Tissue engineering and organ printing

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- Applications of nanotechnology in tissue engineering
- Artificial cells
- Artificial RBC
- Applications of artificial cells
- Organ printing

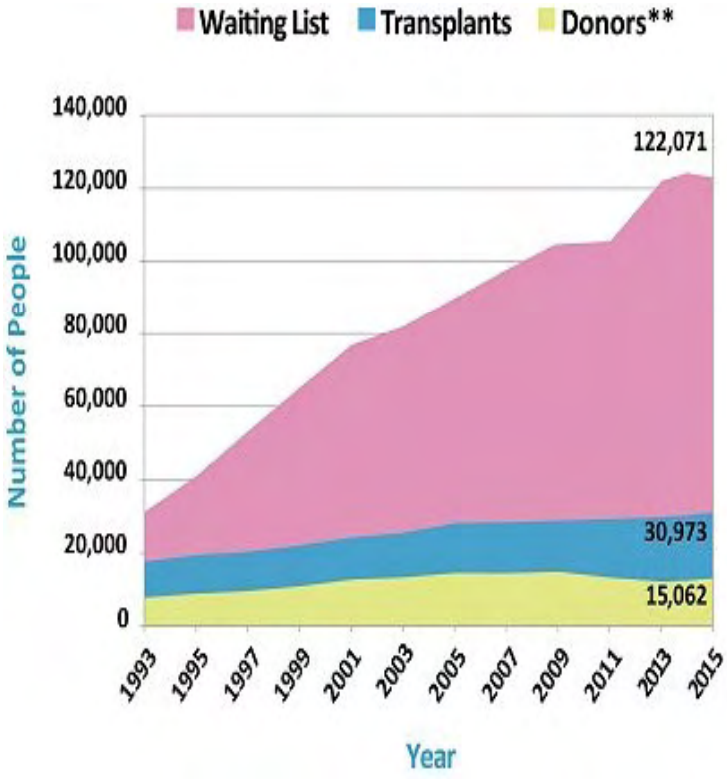
The need for organs today

Statistics at a Glance

119,000+ Current number of men, women and children on the national transplant waiting list.

30,970 transplants were performed in 2015.

22 people die each day waiting for a transplant.



