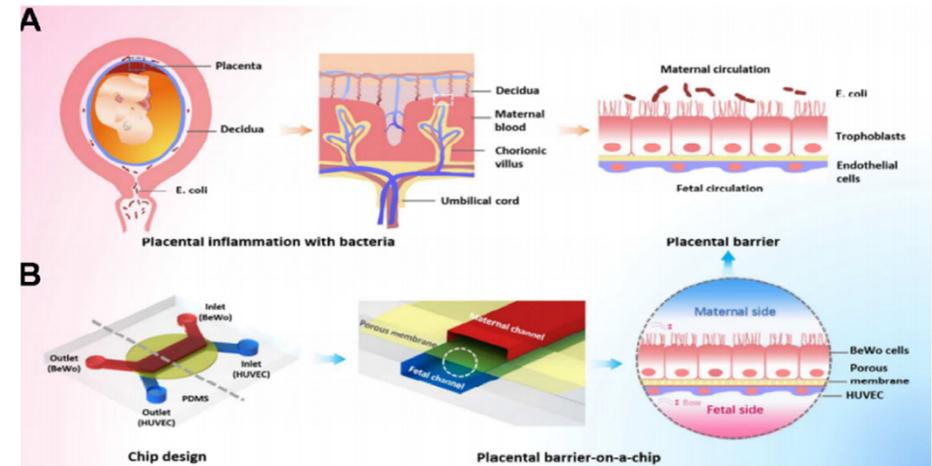
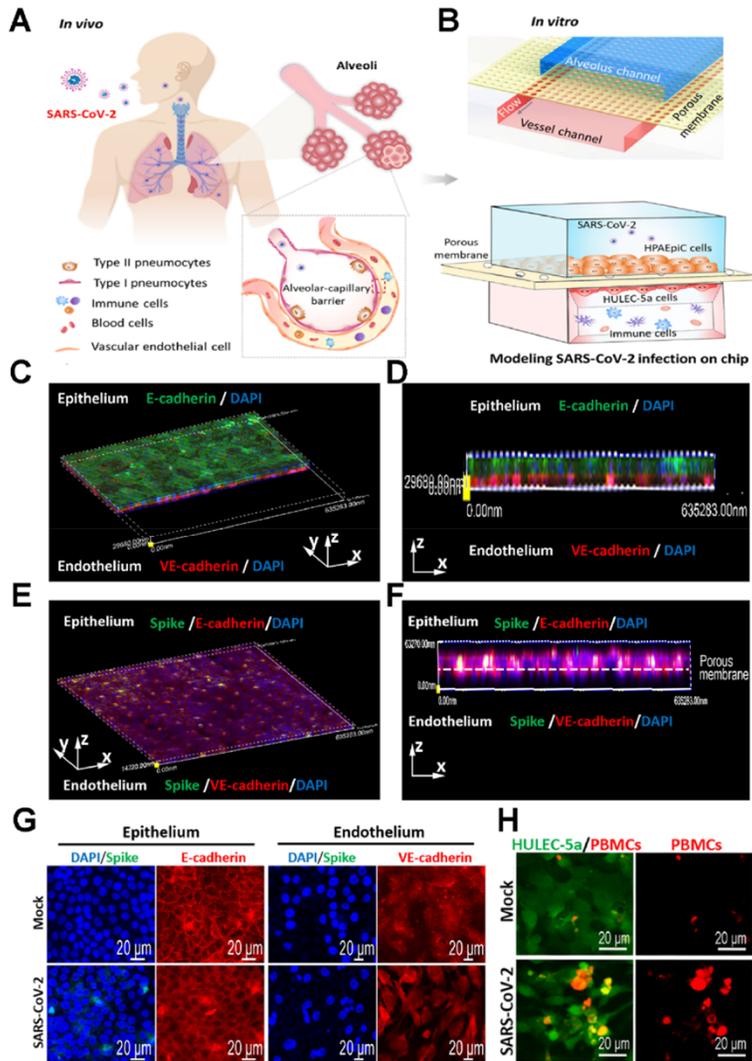


Figure 5. Modeling intrauterine infection caused by bacteria with the placenta-on-a-chip. (A) The placenta is an organ that links the fetus to the maternal uterine wall via the umbilical cord. Bacteria commonly pass by the ascending route in the lower genital tract into the uterus, causing placental inflammation. (B) Diagram of the placenta-on-a-chip consisting of the top maternal layer and the bottom fetal layer separated by a porous membrane. The placental barrier was constructed from a BeWo epithelial layer and a HUVEC layer on the opposite side of the membrane-on-a-chip.

Figure 2. Characterization of SARS-CoV-2-induced lung injury using the human lung-on-a-chip. (A) Illustration of the human alveolar-capillary barrier in vivo. (B) Configuration of the human lung-on-a-chip model with SARS-CoV-2 infection. The microengineered lung chip is composed of an alveolar epithelium in the upper layer and a pulmonary microvascular endothelium in the lower layer, separated by a porous PDMS membrane. It mimics the human alveolar-capillary barrier in vivo by the coculture of HPAEpic and HUVEC-5a cells under fluid flow. (C) Three-dimensional reconstructed confocal image of the alveolar epithelium (E-cadherin) and endothelium (VE-cadherin) on a chip. (D) Side view showing the interface of the alveolar epithelium (E-cadherin) and endothelium (VE-cadherin). (E) Three-dimensional reconstructed confocal image showing the alveolar-capillary barrier on the third day postinfection. (F) Side view showing the alveolar barrier after viral infection. SARS-CoV-2 infection was predominantly identified in the epithelium layer by spike protein expression. (G) Confocal images showing the effects of SARS-CoV-2 infection (spike) on the epithelium (E-cadherin) and the endothelium (VE-cadherin) on the third day postinfection. (H) Confocal immunofluorescence microscopy images showing the recruitment and adhesion of immune cells (PBMCs) (red) to the surface of the HULEC-5a layer (green) after viral infection.

Wang 2021, Microfluidic organs-on-a-chip for modeling human infectious diseases

# Organ-on-a-Chip for Drug Screening

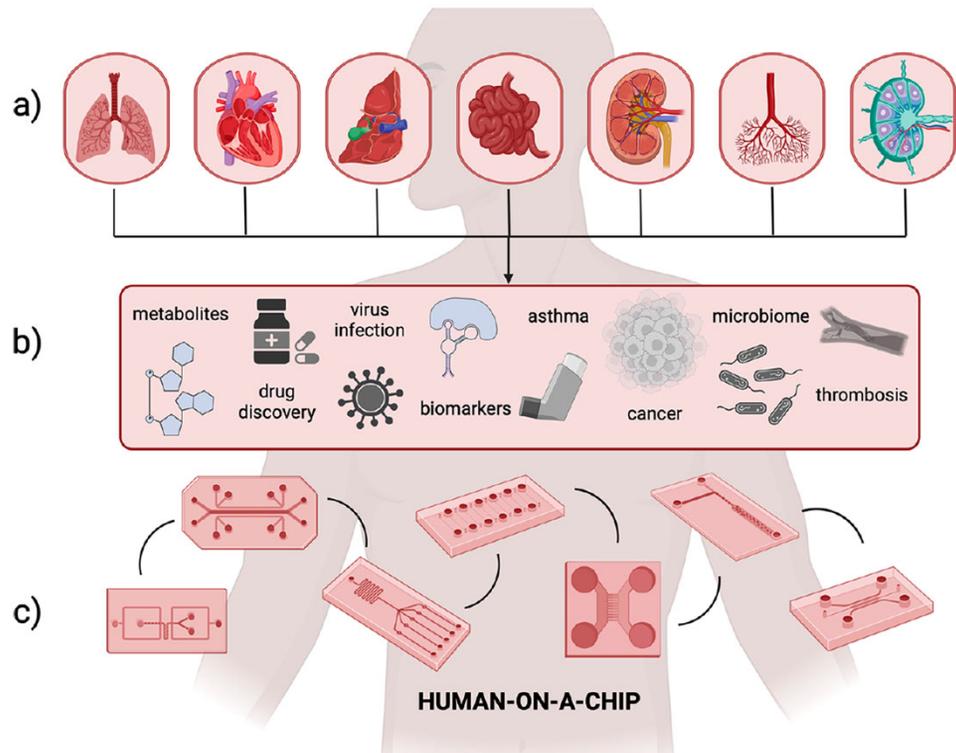


Figure 1. Main organs on chips, their application in the pharmaceutical industry, and their connection to creating a human-on-a-chip. (A) The main organs applied to organ-on-a-chip (OoC) implementation, in order: lung, heart, liver, intestine, kidney, blood vessel, and lymph node. (B) Most typical applications of OoC for the pharmaceutical industry. (C) Multiple OoC designs representing different organs connected, leading to the development of a Human-on-a-chip or a Body-on-a-chip.

## PERSPECTIVES FOR INDUSTRY SCALE

Regardless of the potential for organ-on-a-chip (OoC), the pharmaceutical industry hesitates to adopt this technology as a standard. This hesitation is most likely due to the need for an initial investment in **a standardized and highly reliable OoC production system, which is crucial to provide the necessary reproducibility and accuracy for drug discovery and development.** As a nonstandardized new technology, OoC platforms still need to accurately predict every new drug's *in vivo* behavior, yet recent research and OoC industry efforts are in development to cross this barrier. Ewart and Roth stated that OoC developers and the end-users must cooperate to achieve good acceptance in pharmaceutical companies. This acceptance would allow for **a faster proof of concept, adapting and substituting the traditional tests.** Therefore, new companies and start-ups are rising to fill gaps in OoC technology, mainly by standardizing several aspects of the chips. This includes research involving **substrates, cells, device materials and protocols, interfaces with analytical instruments, and assay protocols.**

Currently, the leaders in the OoC market are still relatively small companies, such as Emulate (USA), Mimetas (NL), Hesperos (USA), Aim Biotech (SG), AxoSim (USA), InSphero (CH), Nortis (USA), Micronit (NL), BEOChip (ES), Mesobiotech (FR), and TissUse GmbH (DE). The fabricants compete for OoC implementation and the development of high-throughput systems and work closely with major pharmaceutical companies to develop targeted solutions. Emulate, for example, has Roche, Takeda Pharmaceuticals, Merck, and Janssen as partners, while TissUse develops OoC for AstraZeneca and Roche.

# From Organ-on-a-Chip to Human-on-a-Chip

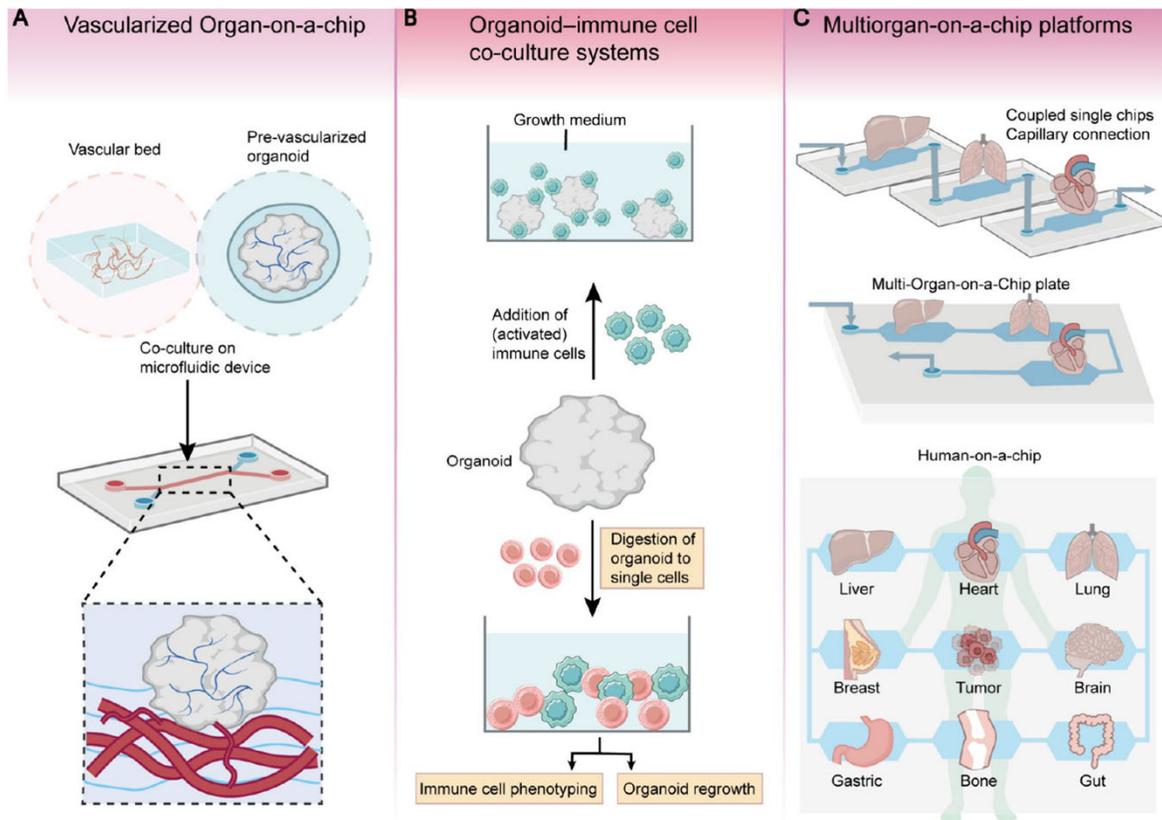


Figure 1. Functionalization of OOCs. (A) Mechanisms for establishing anastomoses between in vitro vascular beds and prevascularized organoids in coculture on microfluidic chips. (B) Two organoid-immune cell coculture systems used in basic research to study immune cell and epithelium interactions. Addition of (activated) immune cells to intact organoids in growth medium (suspension culture) is used to assess the interaction between immune cells and epithelial cells, or the organoids are digested into single cells and then regrown in the presence of immune cells. (C) The primary approaches to the development of multi-OOC platforms. Coupling individual OOC via capillary connections or microfluidic motherboards, each modeling a different organ, or integrating different organoid models on a single plate, which most closely approximates the human-on-a-chip concept.

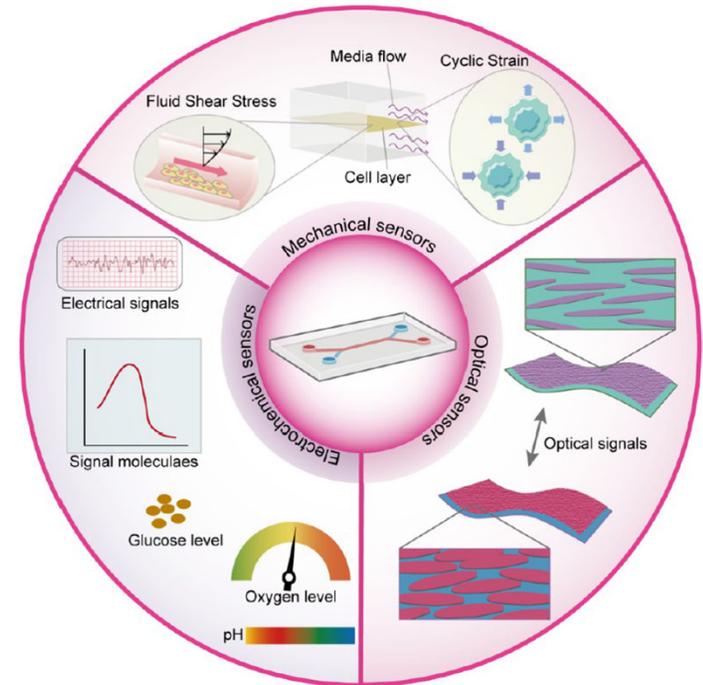


Figure 2. Fundamentals of sensors integrated in OOCs. Sensors are classified based on the characteristics of the target signal and the sensing principle as mechanical, electrochemical, or optical.

Huang 2024, From organ-on-a-chip to human-on-a-chip: A review of research progress and latest applications

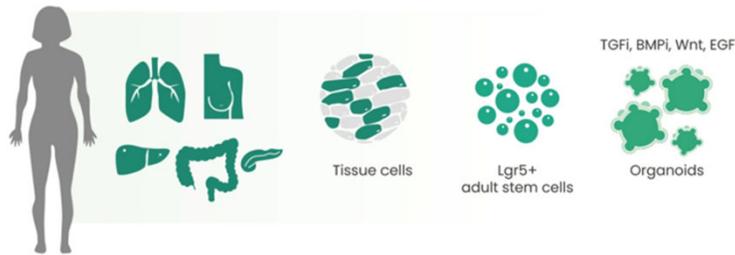
# Exploring New Frontiers: Organoids in Cancer Research

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

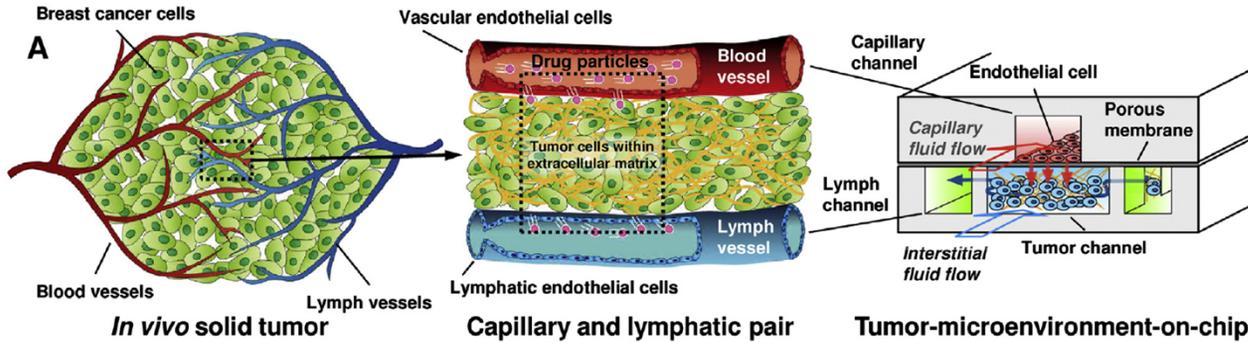
## Cell-Culture Models

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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.

# Tumor-Microenvironment-on-Chip (TMOC)

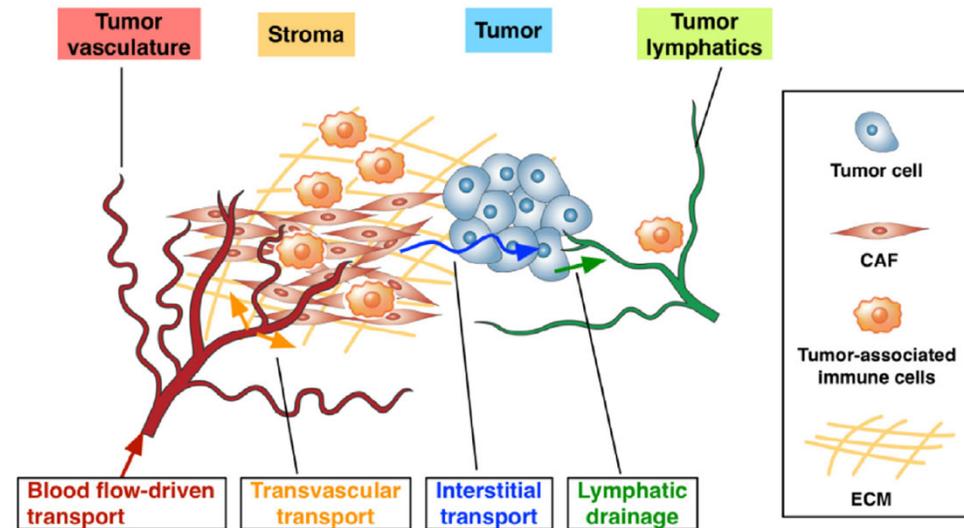


## Cautions

- Cell culture to obtain organoids takes time and is sometimes unpredictable.
- Can it represent the complex in vivo environment?

Fig. 1. Schematic of the tumor-microenvironment-on-chip. (A) Conceptual design: A minimal functional unit of a solid tumor, i.e., a pair of capillary and lymphatic vessels, is mimicked into a 3D microfluidic platform with a 3D structure. The top channel (red) simulates the capillary with endothelium (a monolayer of endothelial cells on a nanoporous membrane). NPs can be introduced along this capillary channel. The bottom layer has a center channel (blue) mimicking a 3D tumor microstructure (i.e., cells in a 3D matrix) and two side channels simulating the lymphatics (green). (B) Fabricated prototype and schematic of the perfusion setup of the T-MOC. The tumor channel will be pressurized to establish the elevated interstitial fluid pressure (IFP).

Fig. 1. Complexity of the tumor microenvironment. TME poses multifaceted barriers to drugs transport owing to the dense stromal tissue, which is composed of collagens, fibronectin, and hyaluronan, an abundance of cancer-associated fibroblasts, and aberrant interactions between infiltrating tumor-associated immune cells, cancer cells, and CAFs.

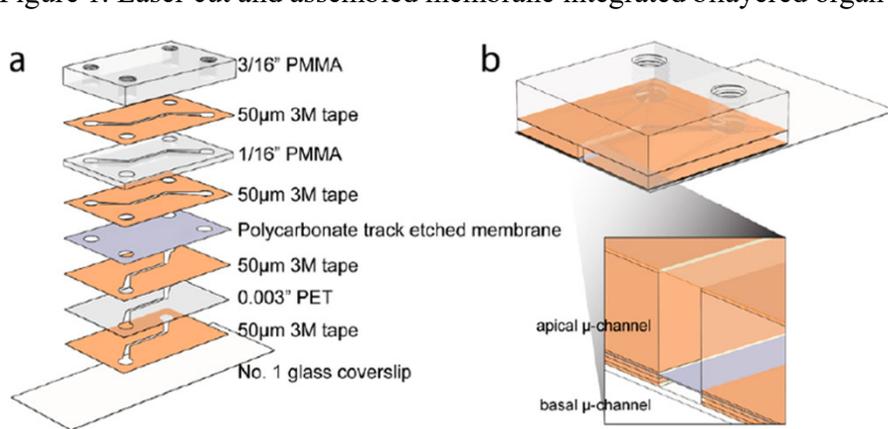


Kwak 2014, Simulation of complex transport of nanoparticles around a tumor using tumor-microenvironment-on-chip

Han 2016, Recapitulation of complex transport and action of drugs at tumor microenvironment using tumor-microenvironment-on-chip

# Multilayer Microphysiological Systems

Figure 1. Laser cut and assembled membrane integrated bilayered organ chip.