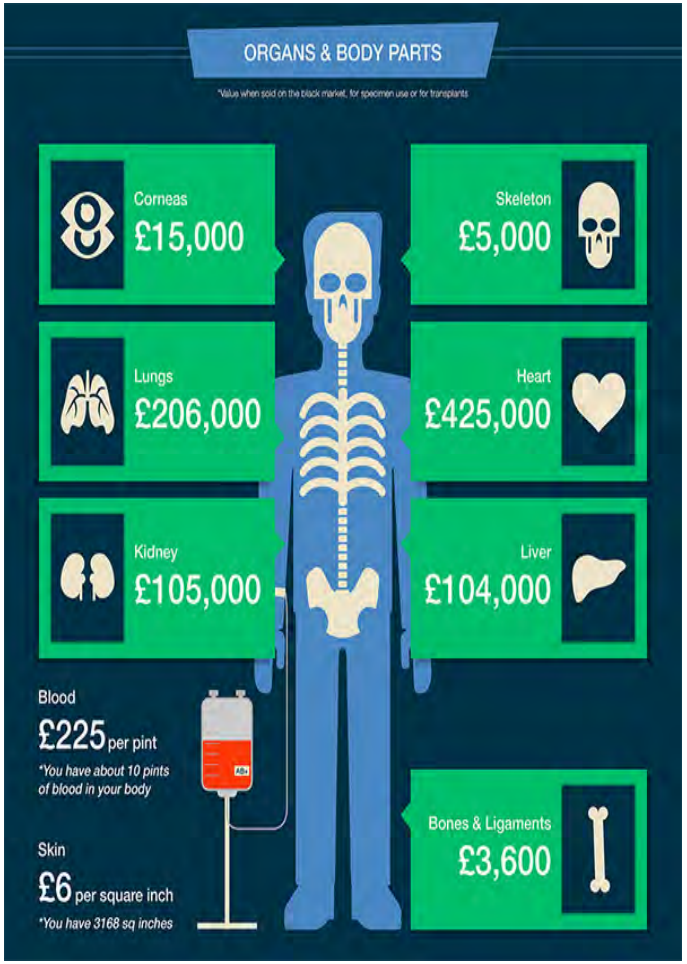
https://organdonor.gov/index.html

Organ donation in India

- Almost 1.5 lakh people in India need a kidney; however, only 3000 of them receive one.
- 90% of people in the waiting list die without getting an organ.
- India's annual liver transplant requirement is 25,000, but we manage only about 800.
- 70% liver transplants are taken care of by a live donor, but 30% are dependent on cadaver donations.

http://notto.nic.in/index.htm *Source: Times of India, DNA India*

Exactly how much is the human body worth?



http://dailyscene.com/exactly-how-much-is-the-human-body-worth-7-photos/

Tissue Engineering

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function

Langer and J. Vacanti "Tissue Engineering". Science 260: 920-6, 1993.

Why tissue engineering?

- To create products that improve tissue function or heal tissue defects.
  - Replace diseased or damaged tissue
- Because……
  - Donor tissues and organs are in short supply
  - We want to minimize immune system response by using our own cells or novel ways to protect transplant.

Introduction

- Tissue engineering is an approach to treat patients who need a new organ or tissue with man-made organs or tissues.

- Tissues are engineered using a combination of a patient's own cells and polymer scaffolds.

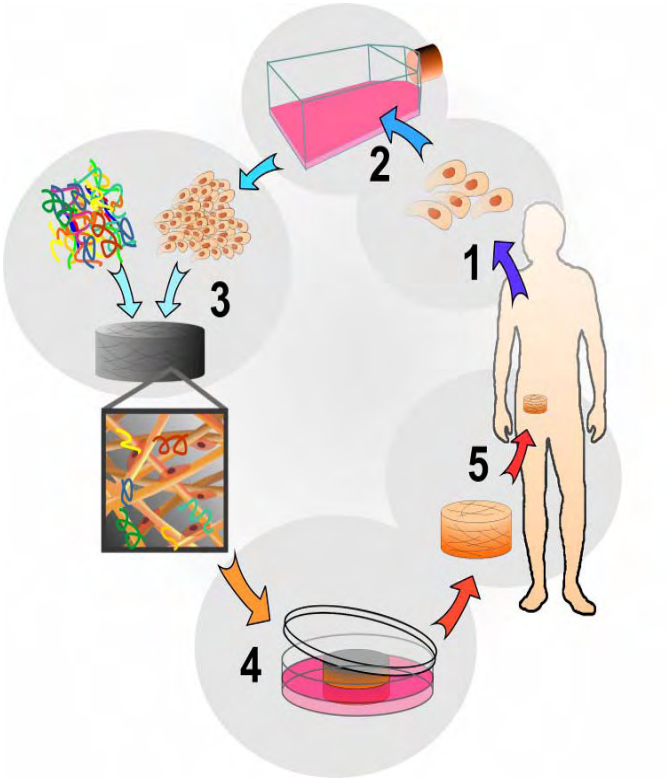
- Tissue specific cells are isolated from the patient and expanded *in vitro.*

- The polymer mimics the natural ECM, which bring cells together and control the tissue structure, regulate the function of the cells, and allow the diffusion of nutrients, metabolites, and soluble factors

Tissue engineering-Overview

Expand number in culture

Seed onto an appropriate scaffold with suitable growth factors and cytokines



Remove cells from the body.

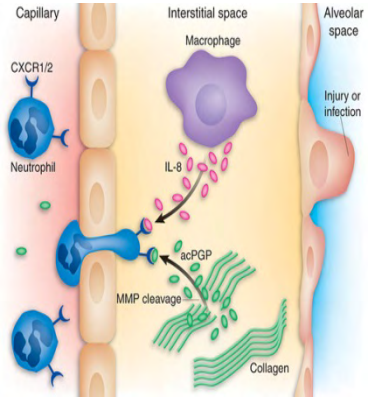
Re-implant engineered tissue repair damaged site

Place into culture

Why 3D?