(a) A schematic showing the integration of nine discrete layers to form a bilayered organ chip.

(b) A schematic of an assembly cutaway view of the bilayered organ chip showing the inner apical and basal microchannels.

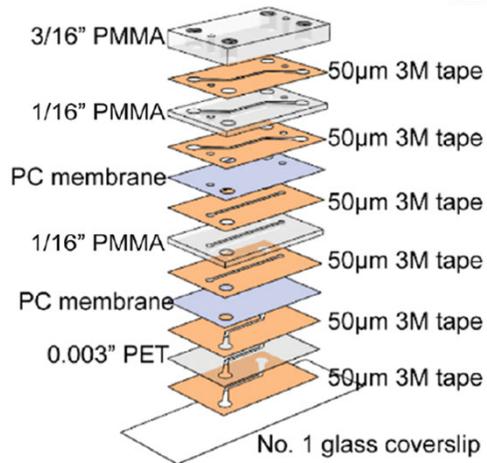
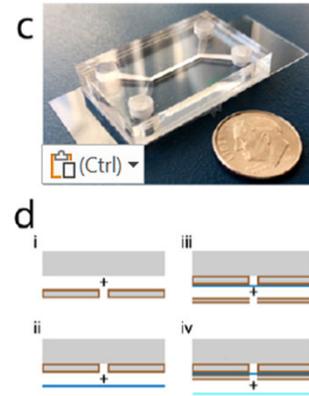


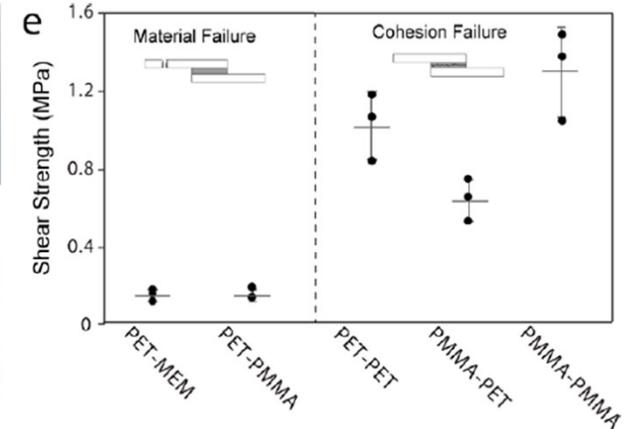
Figure 6. A schematic showing the integration of 13 discrete layers to form a trilayered chip.

A dual membrane, trilayered organ chip integrating a primary intestinal monolayer and organoids. The trilayered organ chip is composed of clear PMMA, acrylic adhesive, polycarbonate track etched membranes, and glass coverslip.



(c) A photograph of the bilayered organ chip composed of clear PMMA, acrylic adhesive, polycarbonate track etched membrane, and glass coverslip.

(d) Five layers are aligned and irreversibly bonded in four steps to form the apical and basal channels separated by a polycarbonate track etched membrane with a pore diameter of 1.0 µm.



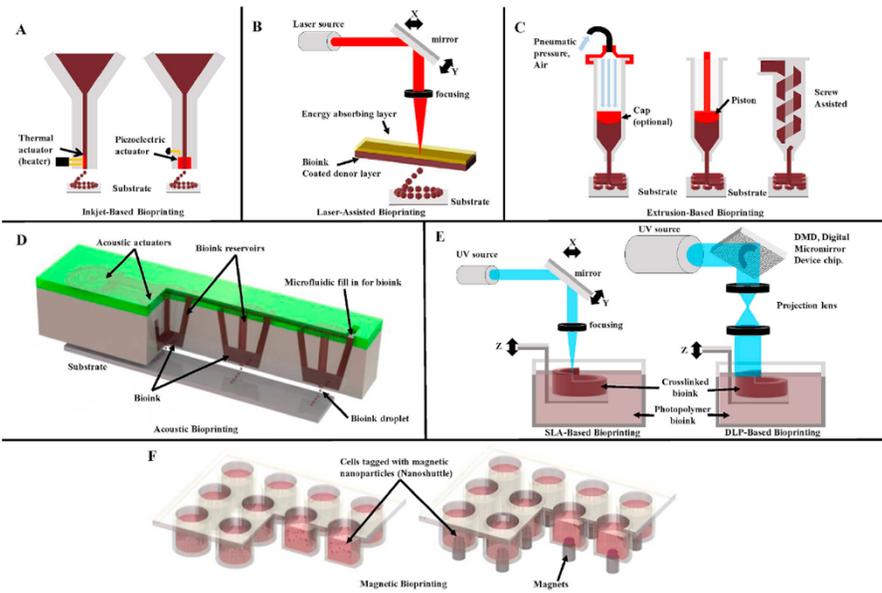
(e) Shear strength of bonded layers from a single-lap shear test indicate mechanical integrity to prevent burst or leaking (horizontal bar = mean, error bars = S.D.).

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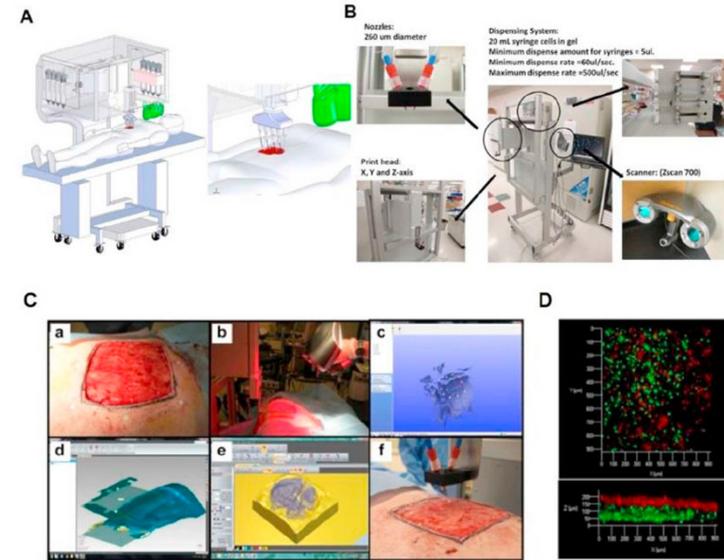
# **3D & 4D Bioprinting**

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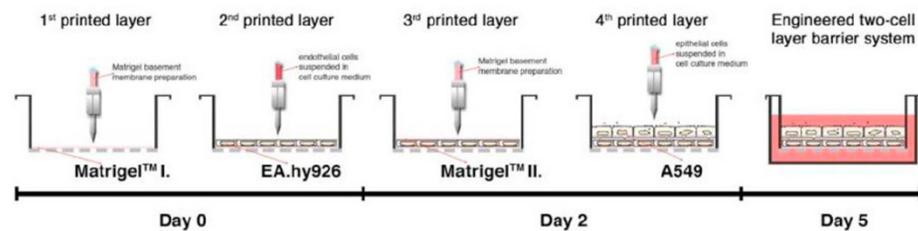
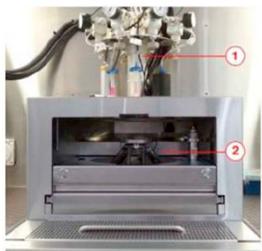
# 3D Bioprinting



**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.

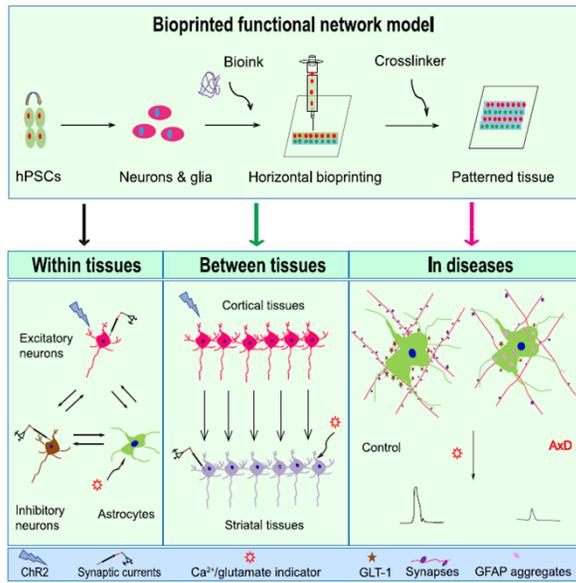


**Fig. 2.** In situ skin bioprinting prototype and gross examination of printed skin in murine full-thickness excision wound repair.



**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.

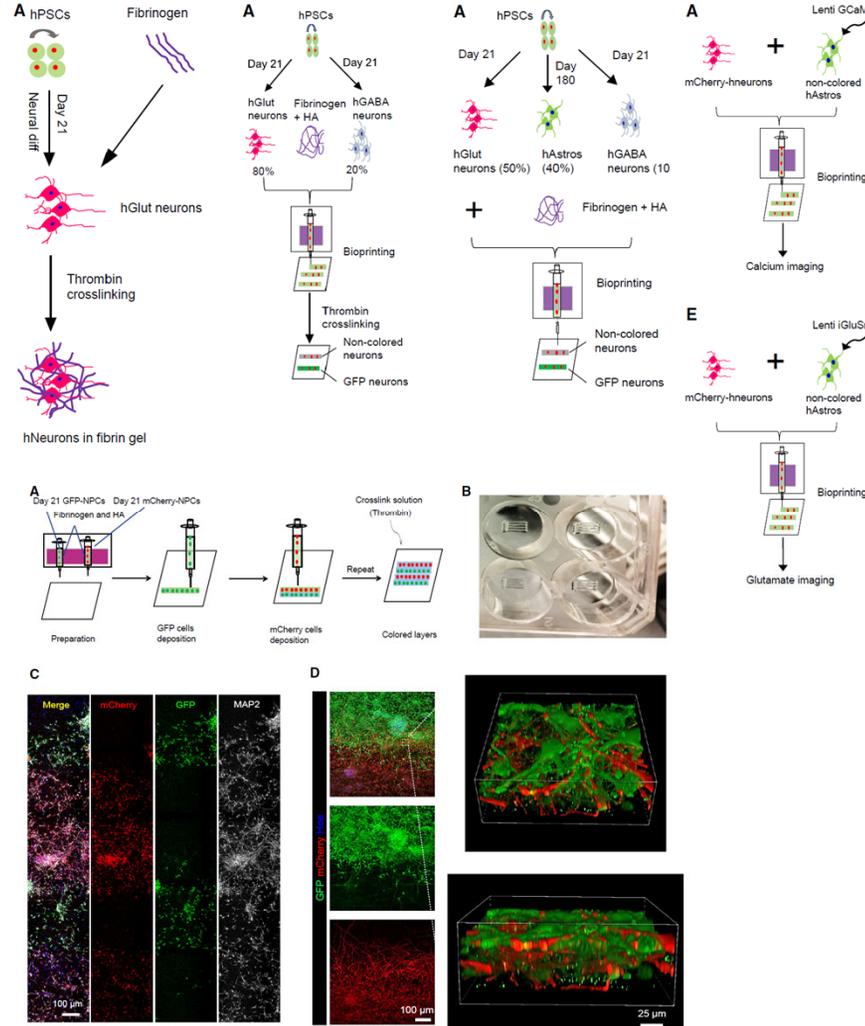
# 3D Bioprinting of Human Neural Tissues



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),

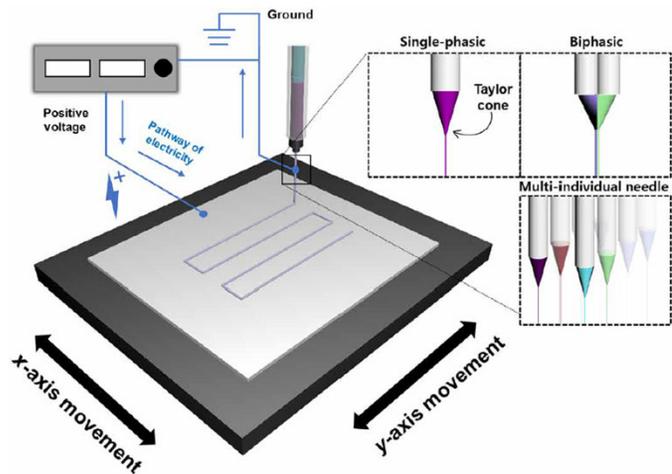
Yan 2024, 3D bioprinting of human neural tissues with functional connectivity



## 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  $\mu\text{m}$  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,<sup>50</sup> 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.

# 3D Jet Writing of Mechanically Actuated Tandem Scaffolds

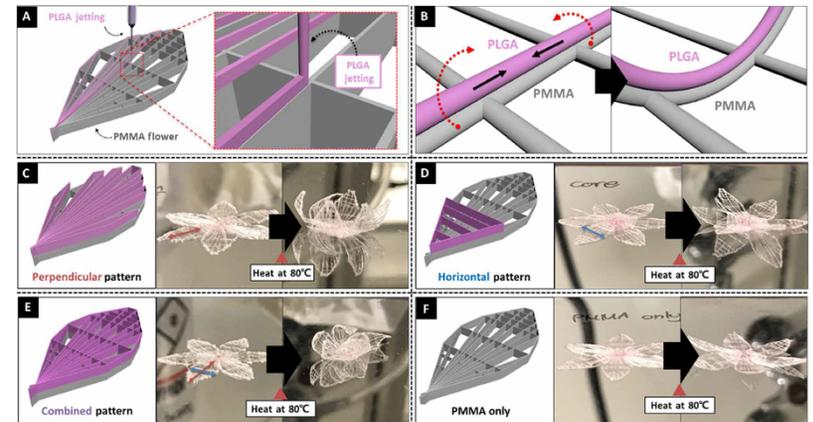
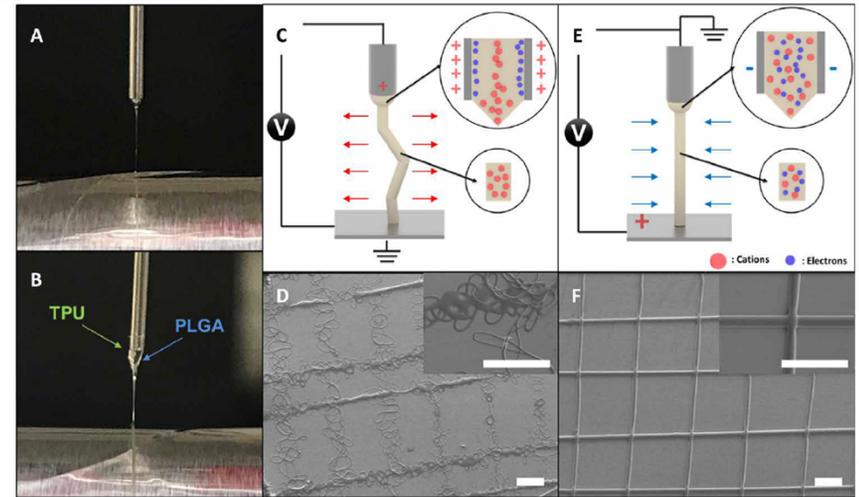


**Fig. 1. Schematic diagram of the CREW system.** The CREW system for fabrication of a fiber-based scaffold using the moving stage. The positive voltages are applied to the conductive collector. The blue lines represent pathway of electricity.

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.**

**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 thermoplastic polyurethane (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  $\mu\text{m}$ .

(CREW: the charge-reversed electro-jet writing)



**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 different thermal expansion of the PLGA and PMMA microfibers at 80°C.

# 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.

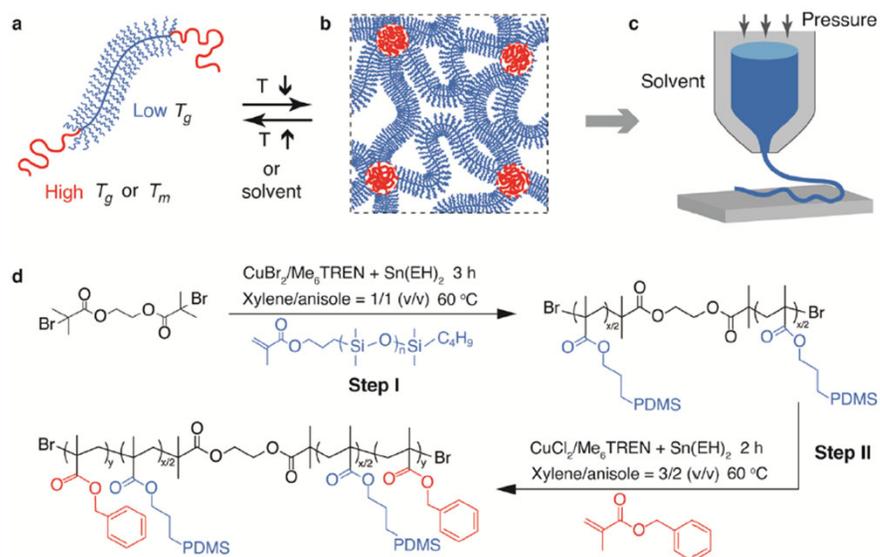


Figure 1. Design concept and synthesis of 3D printable, reversible, ultrasoft, and stretchable elastomers. (a) Schematic of a responsive linear-bottlebrush-linear triblock copolymer. (b) At low temperature, the middle bottlebrush blocks (blue) act as elastic network strands, whereas the high  $T_g$  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 BnMA<sub>y</sub>-b-PDMS<sub>x</sub>-w-b-BnMA<sub>y</sub>, in which  $y$  is the number of repeating BnMA units,  $x$  is the number of PDMS side chains per bottlebrush, and  $w$  represents the MW 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).

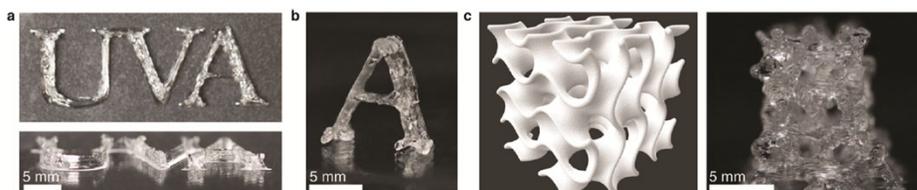


Figure 3. Direct-write printing soft elastomers to create deformable 3D structures. (a) 3D printed UVA initials with a stack thickness of 2 mm. Upper: bird's eye view; lower: side view. (b) Free-standing, 3D printed letter "A". (c) 3D rendering of a cubic gyroid (left) and the corresponding printed product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

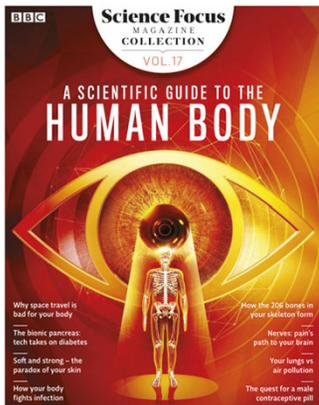
Nian 2021, Three-dimensional printable, extremely soft, stretchable, and reversible elastomers from molecular architecture-directed assembly

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



The 3D-printed 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

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 becomes 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.

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### 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. **SB**

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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) 2019

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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.



2008

## A DEVICE THE SIZE OF AN ESPRESSO MACHINE

quietly whirs to life. The contraption isn't filled with fresh, pungent grounds but, instead, spoonfuls of opaque, sterile goo. Its robotic arm moves briskly: It hovers, lowers, and then repositions a pair of syringes over six petri dishes. In short, rapid-fire bursts, they extrude the milky paste. Soon, three little hexagons form in each dish. After a few minutes, the hexagons grow to honeycomb structures the size of fingernails. No one here is getting a latte anytime soon.



### PART EAR TEAM CORNELL UNIVERSITY

**HOW IT'S MADE** Bioengineers take a 3-D scan of a child's ear, design a seven-part mold in the SolidWorks CAD program, and print the pieces. The mold is injected with a high-density gel made from 250 million bovine cartilage cells and collagen from rat tails (the latter serves as a scaffold). After 15 minutes, the ear is removed and incubated in cell culture for several days. In three months, the cartilage will have propagated enough to replace the collagen.

**BENEFIT** At least one child in 12,500 is born with microtia, a condition characterized by hearing loss due to an underdeveloped or malformed outer ear. Unlike synthetic implants, ears grown from human cells are more likely to be successfully incorporated into the body.

### HOW IT WORKS

## NOVOGEN MMX BIOPRINTER

The first commercial 3-D bioprinter, developed by Organovo, is manufacturing functional liver tissues that will soon help biochemists test new drugs. Here's a look at the printing process.



**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.

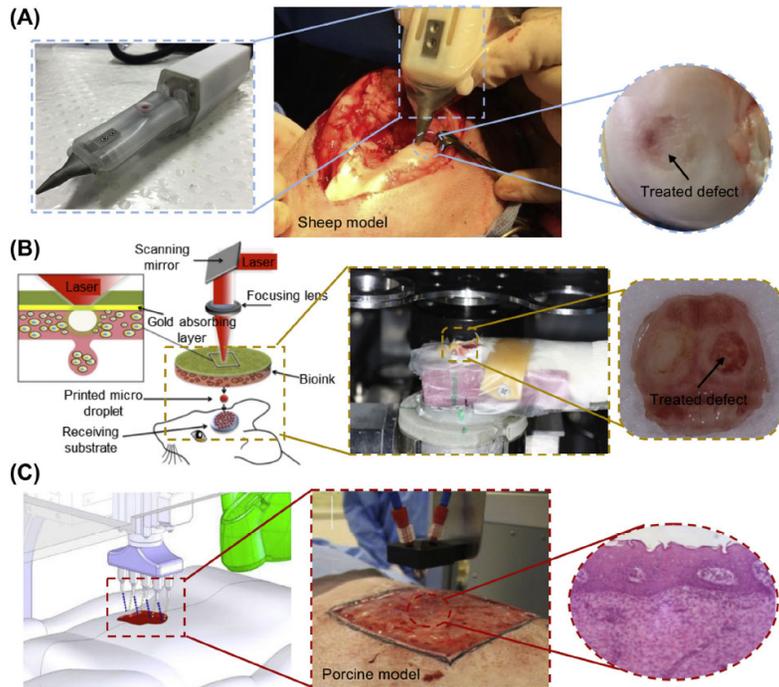


Figure 1. Examples for Intraoperative Bioprinting (IOB). (A) IOB using the biopen for treatment of a full-thickness chondral defect in a sheep. (B) Laser-based IOB for skull repair in a mouse calvarial defect. (C) Droplet-based IOB for skin repair in a porcine model.

Wu 2020, Intraoperative bioprinting

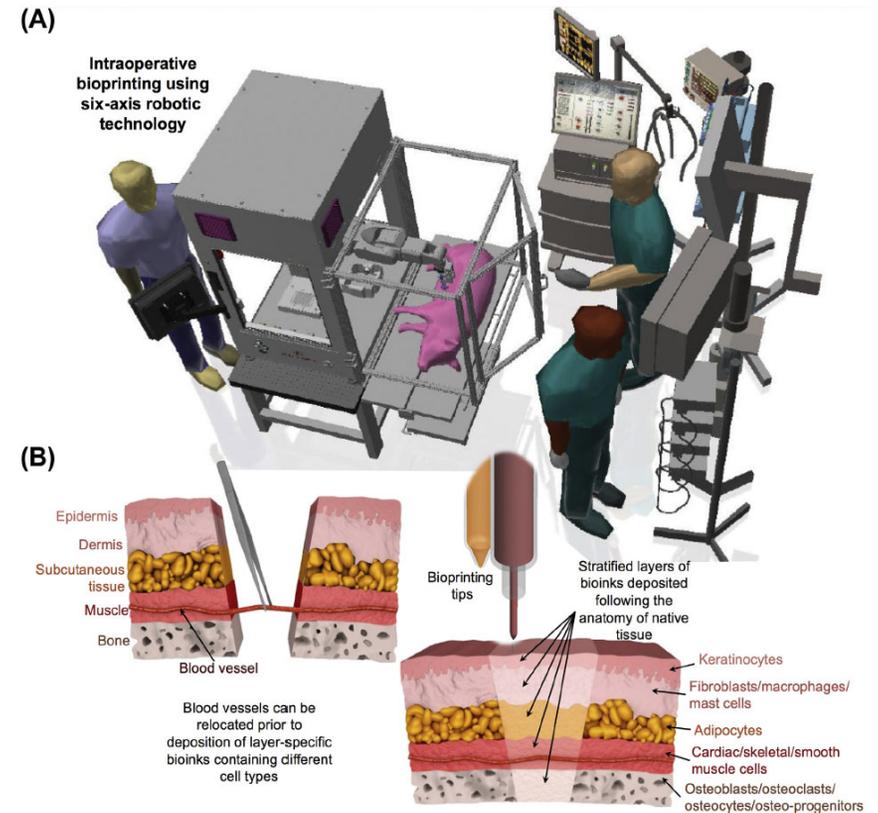


Figure 2. Conceptual schematic diagram of (A) IOB in a surgical setting, and (B) the regeneration of composite tissues in a stratified manner. In the case where blood vessels are retained in host tissue; they can be relocated (left) prior to the deposition of layer-specified bioinks containing different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

# Stimuli-responsive Polymeric Materials

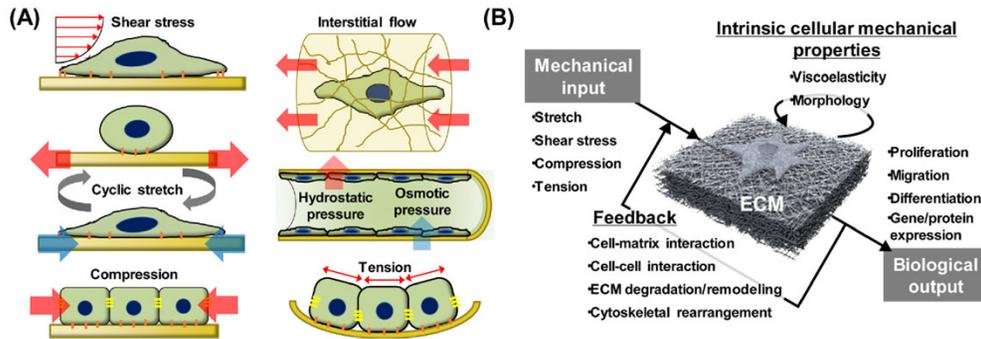


Fig. 1. Mechanical forces in our body and their transduction process into biological output. (A) Mechanical stimuli found at the cell, tissue, and organ level inside the body. (B) Mechanotransduction is the process by which cells convert mechanical inputs into biological responses. Mechanotransduction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

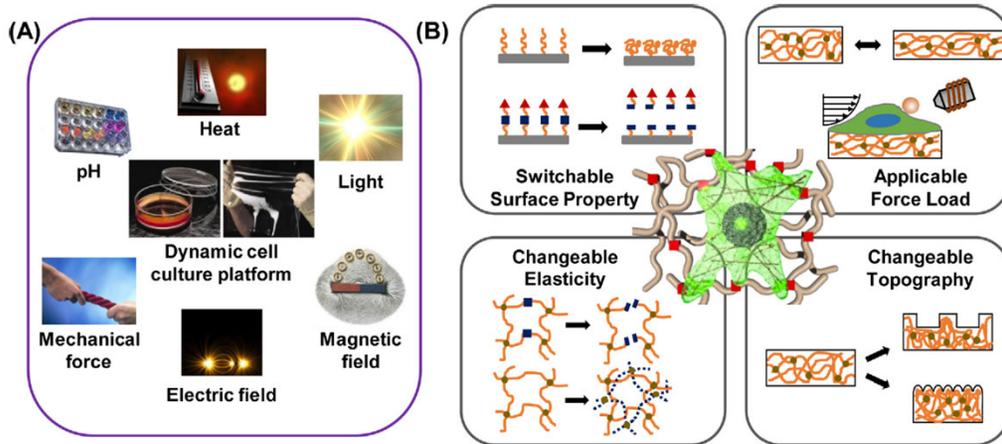


Fig. 3. Stimuli-responsive polymeric materials for dynamic cell culture platforms. (A) Various external stimuli can be utilized for dynamic biomaterials. By modulating chemical, physicochemical and mechano-structural properties, dynamic cell culture platforms can be generated using these materials. (B) 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( <i>N</i> -isopropylacrylamide) (PNIPAAm)		Temperature responsive ability Reversibly changeable hydrophilicity/hydrophobicity or surface energy Functionalizable	Adhesion-detachment manipulation Cell sheet engineering
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 Modifiable	Tissue engineering 3D and 4D tissue culture
Poly( $\epsilon$ -caprolactone) (PCL)		Biocompatibility and biodegradability Semicrystalline nature shape-memory property	Tissue engineering Dynamic cell manipulation (orientation, movement)
		Changeable shape and stiffness	

# 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.

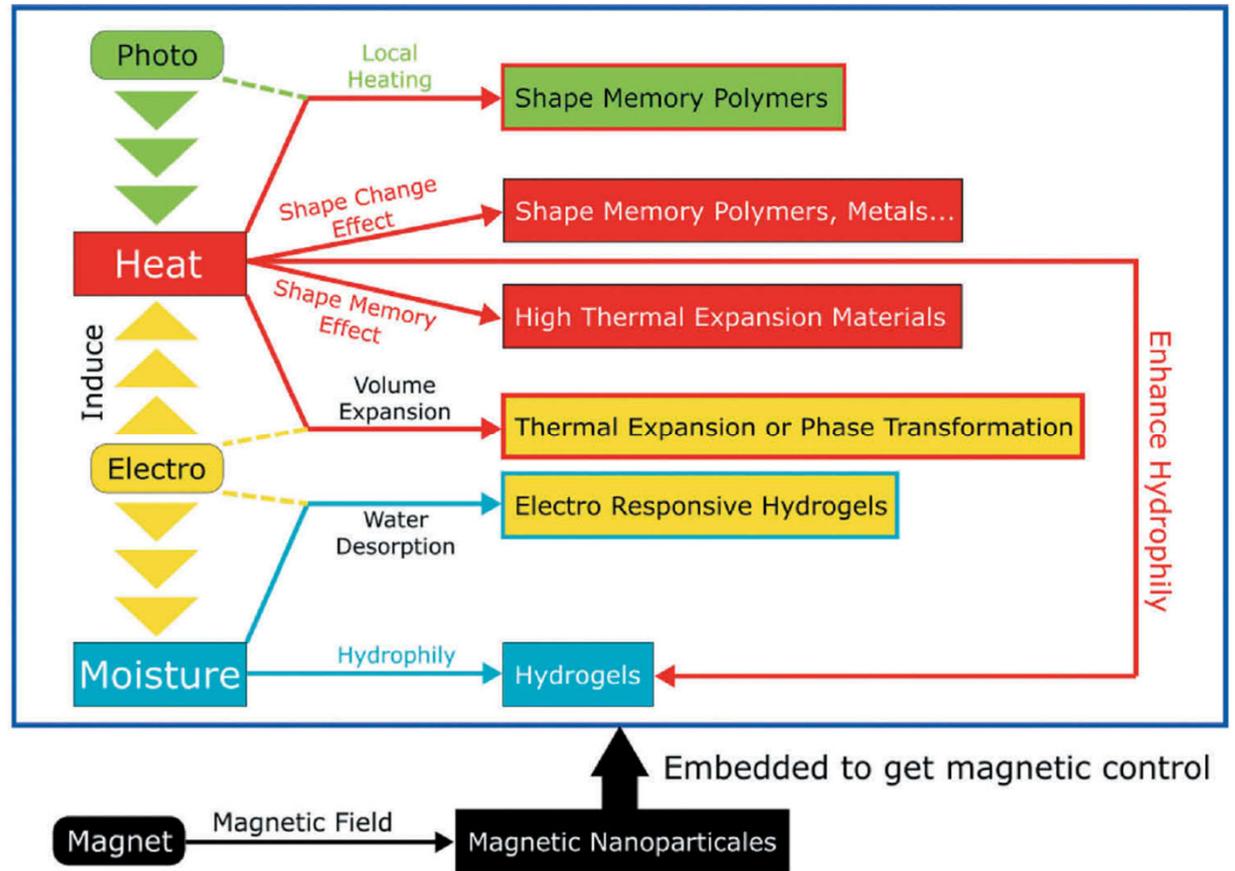
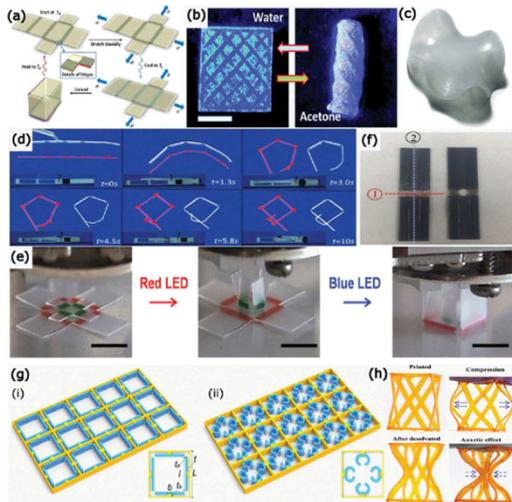


Figure 2. Classes of smart materials that can respond to different types of stimulus including heat, moisture, light, electricity, and magnetic fields.

# 4D-Bioprinting Technologies

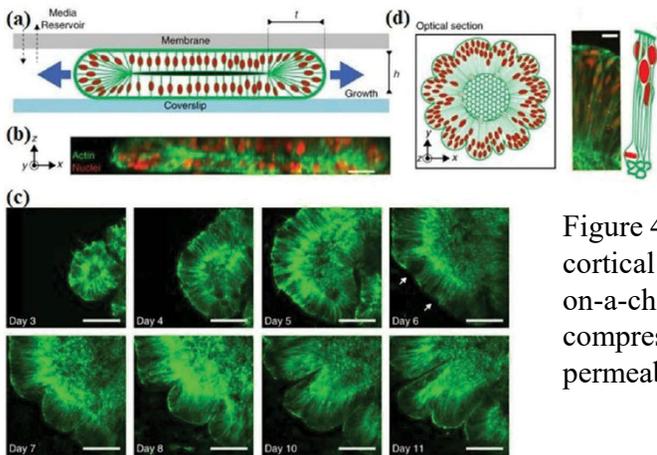
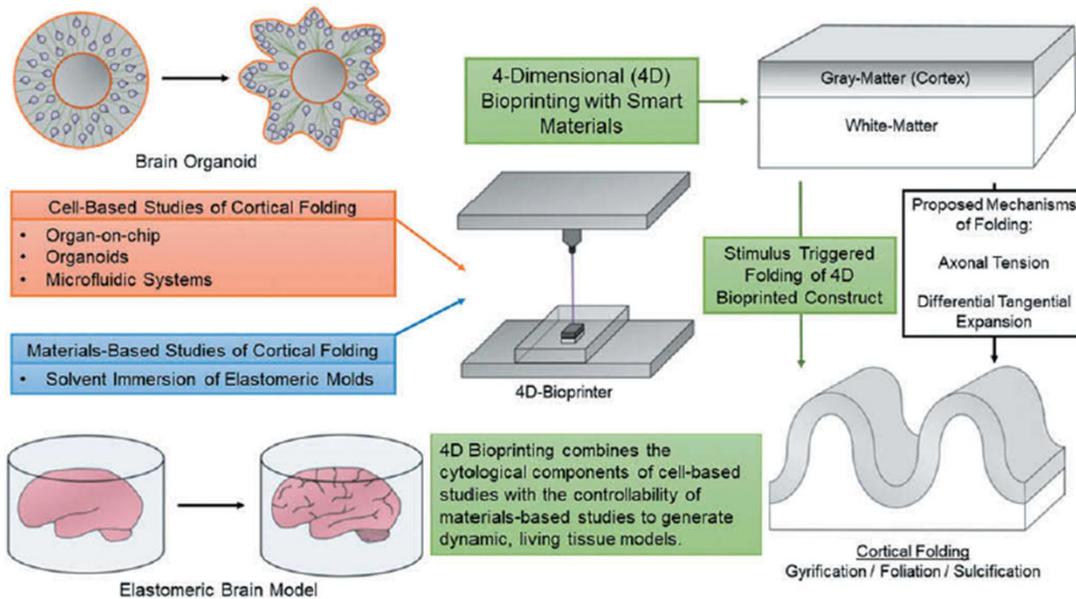


Figure 4. Brain organoid-on-a-chip model of cortical folding. (a) Schematic of neural organoid-on-a-chip system where the organoid is compressed between a coverslip and a media permeable membrane.

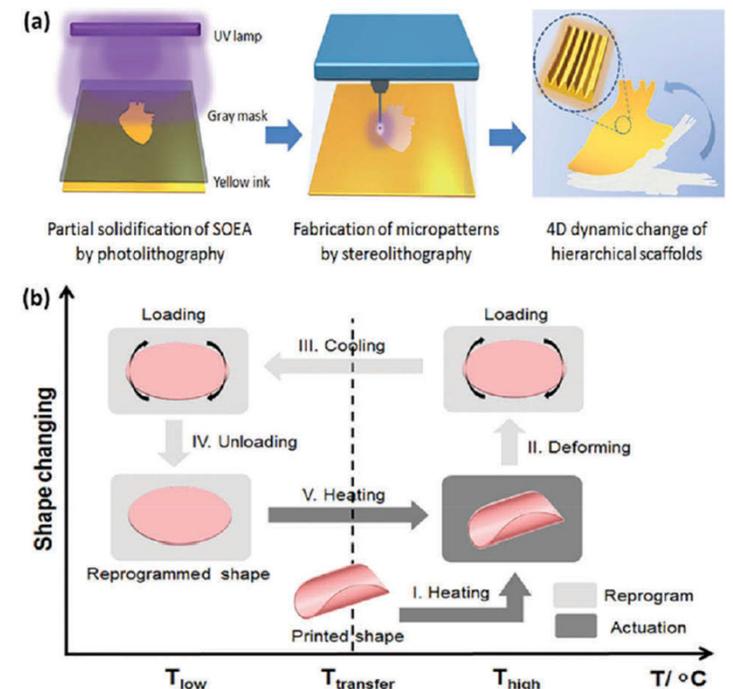


Figure 5. A 4D printed thermally sensitive natural soybean oil epoxidized acrylate (SOEA) constructs developed in our lab. (a) A tandem photolithography-stereolithography process to fabricate heart-shaped constructs from novel soybean oil epoxidized acrylate. (b) Schematic illustration of the 4D shape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

# 4D-Bioprinting Technologies

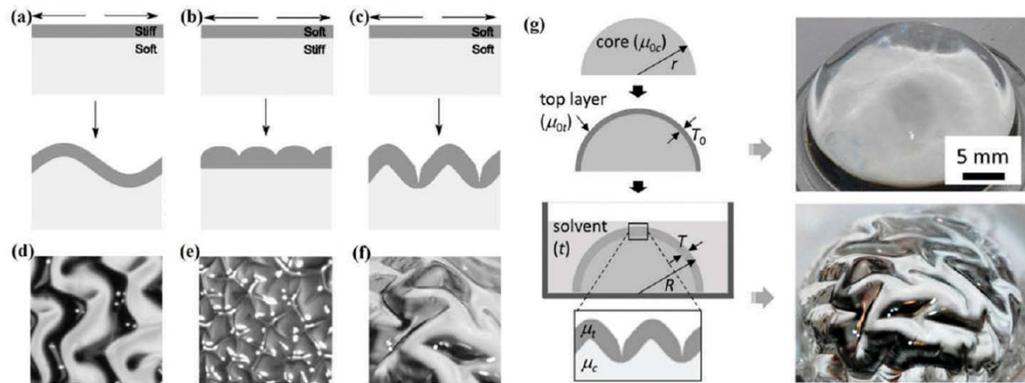


Figure 2. Surface swellability leads to different cortical folding patterns. (a) Sinusoidal folding occurs when the upper layer (gray matter) is **stiffer than the lower layer** (white matter) of a growing bi-layer cortical model. (b) Cupped (**cusped?**) folding occurs when the upper layer is **softer** 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, cupped, 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 ( $\mu_{0c}$ ). **The hemispheric core is coated in a thin layer of polymer with a thickness ( $T_0$ )** which exhibits a shear modulus of ( $\mu_{0t}$ ). The top and core (or upper and lower) layers have a combined radius ( $R$ ). The completed model is then **submerged** 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.

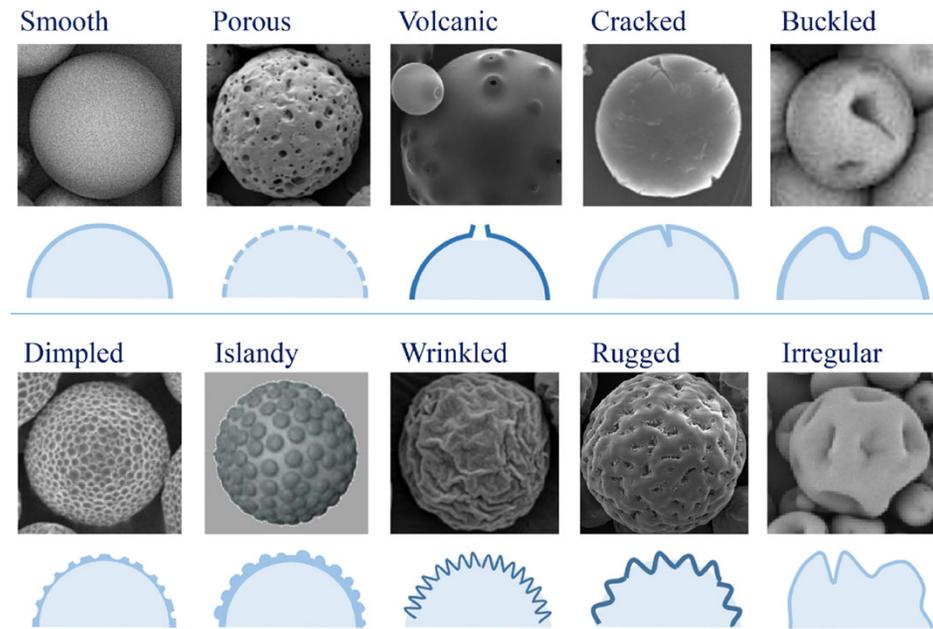


Fig. 4. Examples of different types of surface morphologies observed on microparticles depending on the formulation and/or processing parameters.

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# **Cell Encapsulation & Cell Therapy**

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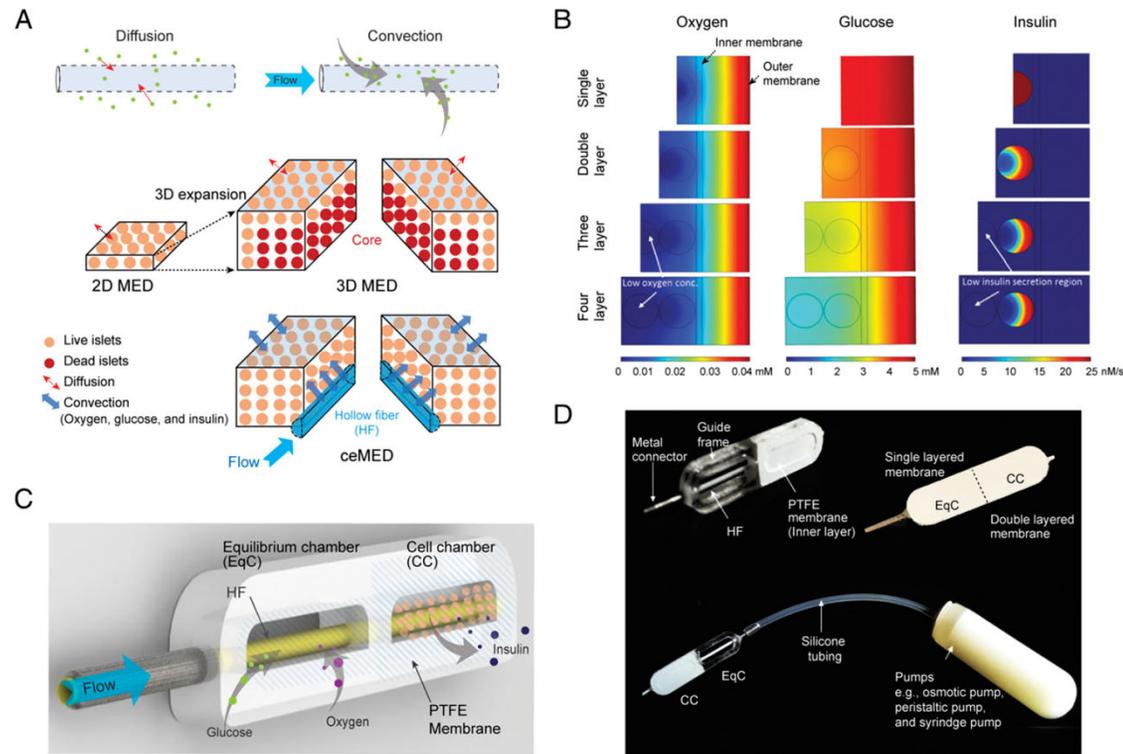


# Cell Macroencapsulation

Type 1 diabetes (T1D) is characterized by the autoimmune destruction of pancreatic  $\beta$  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  $\beta$  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  $\beta$  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,  $\beta$  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  $\beta$  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 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 from 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

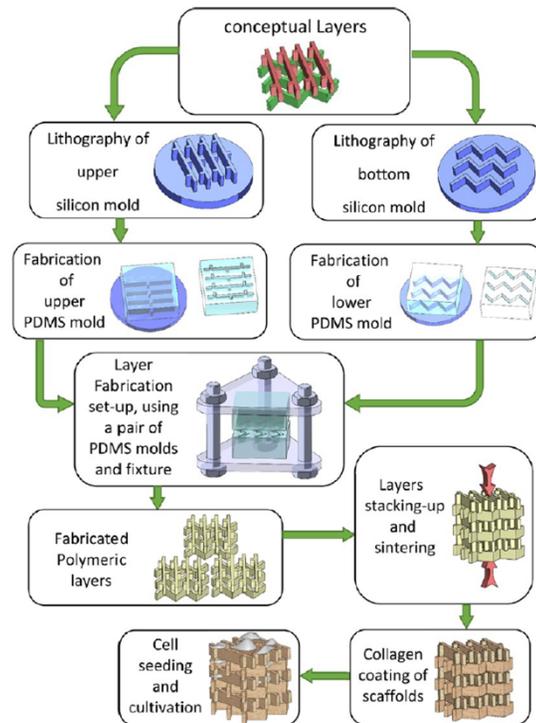


Figure 4. Manufacturing process chart from design to cell cultivation.