- Because we can no longer to view a cell as self contained unit existing in a passive structural network. Thus to properly study the cell interactions it must be in a 3D environment.

In-vitro

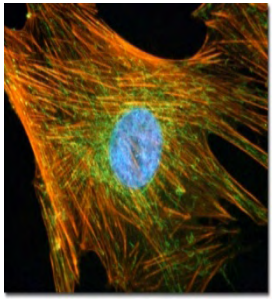


In-vivo

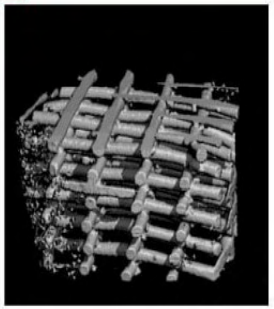
Image source: www.millipore.com/imagesfarm1.static.flickr.com

3 Tools of Tissue Engineering

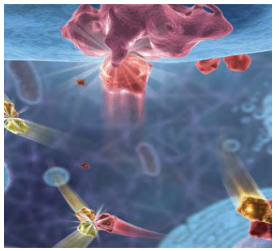
- Cells
  - Living part of tissue
  - Produces protein and provides function of cells
  - Gives tissue reparative properties



- Scaffold
  - Provides structural support and shape to construc
  - Provides place for cell attachment and growth
  - Usually biodegradable and biocompatible

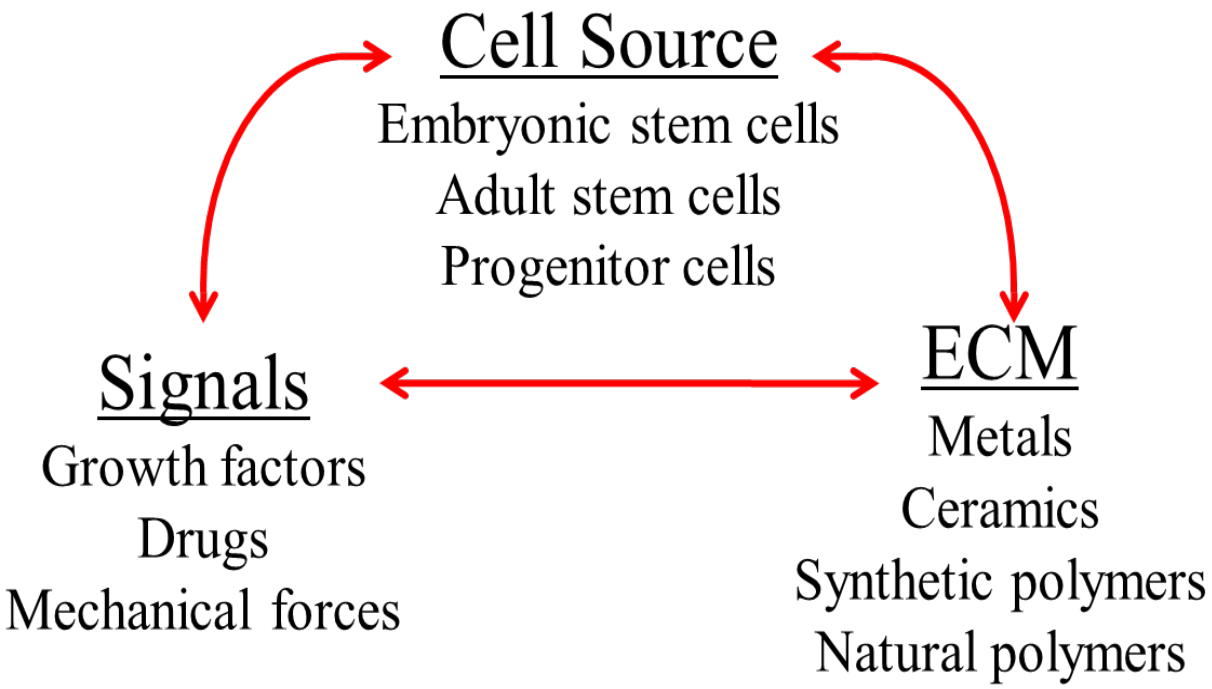


- Cell Signaling
  - Signals that tell the cell what to do
  - Proteins or Mechanical Stimulation