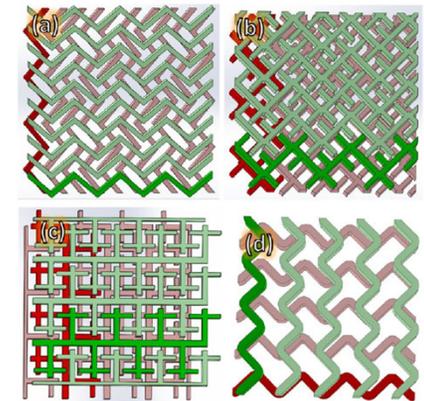
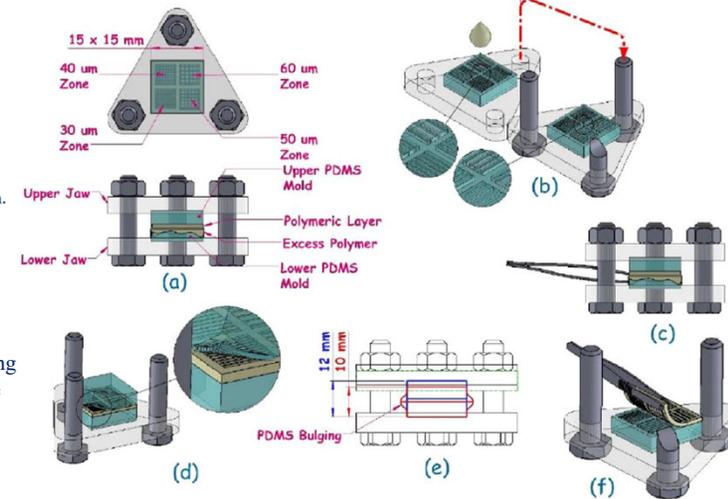
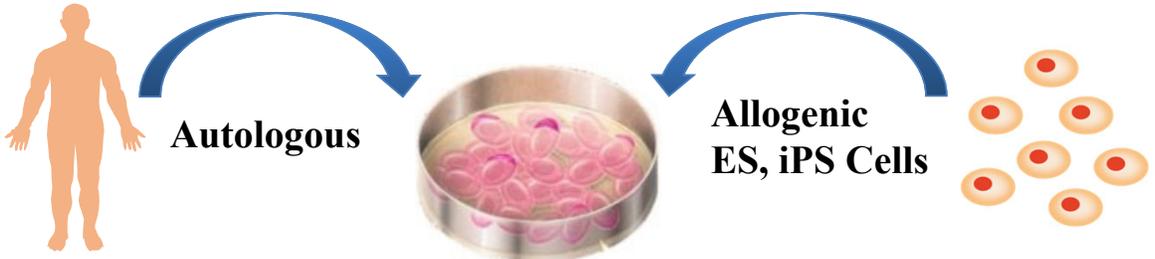


Figure 6. Schematic of the designed scaffold structure with special fiber forms and overall dimension of 20 × 20 mm. Green fibers represent the top level woofs, and the red one represent warps of one polymeric layer. The highlighted woof and warp in each picture can help to understand the pattern design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

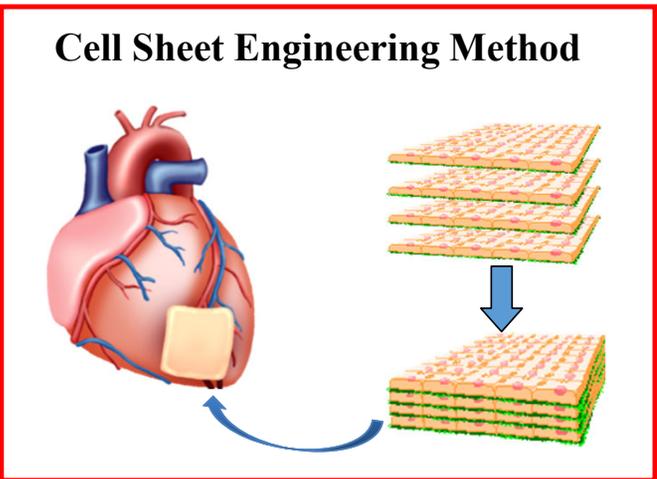
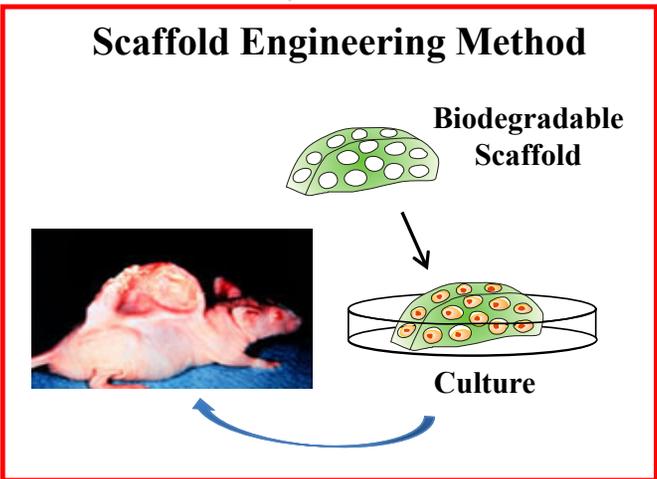
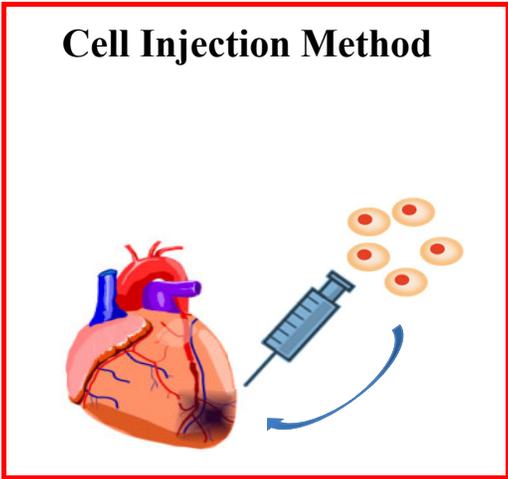


# Cell Therapy / Tissue Engineering Therapy



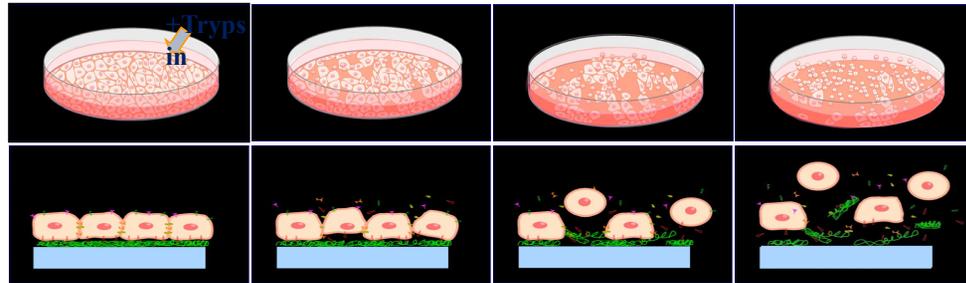
Cell Therapy

Tissue Engineering Therapy

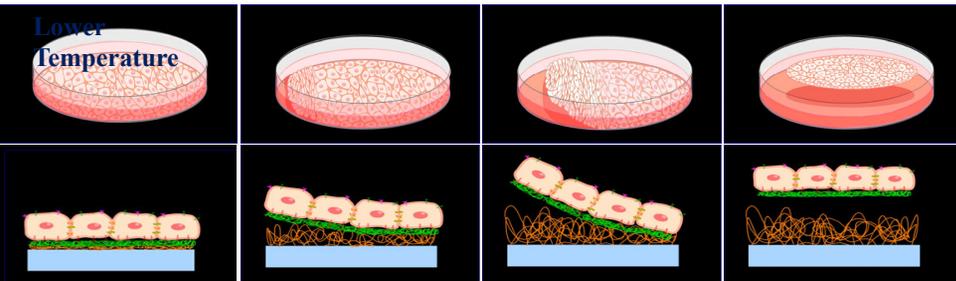


# Cell Sheet Tissue Engineering

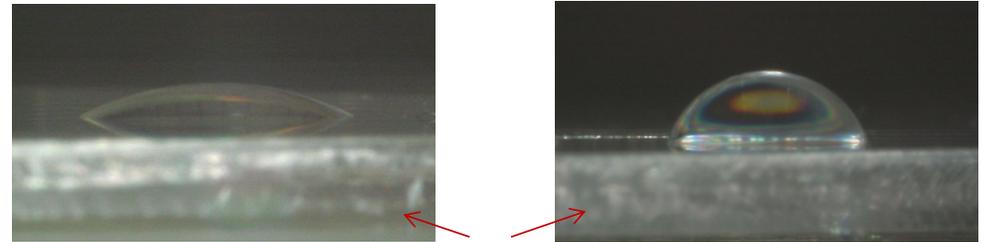
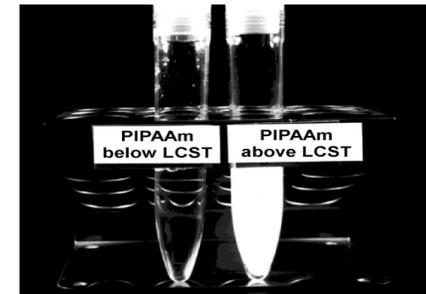
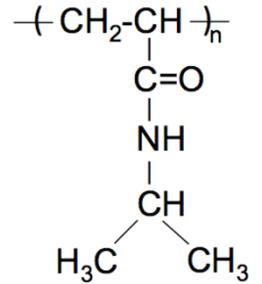
## Conventional Cell Harvest with Proteolytic Enzymes



## Non-Invasive Cell Sheet Harvest by Reducing Temperature

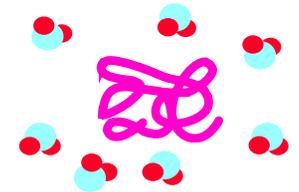
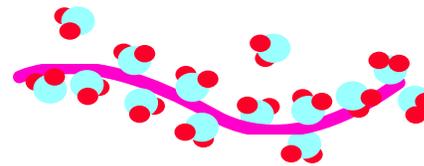


Poly(N-isopropylacrylamide)  
LCST  $\sim 32^\circ\text{C}$



Hydrophilic surface at  $< 32^\circ\text{C}$

Hydrophobic surface at  $< 32^\circ\text{C}$



# Temperature-Responsive Polymer-Grafted Surfaces

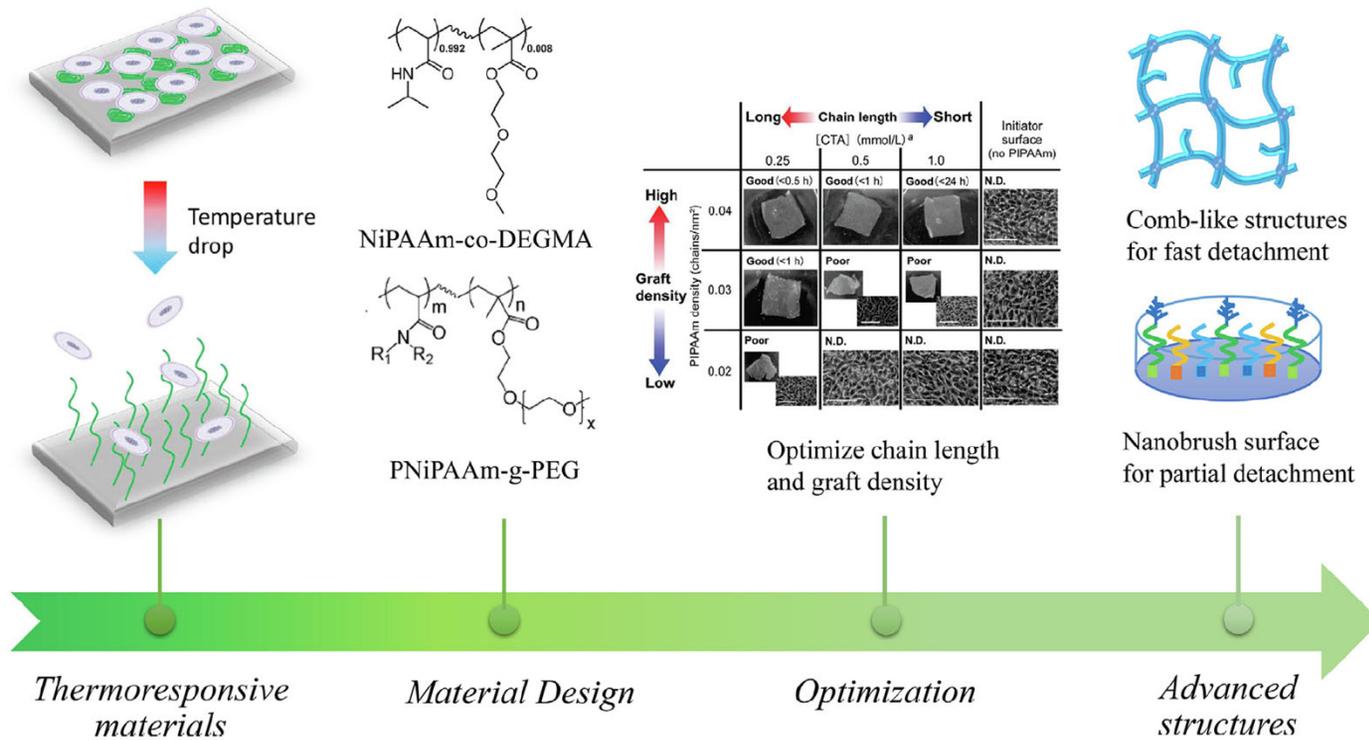


Fig. 3. Thermoresponsive materials for harvesting cells without cell damage. Thermoresponsive materials release cells when temperature is reduced. These materials are designed using copolymers to tune the LCST or other physical properties.

# Vascularization of Tissue-Engineered Skeletal Muscle Constructs

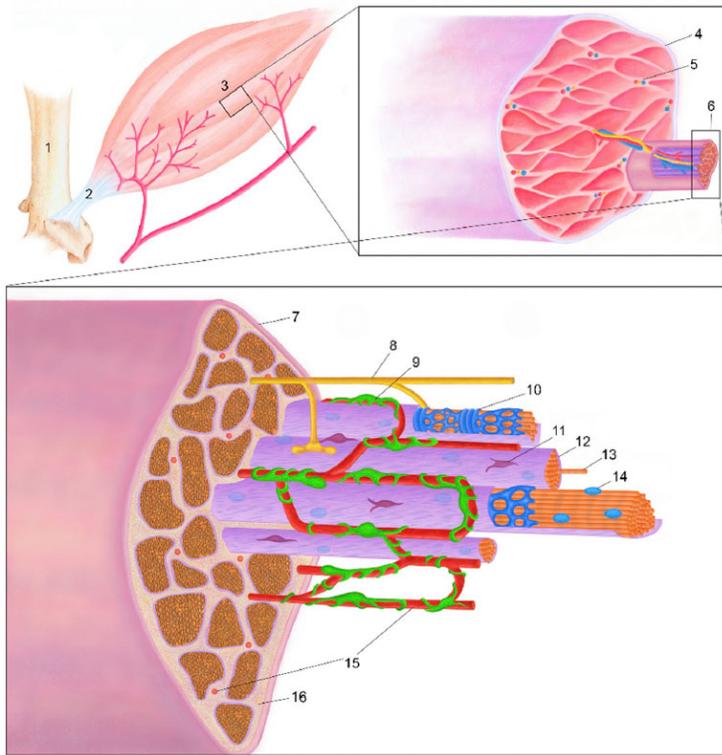


Fig. 1. Skeletal muscle structure. 1. Bone, 2. Tendon, 3. Muscle, 4. Epimysium, 5. Artery (red), vein (blue), nerve (yellow), 6. Fascicle, 7. Perimysium, 8. Nerve, 9. Pericyte, 10. Sarcoplasmic reticulum, 11. Satellite cell, 12. Sarcolemma, 13. Myofibril, 14. Muscle cell nucleus, 15. Capillary, 16. Endomysium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

Gholobova 2020, Vascularization of tissue-engineered skeletal muscle constructs

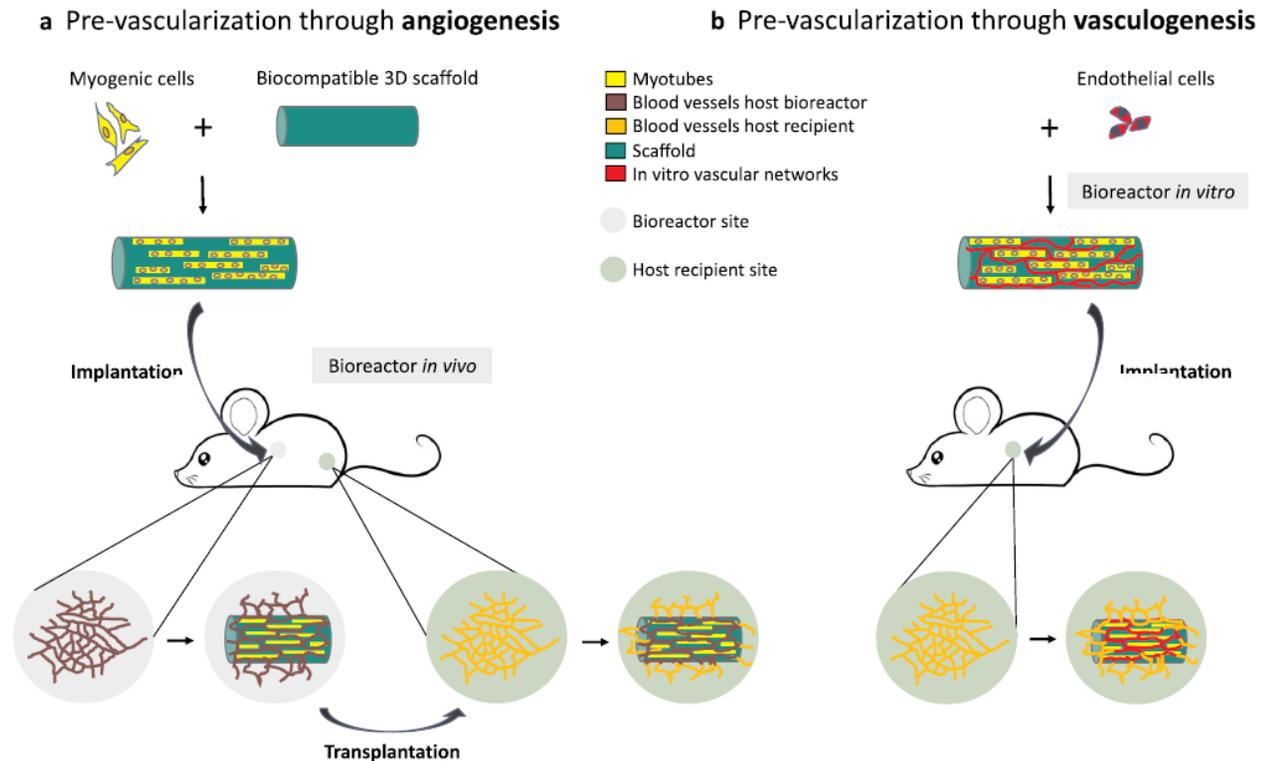


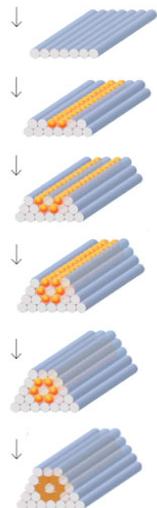
Fig. 2. Pre-vascularization strategies of tissue-engineered constructs. (a) In the pre-vascularization through angiogenesis, a construct with target cells (yellow) is implanted in a bioreactor site *in vivo* allowing for angiogenesis. Through angiogenesis and ingrowth of host vessels (dark red) the construct 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.

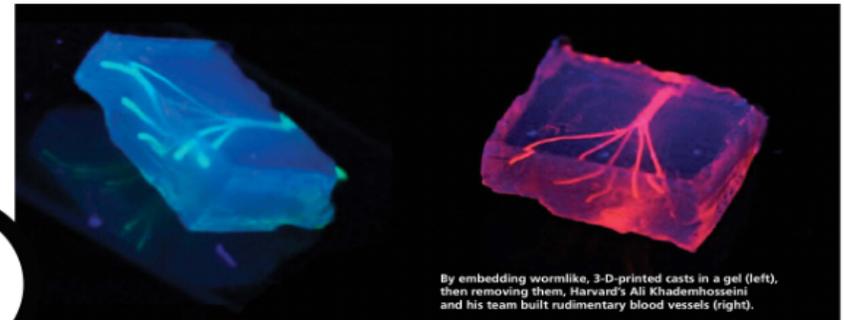


**The difficulty of providing a blood supply has always limited the size of engineered tissues.**

## 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.

67



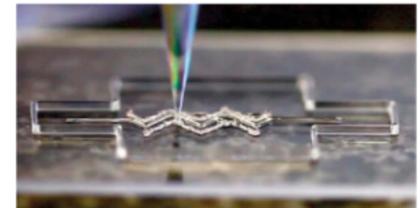
By embedding wormlike, 3-D-printed casts in a gel (left), then removing them, Harvard's Ali Khademhosseini and his team built rudimentary blood vessels (right).

## Blood Vessels Via Printer

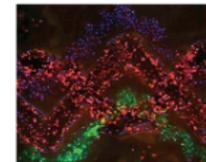
➔ Could tomorrow's surgeons create customized replacement tissue for patients just by hitting print? Two teams of Harvard bioengineers made big strides toward that goal in 2014, reporting two new 3-D-printing methods that help construct rudimentary blood vessels.

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.

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



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.



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

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.

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

# How to Grow Blood Vessels

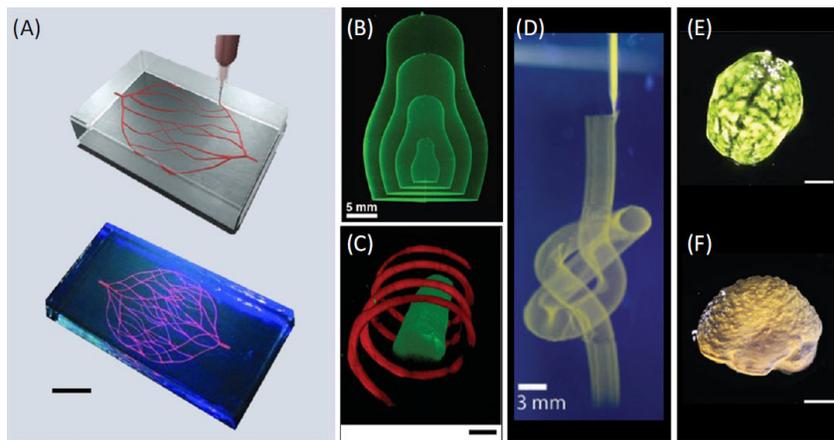


Figure 2. Advancement of Omnidirectional 3D Printing: From Yield Stress Fluids to Suspension Media. (A) Omnidirectional printing in a non-self-healing yield stress fluid. (Top) Schematic for 3D-printed vascular network using a removable ink. (Bottom) A fluorescence image of a 3D microvascular network 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  $\mu\text{m}$ . (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

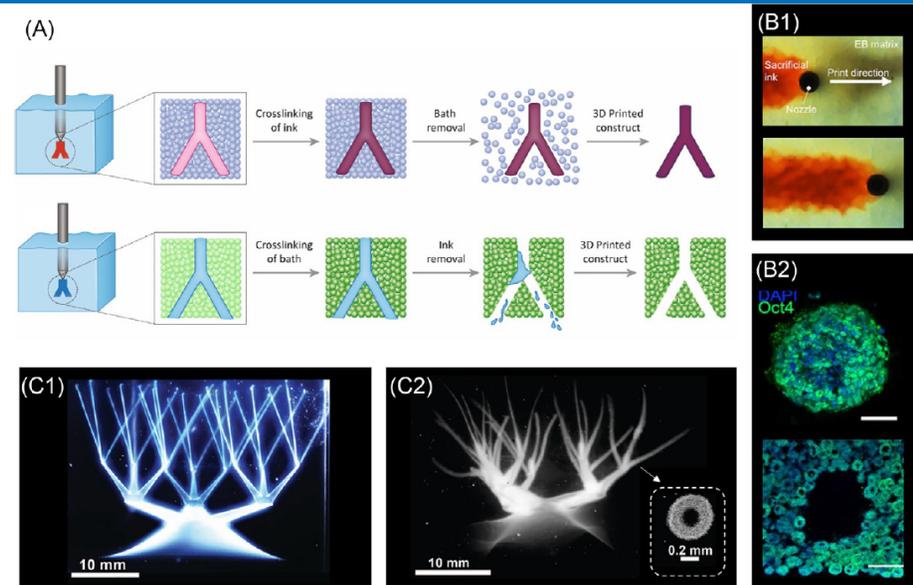


Figure 3. Suspension Media Used as a Strategy to Aid Better Biomimicry in the 3D-Bioprinting Field. (A) Overview of the 3D-printing pathways that can be followed when printing in a suspension medium. (Top) Pathway is defined by removal of the medium after printing. Suspension medium provides mechanical 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  $\mu\text{m}$ . Row 2: Cross-section of channel printed in an embryoid body suspension medium following removal of the sacrificial printed ink. Scale bar, 500  $\mu\text{m}$  [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 **Brainware-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 “Brainware”) was quickly trained to accurately recognize speech patterns, as well as perform certain complex math predictions. Researchers treated their Brainware 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 Brainware’s part, they do provide enough computational power for some interesting results.

To test out Brainware’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 Brainware 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, Brainware 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, Brainware’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/brainware-brain-organoid-chip/>

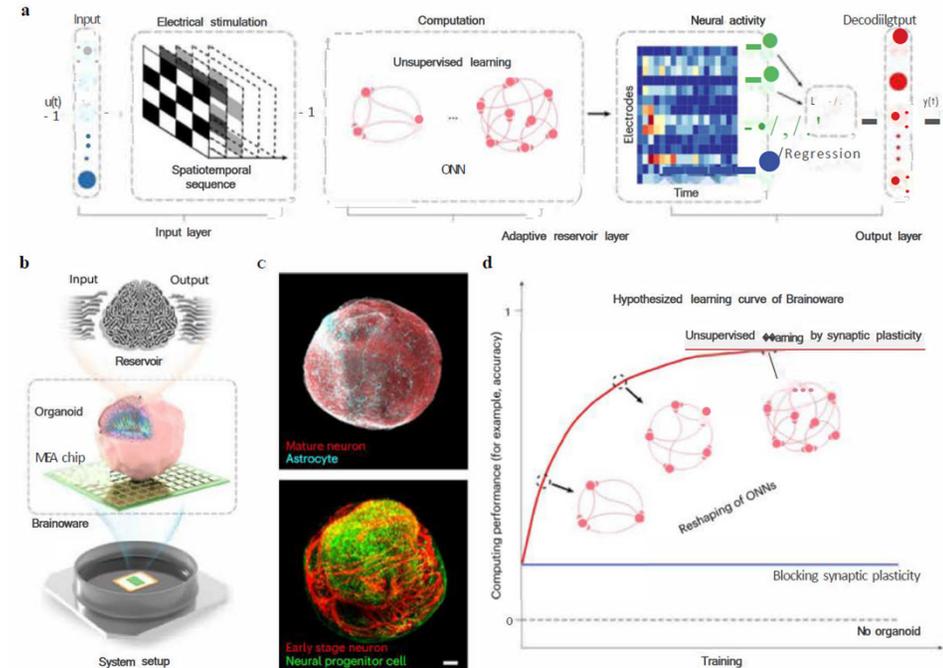


Fig. 1. Brainware with unsupervised learning for AI computing. a, Schematic of an adaptive reservoir computing framework using Brainware. b, Schematic of the paradigm of Brainware setup that mounts a single brain organoid onto a high-density MEA for receiving inputs and sending outputs. c, Whole-mount 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, Tuj1; neural progenitor cells, SOX2). d, Schematic demonstrating the hypothesized, unsupervised learning of Brainware by reshaping the BNN during training, and the inhibition of unsupervised learning after synaptic plasticity is blocked. Scale bar, 100  $\mu\text{m}$ .

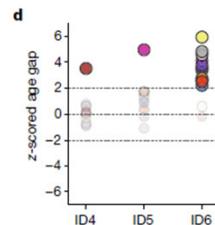
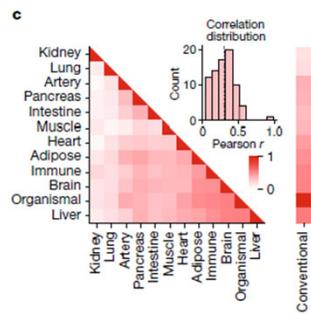
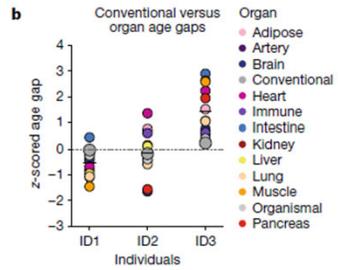
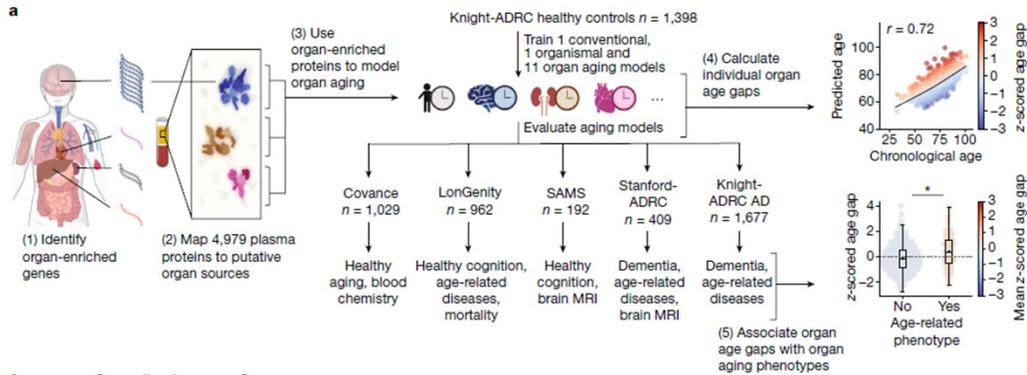
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# **Tissue Regeneration**

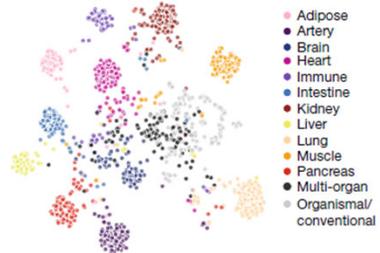
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# Organ Aging

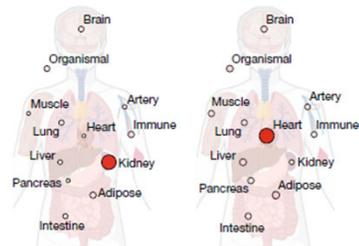
Oh 2023, Organ aging signatures in the plasma proteome track health and disease



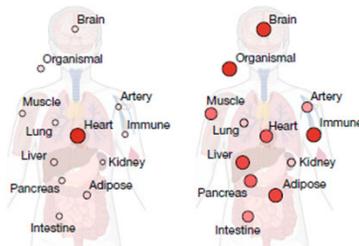
**e** All extreme organ agers ( $n = 23\%$ )



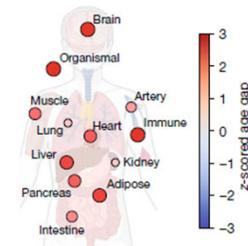
Kidney agers ( $n = 2.01\%$ )



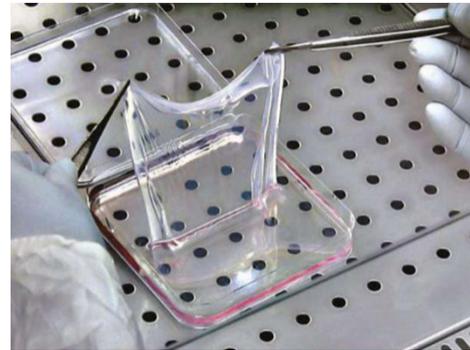
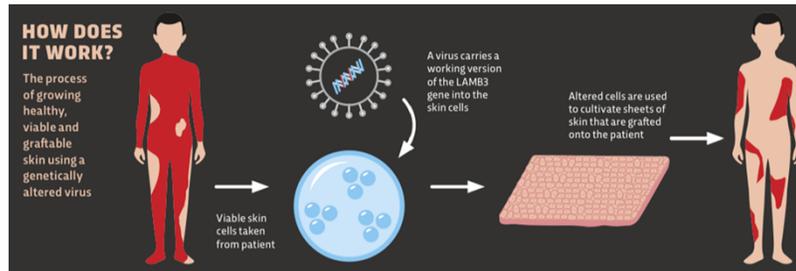
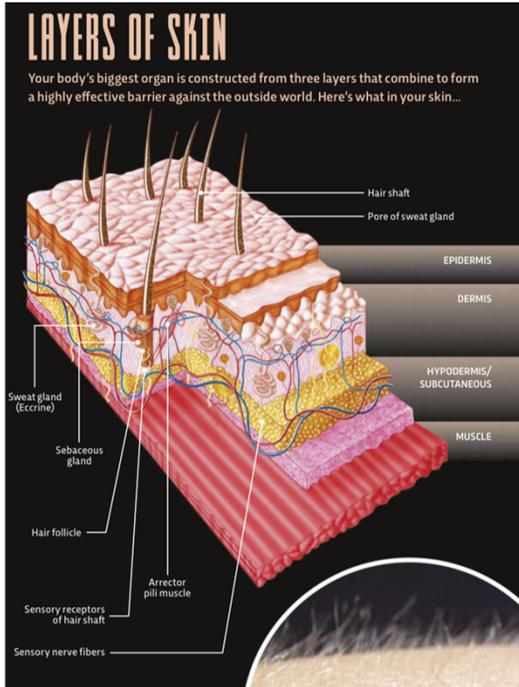
Heart agers ( $n = 2.04\%$ )



Multi-organ agers ( $n = 1.71\%$ )



# Fixing Broken Skin



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

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 after 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

The process of growing healthy, viable and graftable skin using a genetically altered virus.

A virus carries a working version of the LAMB3 gene into the skin cells.

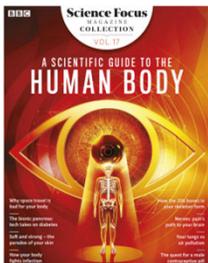
Altered cells are used to cultivate sheets of skin that are grafted onto the patient.

Viable skin cells taken from patient.

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 effective at treating autoimmune and inflammatory 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 off the epidermis of his hand, causing immense pain and breaking the all-important barrier, letting water out and microbes in.



# Immortal Man by Year 2045

# Biomask Regeneration System



September 2014

## THE WOUNDED SOLDIER BIOMASK REGENERATION SYSTEM

This prototype of a mask that both heals and reduces scarring is central to a facial reconstruction effort at the U.S. Army Institute of Surgical Research in San Antonio.

**STEP 1** Patient with severe facial burns and wounds is evaluated for treatment.

**STEP 2** Thorough preparation of the wounded area, including cleaning and the removal of dead tissue.

**STEP 3** Patient's head is sealed inside a standard-size polyurethane Biomask. Antibiotics, analgesics and anti-inflammatories are pumped in for the first 24 hours, to prevent infection and reduce pain. The mask is then connected to a negative-pressure wound therapy (NPWT) device.

Single-layer polyurethane mask

Embossed inner microtexture creates tiny channels (shown above in detail) that help guide fluids in or out

A Ziploc-style seal could replace the current adhesive rim

Container collects drainage

NPWT device

Depending on stage of treatment, connecting tubes can plug in to different devices, to either:

- Apply atmospheric pressure
- Drain wound
- Deliver therapeutic agents

Negative-pressure wound therapy (NPWT) is also known as vacuum-assisted closure. It speeds up the healing process by:

- stimulating blood flow and new blood vessel growth
- biomechanically stimulating cells, encouraging them to divide and proliferate
- removing factors that might inhibit healing, such as bacteria

Placement of tracheostomy (neck) and gastrostomy (stomach) tubes is required to allow breathing and feeding while mask is fully sealed

Conventional NPWT treatment requires a sponge and a layer of special foam, while Biomask interfaces directly with wound area

Biomask provides protective layer during healing

Day 0: Grafted skin or skin substitute, Blood vessels

Day 6: Cell infiltration, Ingrowing blood vessels

Day 10-14: Healing

Epidermis, Dermis, Hypodermis (subcutaneous fatty tissue), Muscle

**STEP 4** Negative pressure is applied for 48 to 72 hours, until skin can be grafted onto wound area.

**STEP 5** Mask is removed to apply skin graft or artificial skin, and then placed again, this time for seven to 14 days. The shell serves as protection and holds the graft in place, while low negative pressure stimulates cells and blood vessels to help the graft firmly take.

# Recipes for Limb Renewal

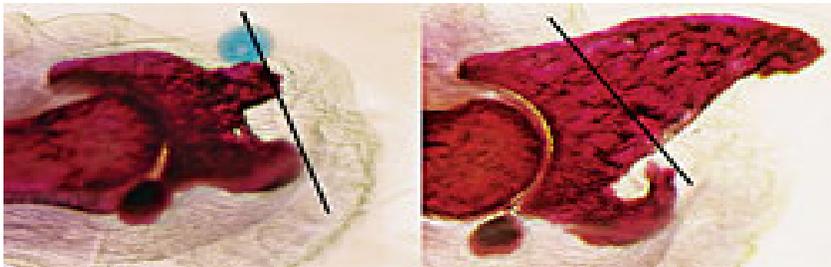
Salamanders and other creatures that regrow lost body parts provide clues for ways to regenerate human limbs (Sophie L. Rovner)



**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.

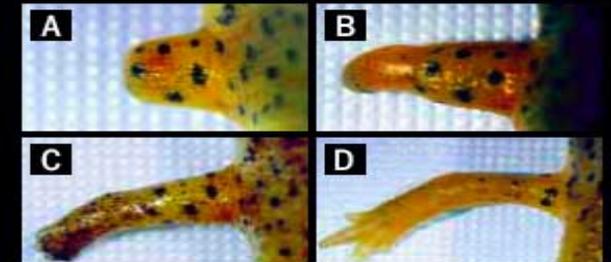


**REGENERATION FERTILIZER** A mouse can regrow an amputated digit tip, but if enough of the digit is removed—as shown in the image at left, in which the black bar indicates the line of amputation—the digit won't grow back. However, Muneoka found that treating such an amputation site with bone morphogenetic 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>

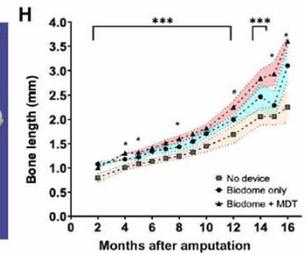
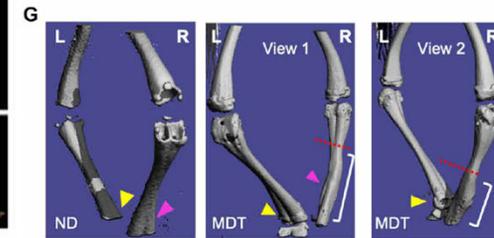
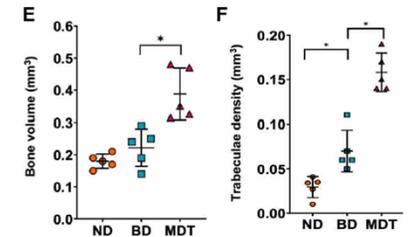
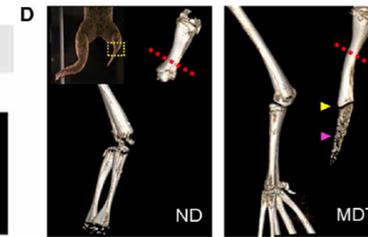
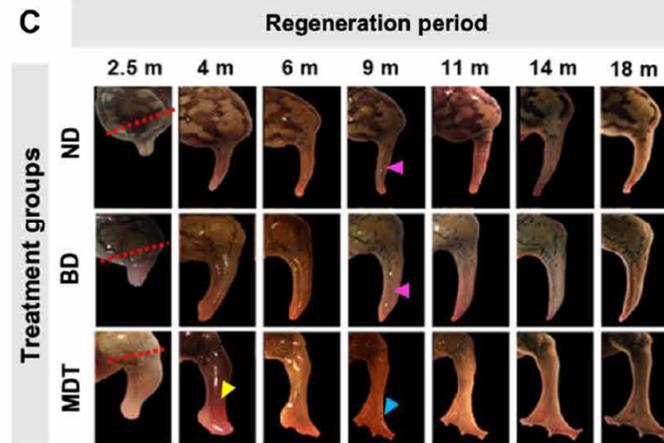
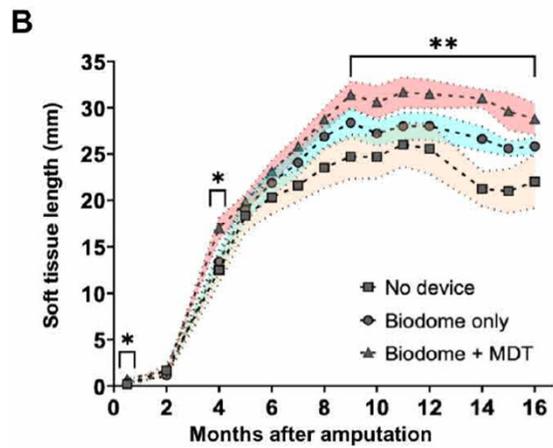
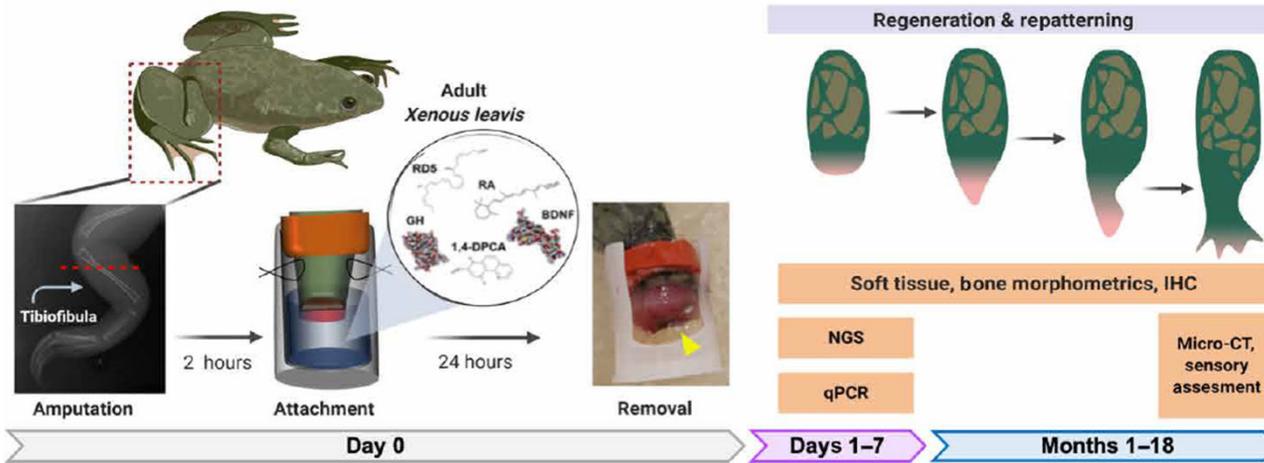
## Newt Knowhow

Salamanders can regrow legs, tails, jaws, spines—even parts of their eyes



Over 10 weeks, cells at an amputation site revert to a biological blank slate, with the potential to become skin, bone, or cartilage. Then they proliferate, form a cone, specialize, and grow into a limb.