Tissue engineering requirements

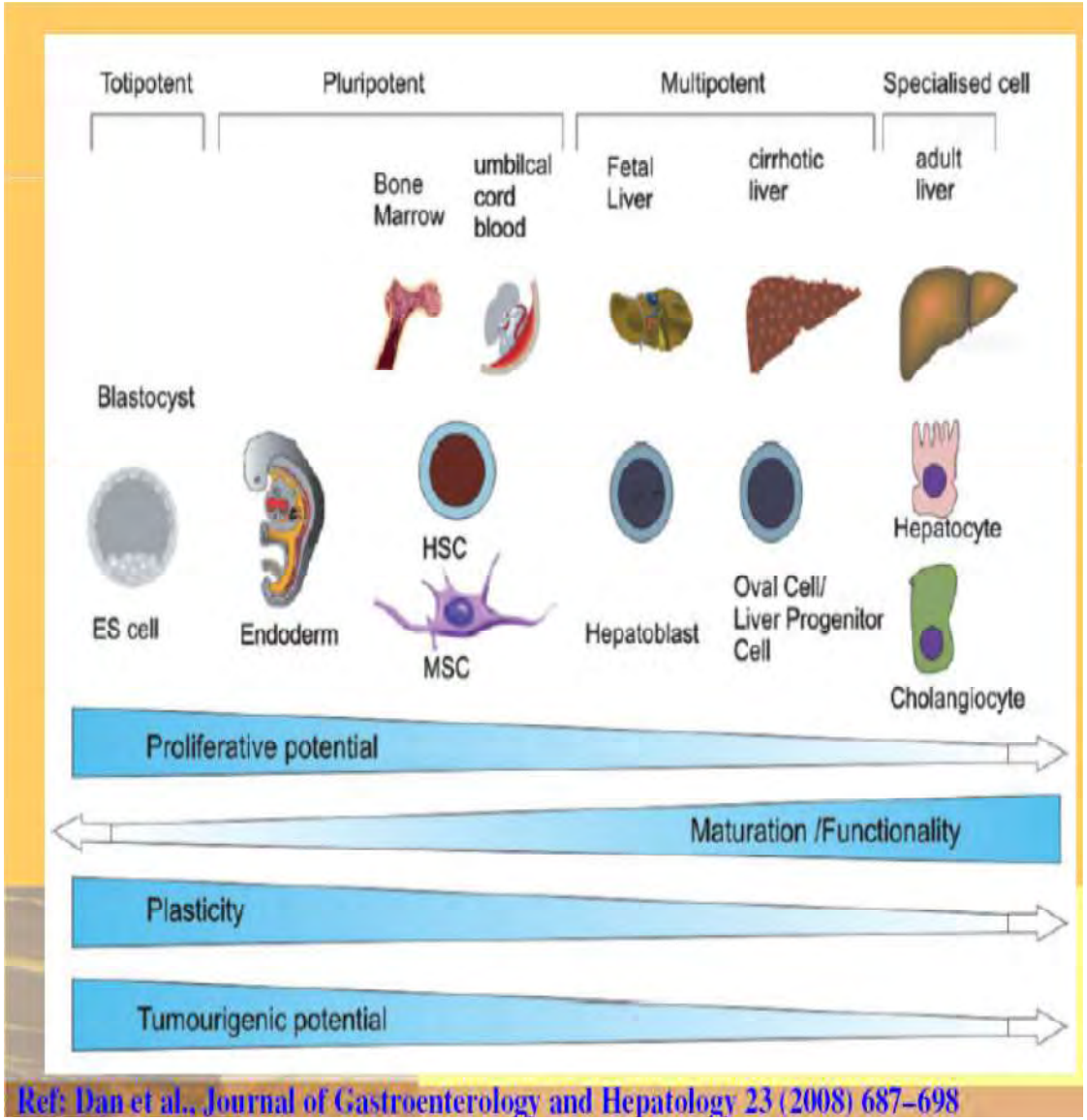
Tissue engineering requires three things:



What is a stem cell?