# 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 laevis*

## 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.

**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



## Problems of Extra Blood Vessels

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



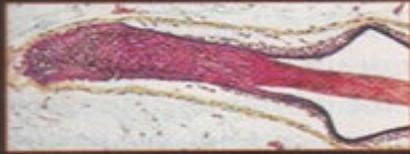
**OBESITY**  
Fat requires miles of blood vessels, which could be trimmed by angiogenesis inhibitors



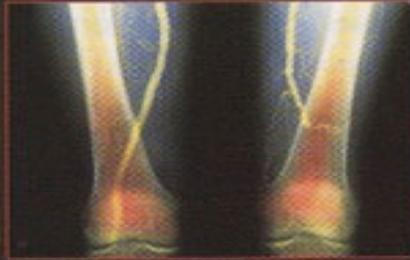
Proangiogenic agents can stimulate vessel development to treat certain diseases.

## Benefit of Extra Blood Vessel

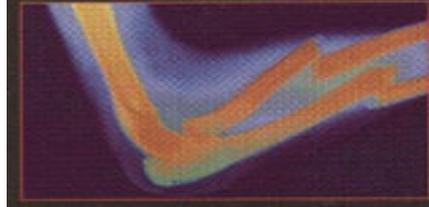
**BALDNESS**  
Hair follicles depend on a good blood supply



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



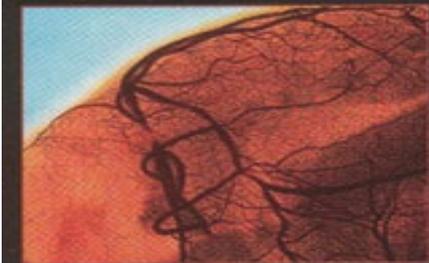
**LIMB FRACTURES**  
New blood vessels could help repair broken bones



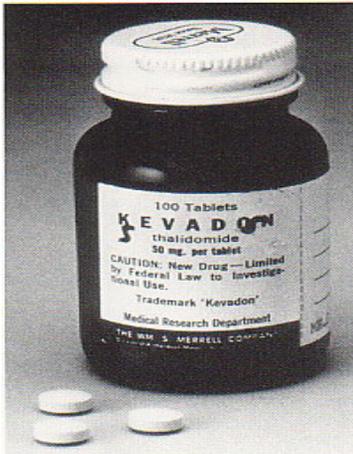
**NEURODEGENERATIVE ILLS**  
An increased blood supply could minimize neuronal damage in the brain



**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.

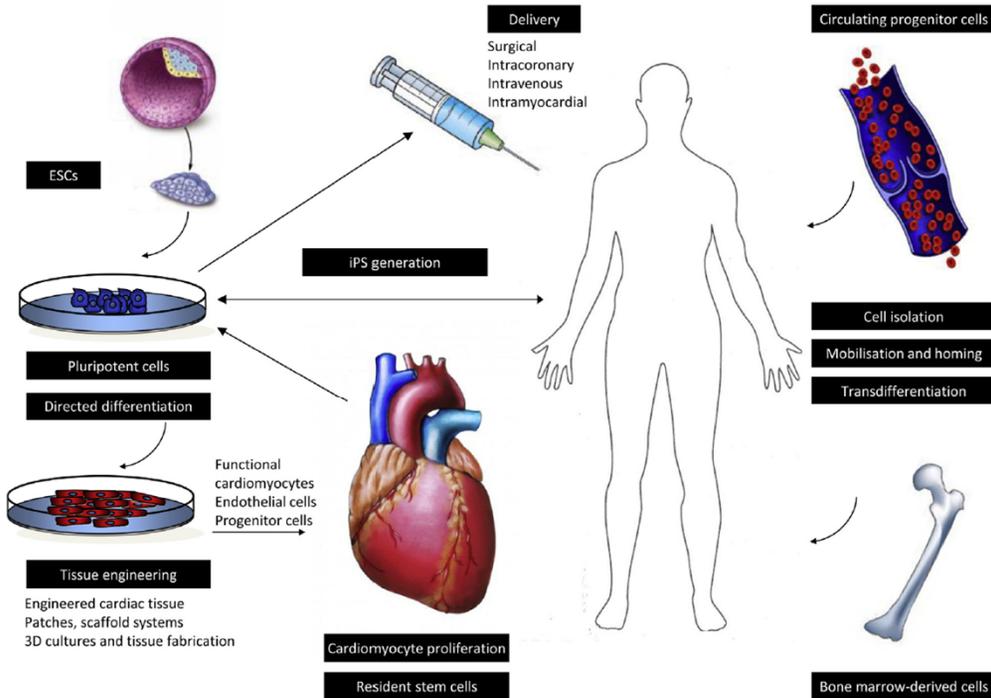


**Thalidomide's horrifying effects on newborns became known in 1962. Distribution of two million tablets by Merrell for investigational use.**



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

# Stem Cells



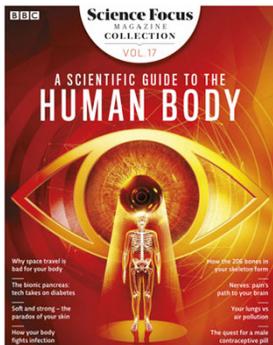
**Figure 1** Experimental approaches to cell therapy in heart failure. Clinical trials have been performed with bone marrow-derived cells, circulating progenitor cells and skeletal myoblasts that have been delivered to the heart. Conceptually it would be also possible to achieve by purifying cells of 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 neovascularization 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 of 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

# 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/2GfCkCk](http://bbc.in/2GfCkCk)

## 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

**H** eart 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

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**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 RIGHT:** Ultra sound scans are used to check on the heart beat of babies as they develop in the womb  
**MIDDLE:** Atherosclerosis, the accumulation of fatty plaque in your arteries, is a common cause of heart attacks and strokes

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# Stem Cells

Research on stem 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.



## Voices of Innovation

### Stem Cell Sleuth

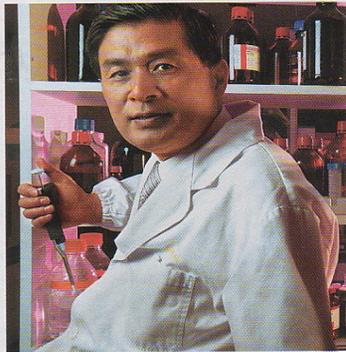
**HE'S NOT A POLITICIAN,** a tycoon, or a pop star. But these days, Hwang Woo Suk may enjoy more popularity and respect in South Korea than the hottest celebrity. He is a pioneer of embryonic stem cell research—and a national hero. The government even issued a postage stamp in his honor in February that 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



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

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.

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 Hwang  
[BusinessWeek online](http://www.businessweek.com/extra) For an interview with Hwang Woo Suk, go to [www.businessweek.com/extra](http://www.businessweek.com/extra)

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."



**FIGHTING FOR LIFE** Shares in Lanza's ACT have stalled

## 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

**O**N 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 comm-

# Embryonic Stem Cells

## The Origins and Fates of Embryonic Stem Cells

Embryonic stem (ES) cells are derived from the portion of a very early stage embryo that would eventually give rise to an entire body. Because ES cells originate in this primordial stage, they retain the "pluripotent" ability to form any cell type in the body.

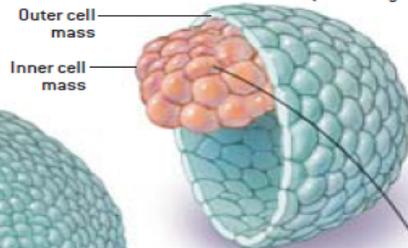
### CELL FATE

Less than a week after a human egg is fertilised, the developing embryo contains about 100 to 150 cells. The embryo is a hollow ball, called a blastocyst, consisting only of an outer cell mass, which in a pregnancy would later form the placenta, and an inner cell mass, which would become the foetus. Inside a womb, these cells would continue multiplying, beginning to specialise by the third week. The embryo, then called a gastrula, would contain three distinctive germ layers whose descendants would ultimately form hundreds of different types of tissues.

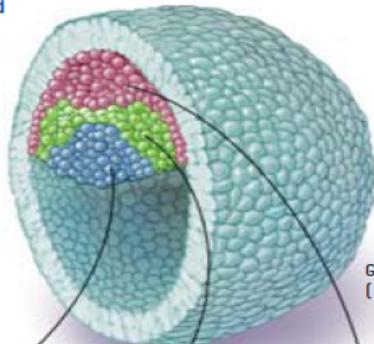
### EMBRYONIC GERM LAYERS AND SOME OF THE TISSUES THAT THEY YIELD



BLASTOCYST (5 to 6 days)



GASTRULA (14 to 16 days)

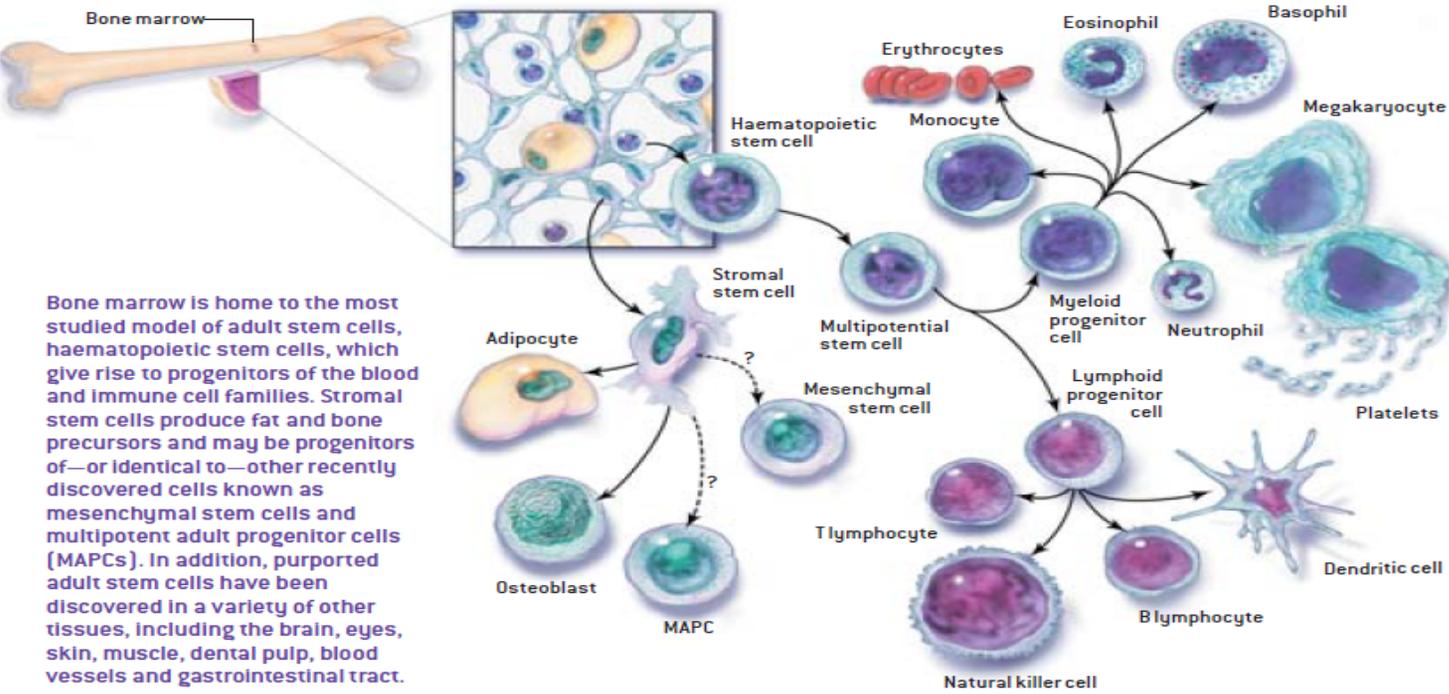


Grow your own eye: biologists have coaxed cells to form a retina, a step toward growing replacement organs outside the body.

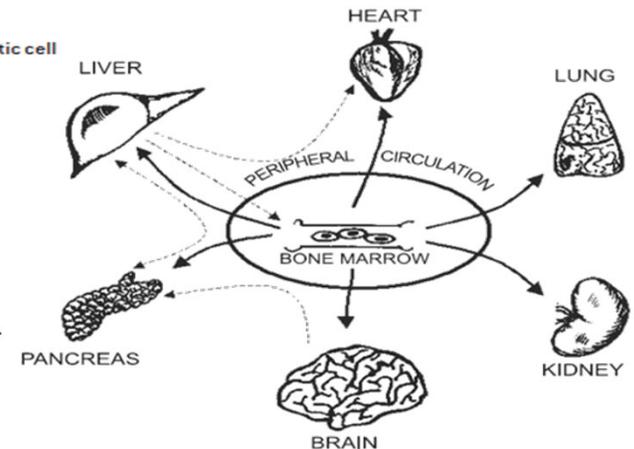
Sasai, Y. Scientific American 307(5): 44-49. November 2012.

# Haematopoietic Stem Cells

## Stem Cell Storehouse



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.

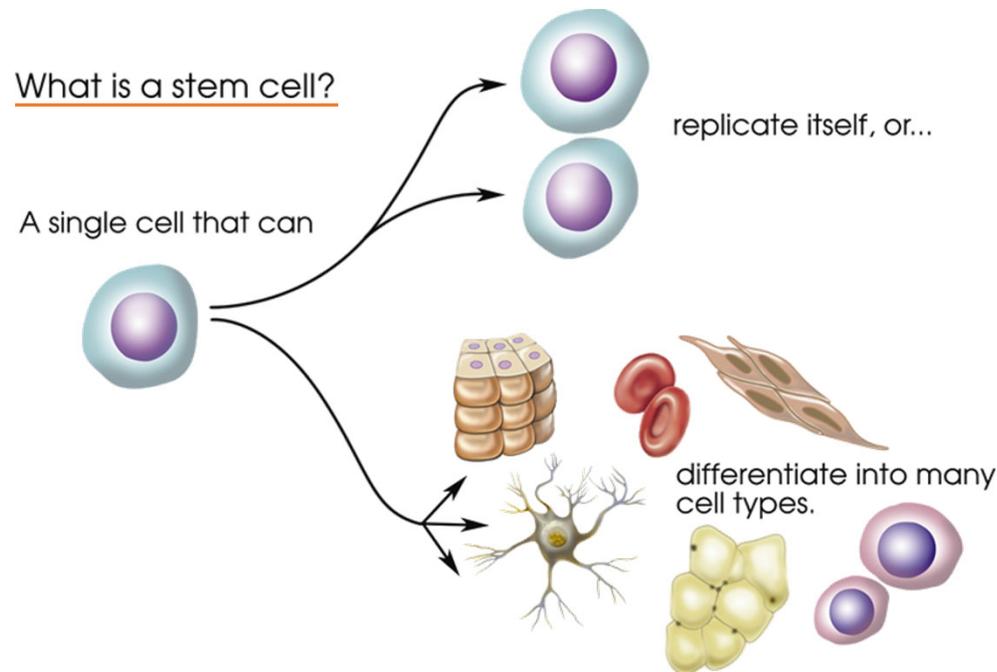


Image prepared by Catherine Twomey for the National Academies, Understanding Stem Cells: An Overview of the Science and Issues from the National Academies, <http://www.nationalacademies.org/stemcells>.

# Reprogramming Cells

MEDICINE



## 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 HOCHDELINGER

### 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.

Reprogramming the normal body cells of any individual—then converting them to any of the 220 human cell types—could yield new disease treatments and custom replacement tissues.

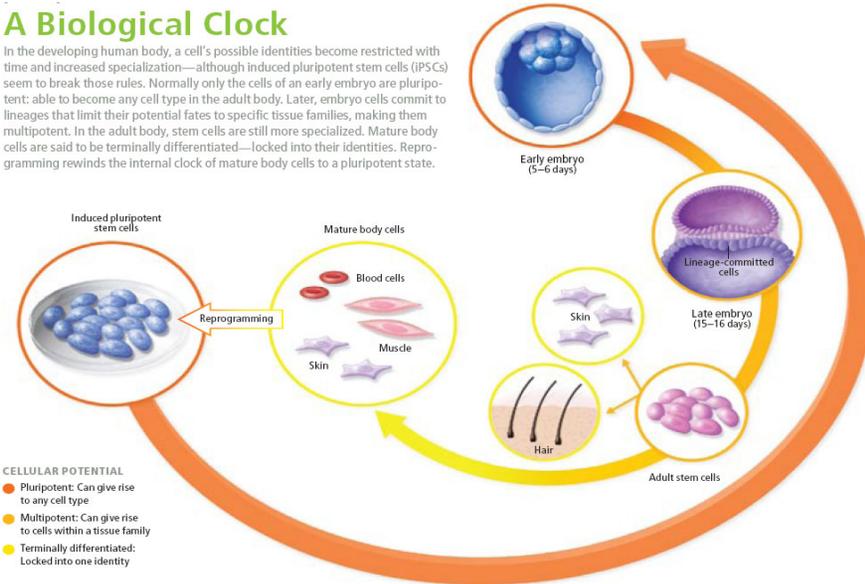
Scientists are now working to understand how these cells are able to reverse their biological clocks and whether the lowest kind of stem cell will prove as powerful as embryonic cells.

—The Editors

SCIENTIFIC AMERICAN 47

## A Biological Clock

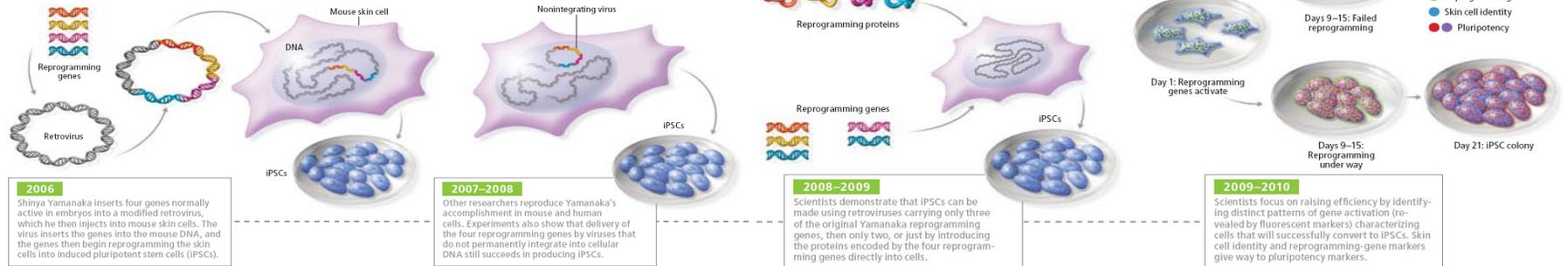
In the developing human body, a cell's possible identities become restricted with time and increased specialization—although induced pluripotent stem cells (iPSCs) seem to break those rules. Normally only the cells of an early embryo are pluripotent: able to become any cell type in the adult body. Later, 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.

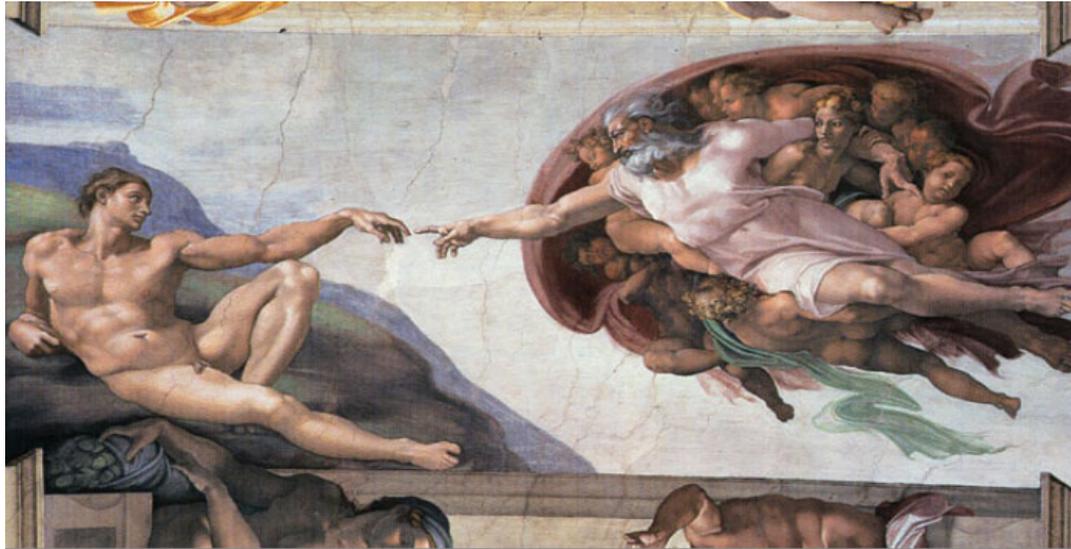
**2009–2010**  
Scientists focus on raising efficiency by identifying distinct patterns of gene activation (revealed by fluorescent markers) characterizing cells that will successfully convert to iPSCs. Skin cell identity and reprogramming-gene markers give way to pluripotency markers.