- A stem cell is a cell that has the ability to divide (self replicate or self renew) for indefinite periods—often throughout the life of the organism.
- Under the right conditions, or given the right signals, stem cells can give rise (differentiate) to the many different cell types that make up the organism.
- Totipotent –Embryonic stem cells
- Pluripotent/multipotent – Mesenchymal stem cells
- Unipotent

Potency of stem cells



Scaffolds

- Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds

- Scaffolds usually serve at least one of the following purposes:

➢ Allow cell attachment and migration

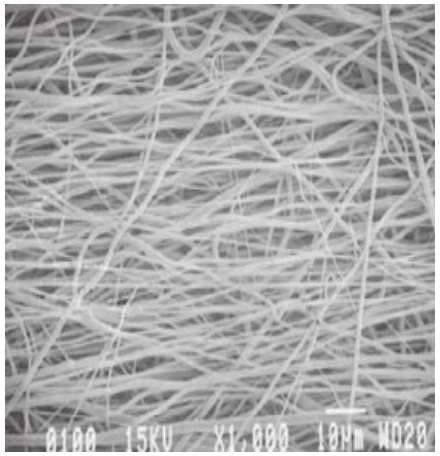
➢ Deliver and retain cells and biochemical factors

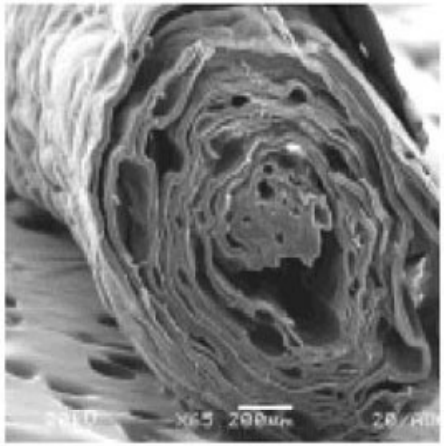
➢ Enable diffusion of vital cell nutrients and expressed products

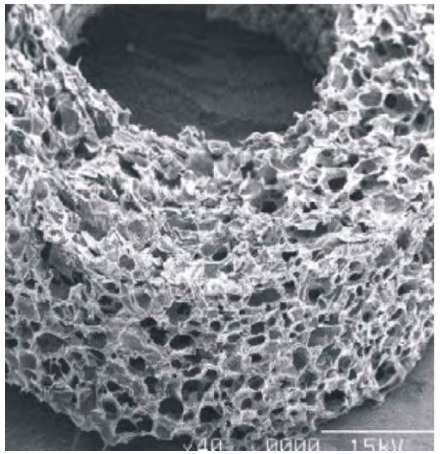
➢ Exert certain mechanical and biological influences to modify the behaviour of the cell phase

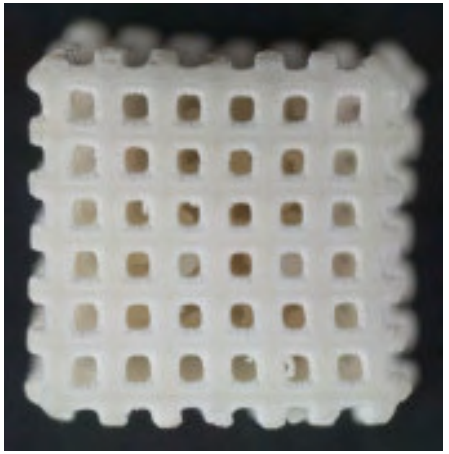
Scaffolds

- Various textures and materials
- Encourage cells to grow
- Allow nutrients to permeate
- Won't harm the patient