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# Cloning

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# Creation of Adam



The Creation of Adam by Michelangelo Buonarroti

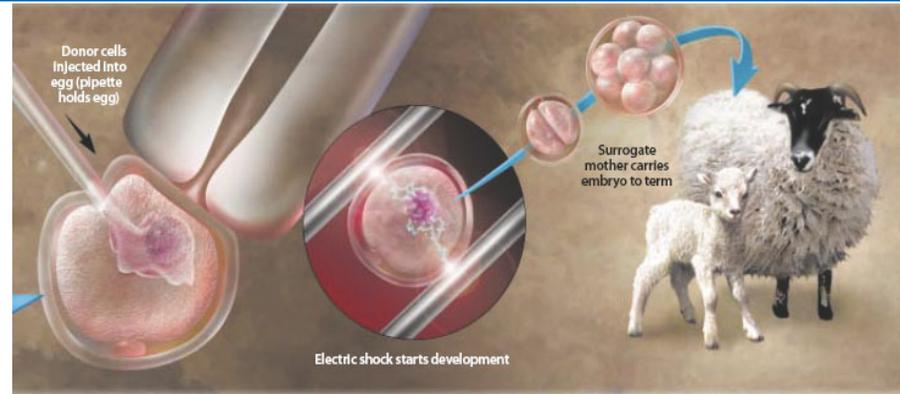


Creation of Life  
Synthetic biology remakes organisms,  
but can it bring inanimate matter to life?

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



Copyright 1998 Scientific American, Inc.

## Cloning for Medicine

by Ian Wilmut

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 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 provide donor nuclei for nuclear transfer cloning. Because **scientists had already learned to transfer genes into cultured cells, this experiment suggested it might be possible to use such modified cells to create transgenic animals—such as cows that could produce insulin for diabetics in their milk.**



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.

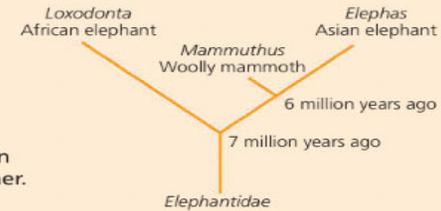
# Jurassic Ark



# 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.



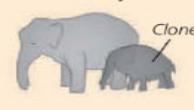
**2** Electrically shock the egg to stimulate cell division, creating an embryo.



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

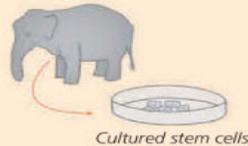


**4** After gestation, Asian elephant mother gives birth to a cloned baby mammoth.

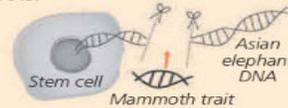


### Building a Hybrid With Genome Engineering

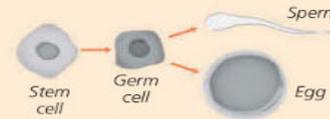
**1** Cultivate Asian elephant stem cells.



**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.

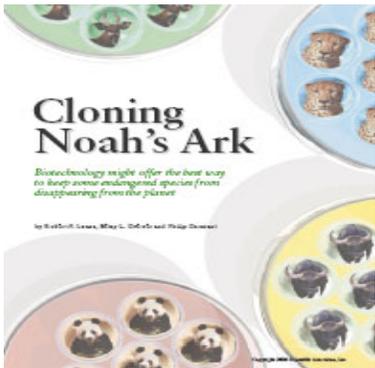


**6** See if the baby has mammoth cold-resistance traits. Make additional changes, if needed, creating a herd of mammoth-elephant hybrids that can flourish on the tundra.

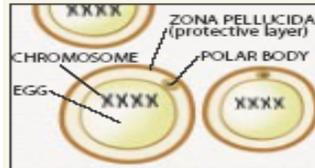


The elephant genome is modified with mammoth genes for cold resistance, such as long hair, extra subcutaneous fat and an altered blood protein.

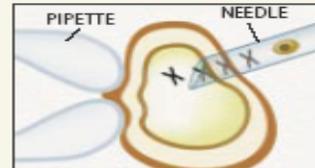
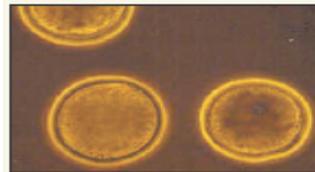
# Cloning



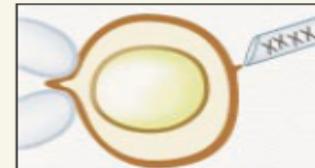
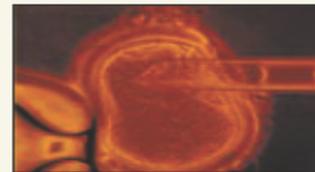
## THE NUCLEAR TRANSFER (CLONING) PROCESS



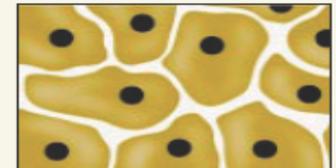
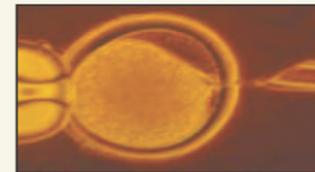
Recipient eggs are coaxed to mature in a culture dish. Each has a remnant egg cell called the polar body.



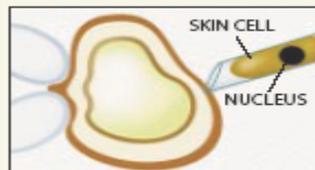
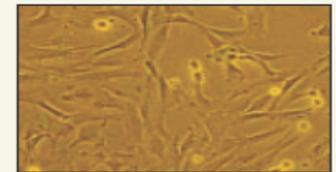
The polar bodies and chromosomes of each egg are drawn into a needle. A pipette holds the egg still.



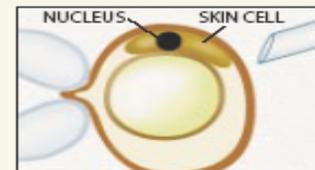
Once the chromosomes and polar body are removed, all that remains inside the zona pellucida is cytoplasm.



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



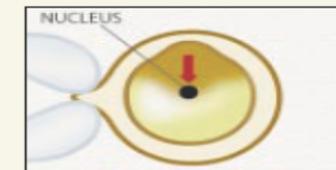
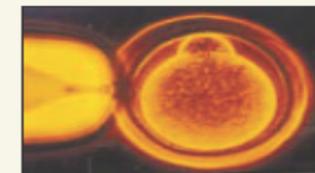
An entire skin cell is taken up into the needle, which is again punched through the zona pellucida.



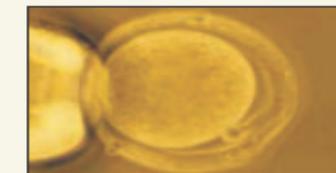
The skin cell is injected underneath the zona pellucida, where it remains separate from the egg cytoplasm.



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

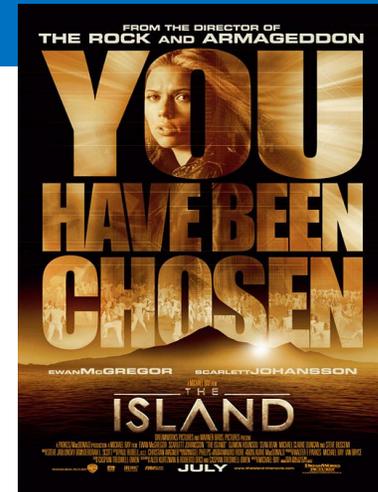


The skin cell's nucleus, with its genes, enters the egg cytoplasm. Within a few hours, the fused cell begins to divide.



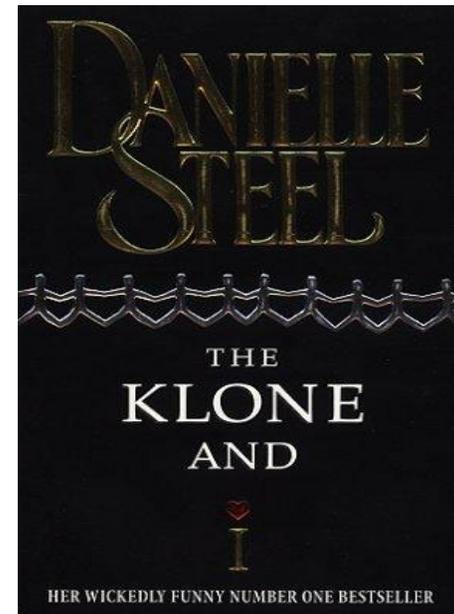
# Make Your Own Bank of Organs through Cloning

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 refers to the clone of the main character. The cloned you may do the job for you when you are so busy. Your productivity will increase, and you can be in 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.



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# **Tissue Regeneration: FDA**

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# 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>

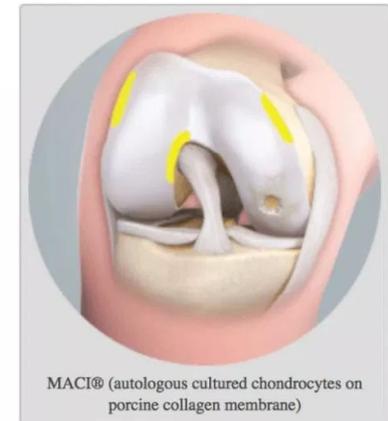
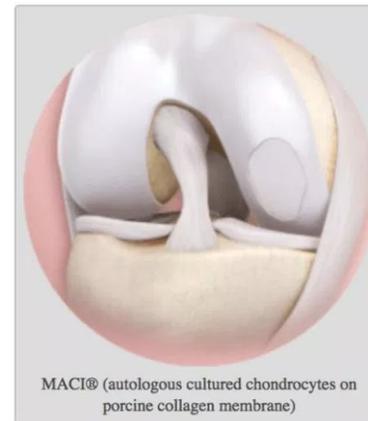
# Licensed Cellular Products

- **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 (Axficel-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

# 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.

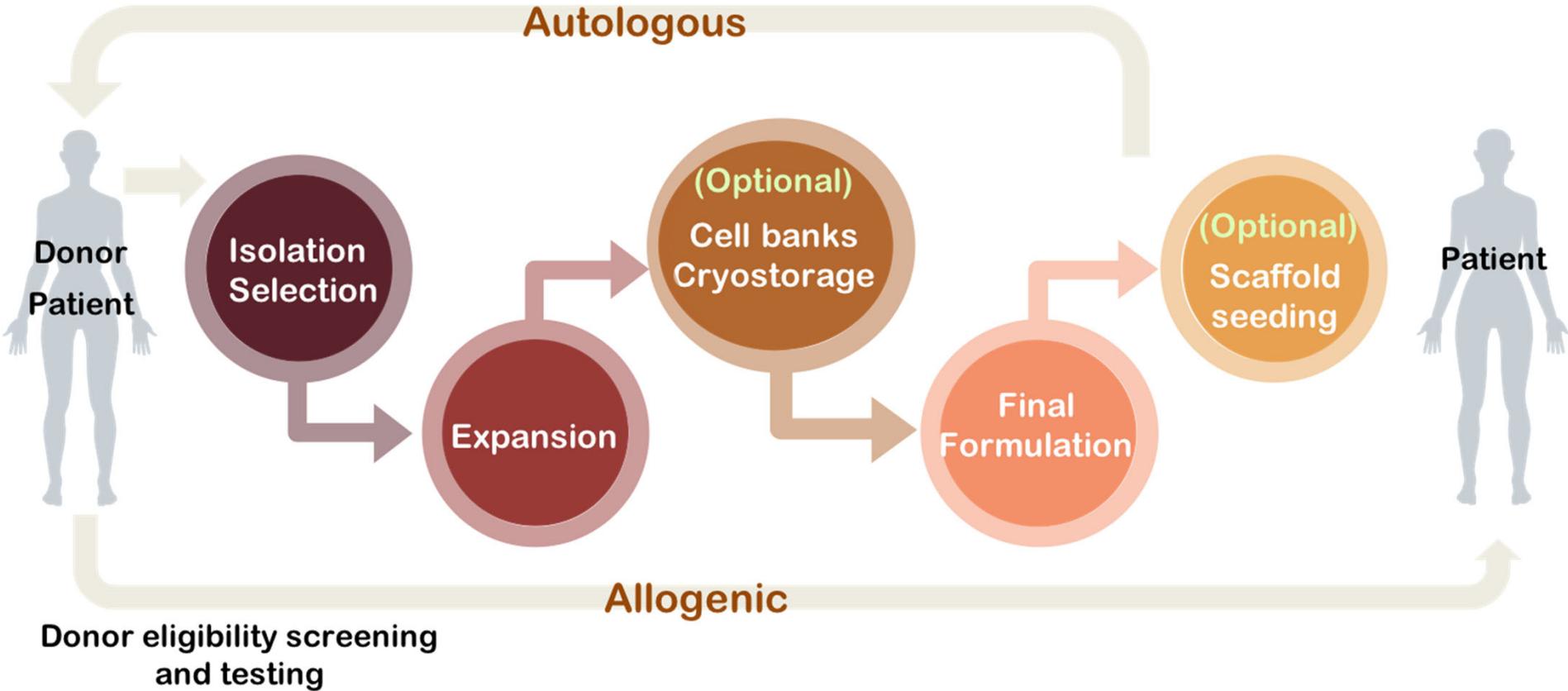


# Chemistry, Manufacturing, and Controls (CMC)

- **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



# Cellular product manufacturing process



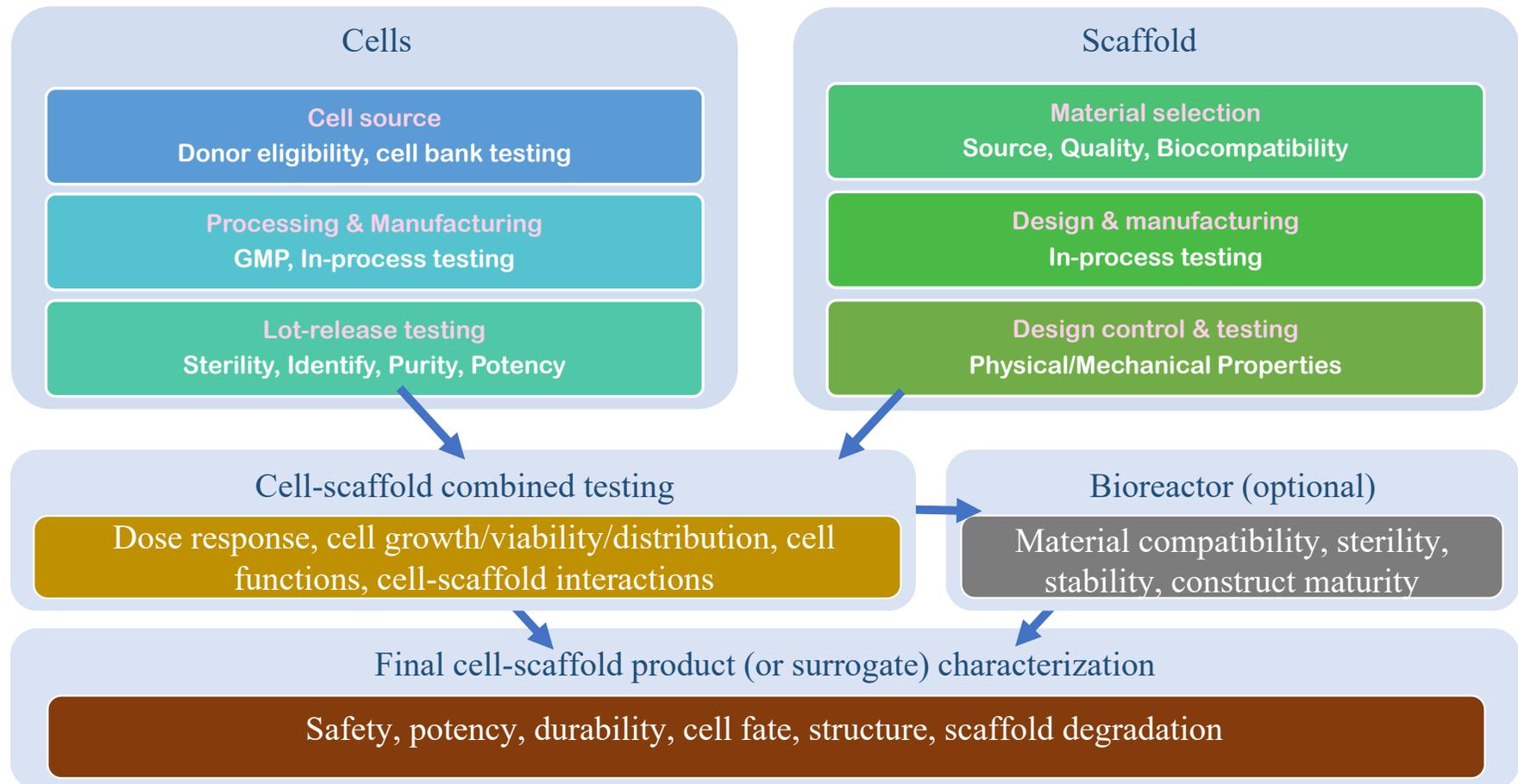
# An Example of Testing for Cellular Products including Cell-Scaffold Combination Products

Source material	Cell banks	In-process	Final product
<ul style="list-style-type: none"> <li>Donor eligibility (21 CFR Part 1271)</li> <li>Adventitious agents on reagents</li> </ul>	<ul style="list-style-type: none"> <li>Adventitious agents (21 CFR 610.18)</li> <li>Sterility (21 CFR 610.18)</li> <li>Identity (21 CFR 610.18)</li> <li>Mycoplasma (21 CFR 610.18)</li> <li>Cytogenetic characterization (21 CFR 610.18)</li> <li>In vitro growth characteristics (21 CFR 610.18)</li> <li>Stability</li> <li>Purity</li> <li>Cell activity/maturation</li> </ul>	<ul style="list-style-type: none"> <li>Sterility</li> <li>Stability</li> <li>Viability</li> <li>Identity</li> <li>Mycoplasma</li> <li>Biocompatibility</li> <li>Physical, chemical and mechanical properties</li> </ul>	<ul style="list-style-type: none"> <li>Identity (21 CFR 610.14)</li> <li>Sterility (21 CFR 610.12)</li> <li>Stability (21 CFR 211.166)</li> <li>Purity (21 CFR 610.13)</li> <li>Potency (21 CFR 610.10)</li> <li>Viability</li> <li>Appearance</li> <li>Cell concentration</li> <li>Physical, chemical and mechanical properties</li> </ul>

- Described in regulations
- Described in guidance documents
- Additional considerations For combination product

The type and level of testing are product dependent and could be improved with advances in regulatory science.

# Safety and Effectiveness Consideration for a Cell-Scaffold Product



# Cell Therapy Challenges and Regulatory Science Goals

## Challenges

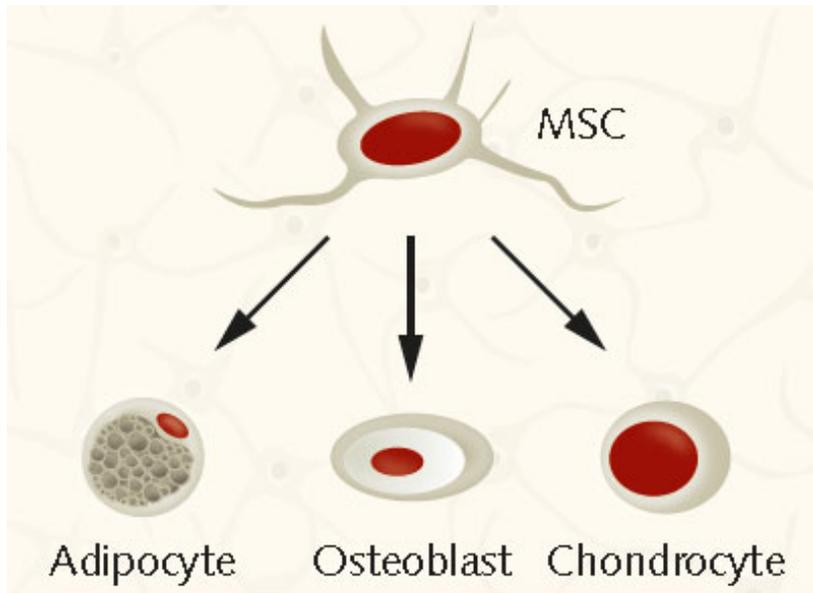
- Inadequate markers predictive of **cell state and cell fate**
- Poor understanding of how cells **interact with their microenvironment**
- Poor understanding regarding the **impact of manufacturing parameters** on product quality

## Regulatory science goals

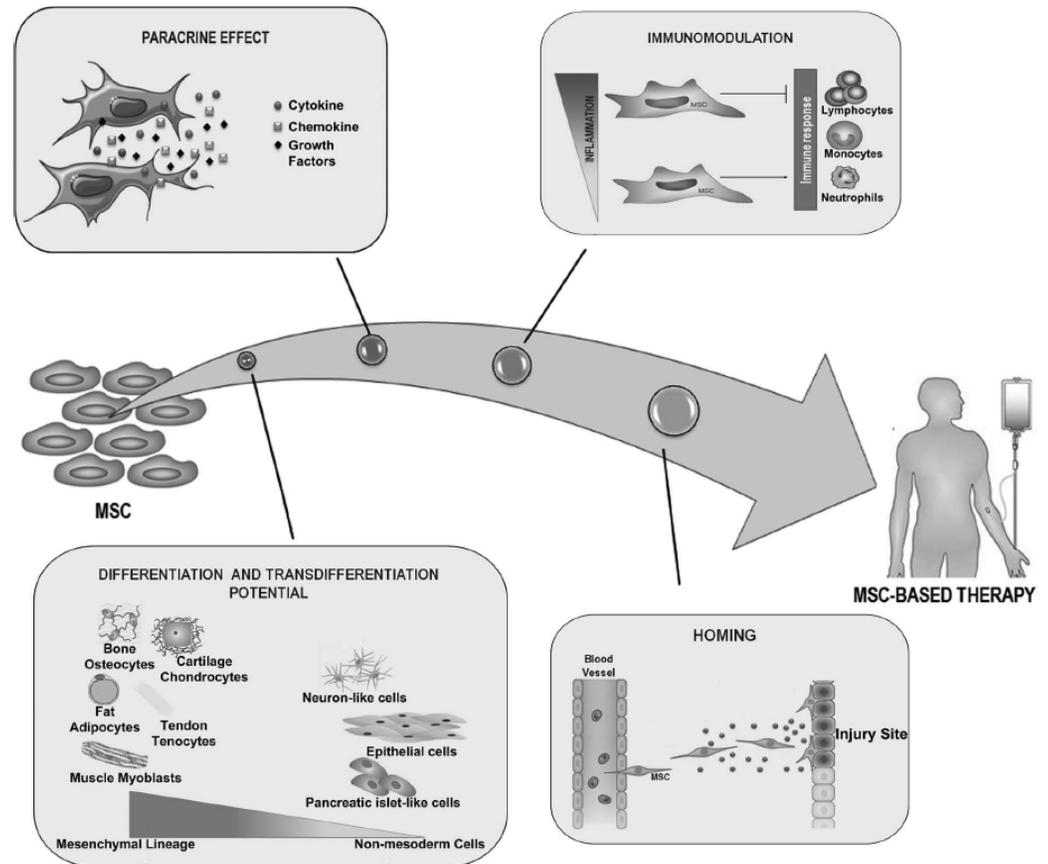
- Develop and improve test methods for cell product characterization
- Identify product attributes that are quantifiable and predictive of safety and effectiveness
- Identify product quality attributes that are predictive of safety and effectiveness

# 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

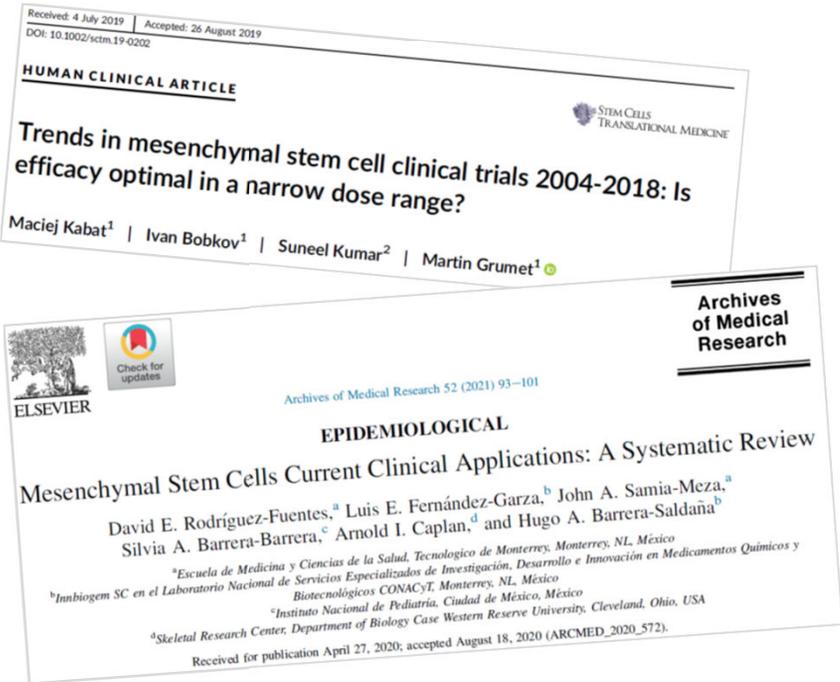
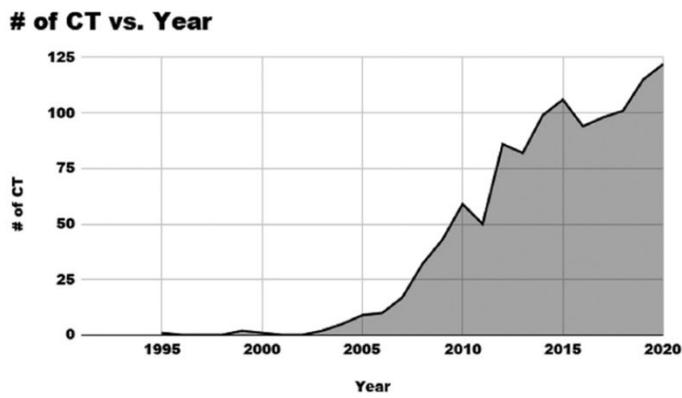
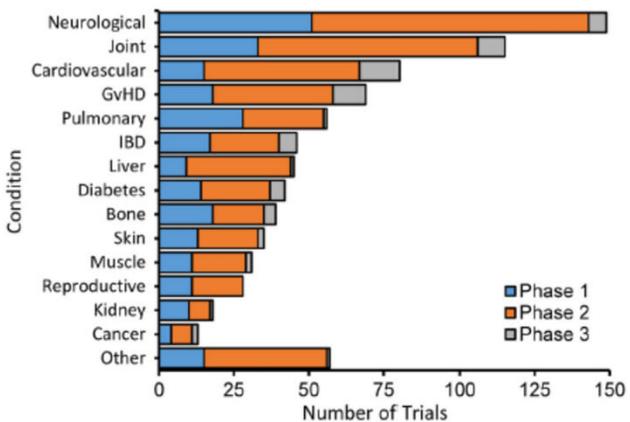
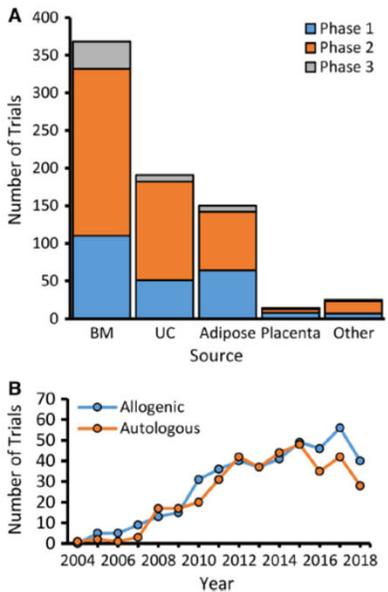
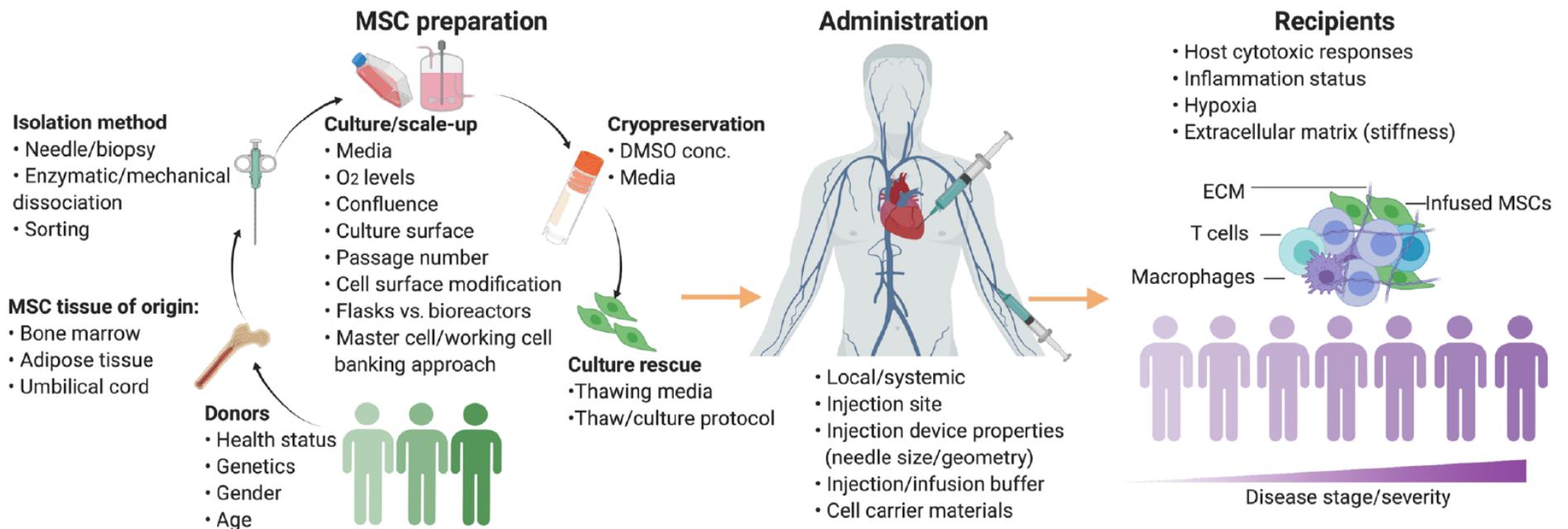
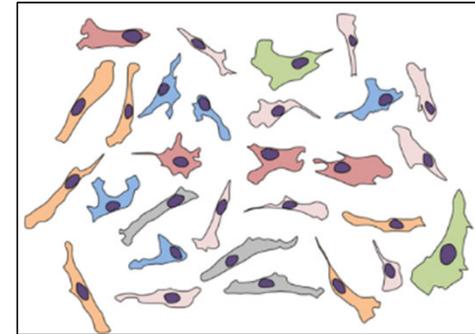


Table 2. Listed MSC Clinical Trials around the world

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



# MSC Functional Heterogeneity

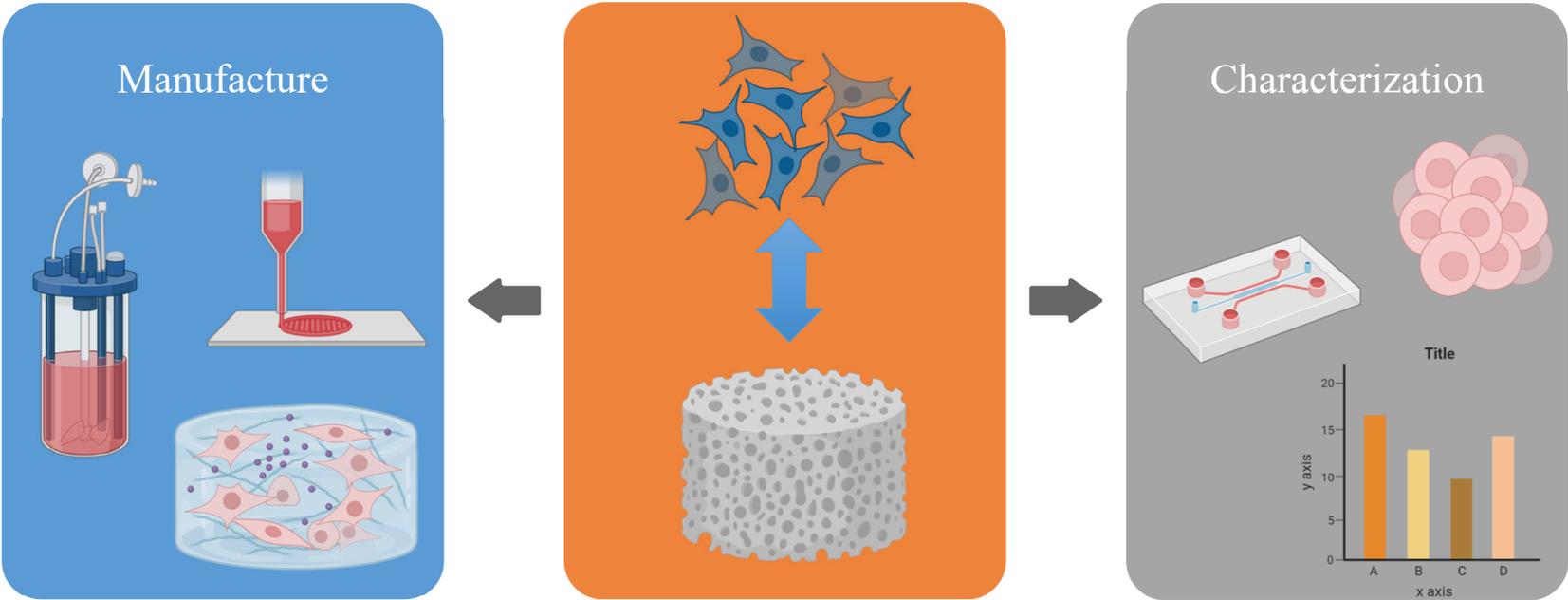


Levy et al, *Science Advances*, 2020

Slide by Kyung Sung, Ph.D., FDA

# Cell-Materials Interactions

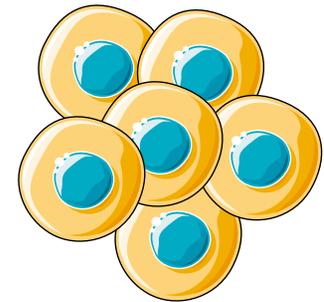
## Investigating the effect of cell-materials interactions on the safety and effectiveness of cell-based products



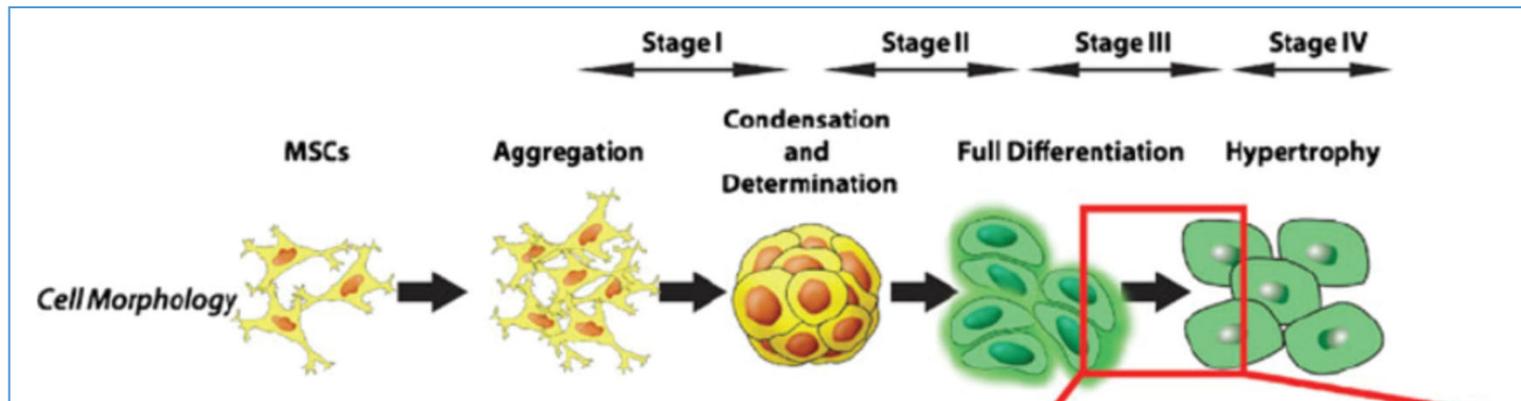
# 3D In Vitro System for MSC Chondrogenesis

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

3D aggregates



Recapitulate cell-cell contacts during condensation

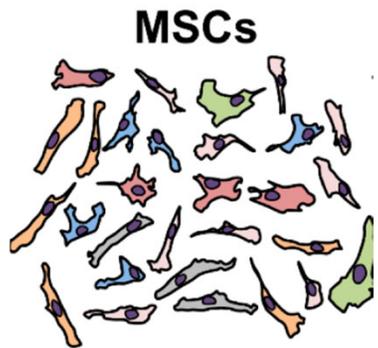


*Gadjanski et al. Stem Cell Rev and Rep (2011)*

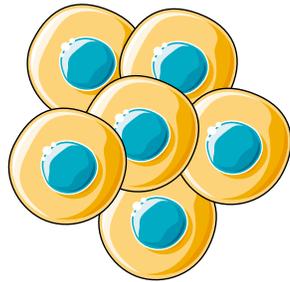
*Lam et al. Stem Cell Transl Med (2018)*

Slide by Kyung Sung, Ph.D., FDA

# Experimental Scheme



MSC aggregates



8 MSC lines

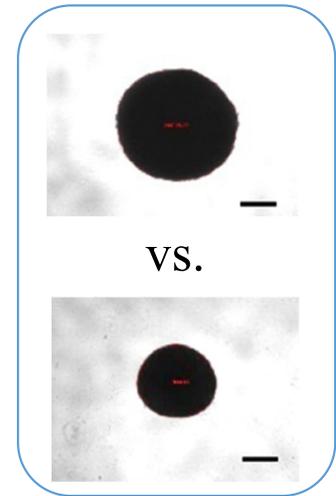
Early passage: P2/P3

Late passage: P5



- 21 days of culture
- 10ng/mL TGF- $\beta$ 3

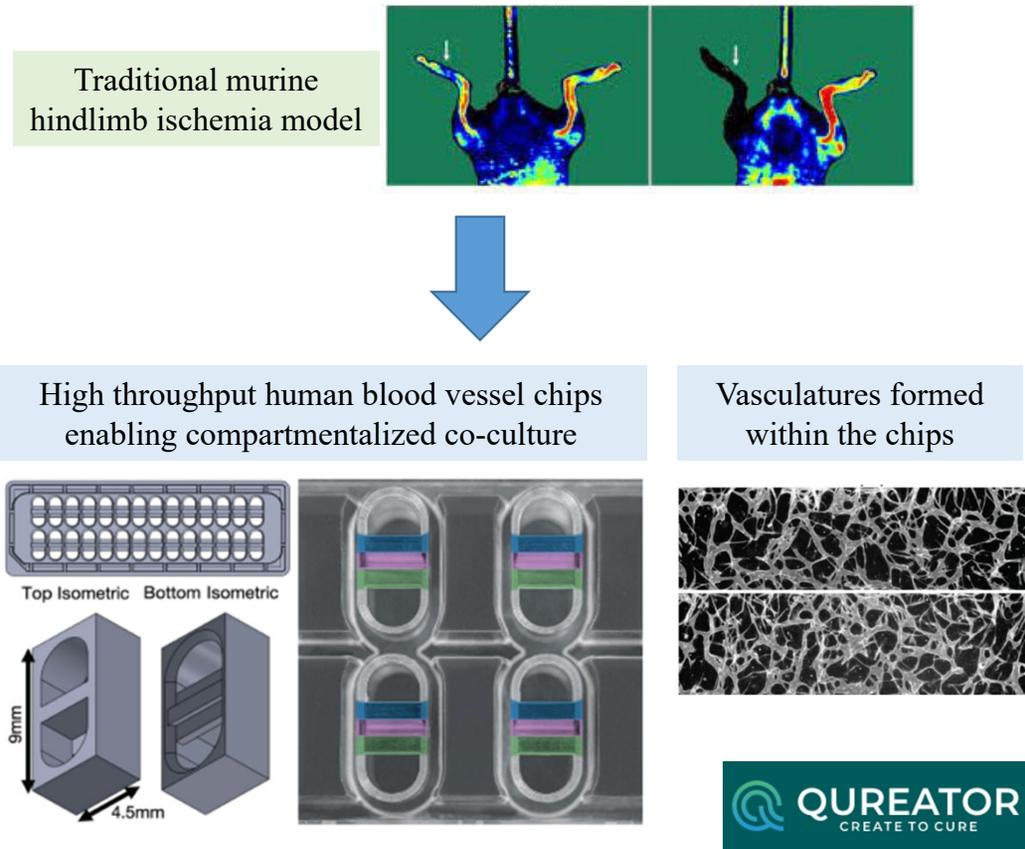
- Image acquisition at days 1, 4, 7, 11, 14, 18, and 21



- **Spheroid morphology quantification:** 14 shape and size features
- **Matrix deposition:** Sulfated glycosaminoglycan (GAG) normalized to the total DNA amount
- **Chondrogenic gene expression:** type II $\alpha$ 1 collagen, aggrecan, and type II $\alpha$ 1/type I collagen fold ratio
- **Histology:** GAG and collagen

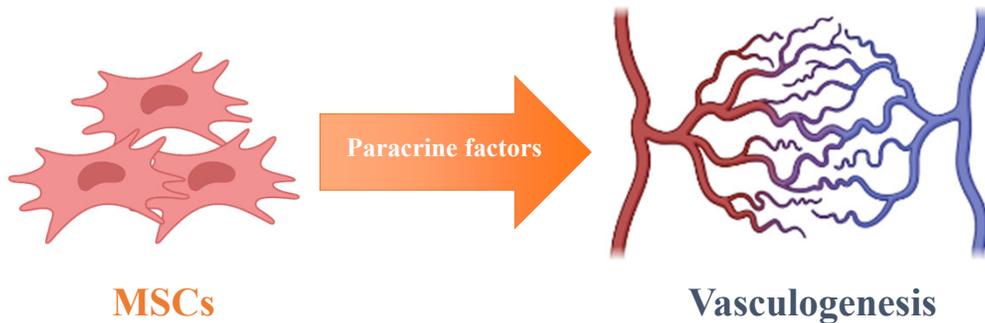
# Human Blood Vessel Chips to Predict Vasculogenic Potential of MSCs

- 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.



# MPS for Evaluating MSC-Vasculogenic Bioactivity

Microphysiological systems (MPS)



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

## Experimental Overview

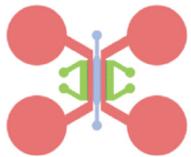
MPS Selection

MPS Platform Optimization

Assay Design

Assay Qualification

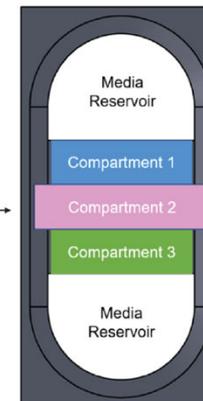
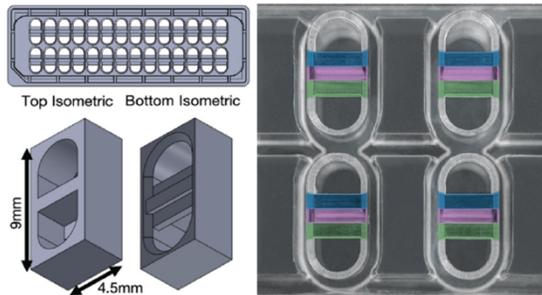
PDMS-based Platform



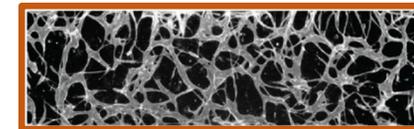
- Low throughput
- Batch-to-batch variation
- Incompatible with automation

Injection-Molded Polystyrene-Based Device

- High throughput
- Batch-to-batch consistency
- Compatible with automation



Vasculature formed within MPS



Slide by Kyung Sung, Ph.D., FDA