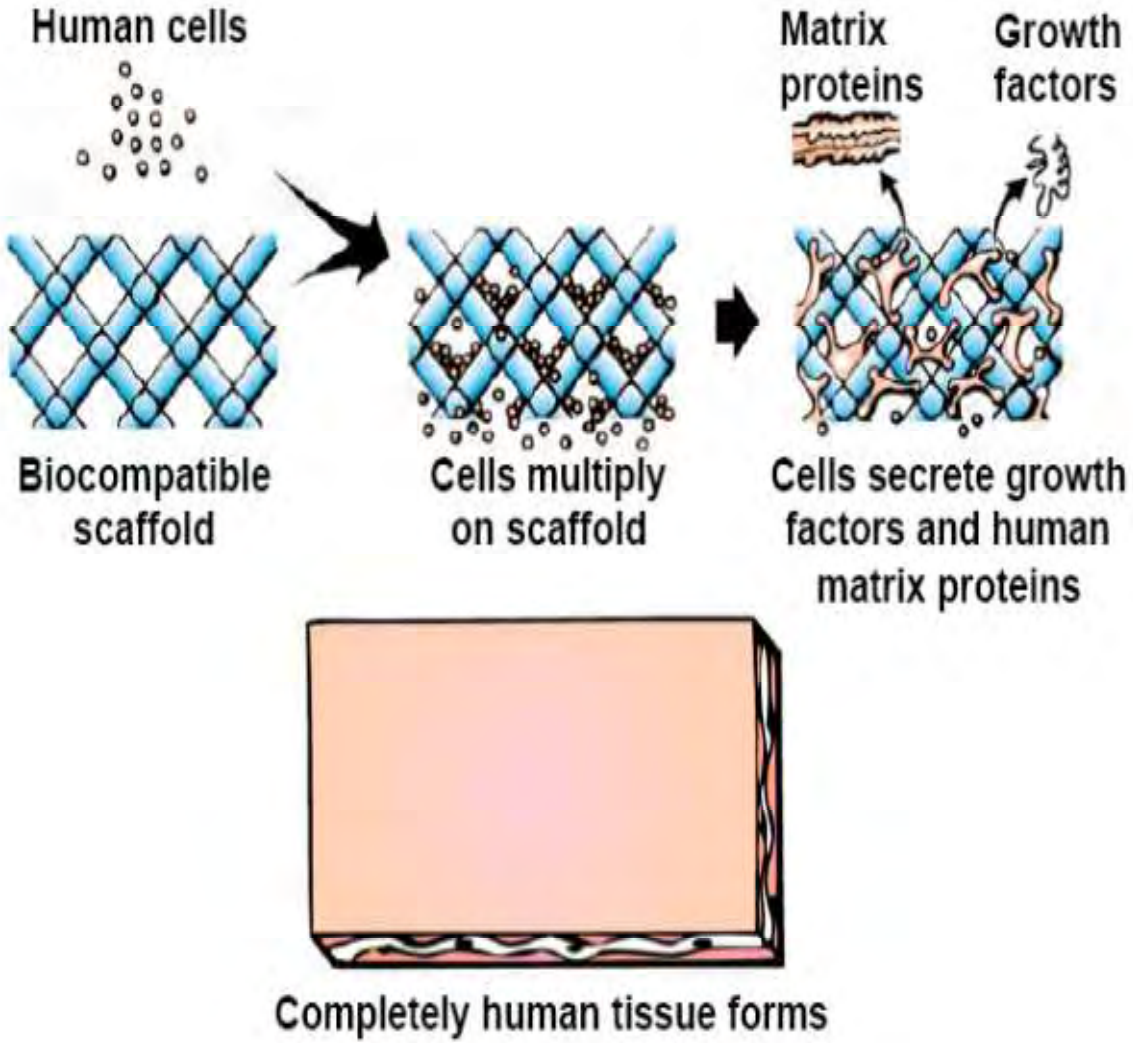




Steps in tissue engineering

- Appropriate cell source must be identified, isolated and produced in sufficient numbers
- Appropriate biocompatible material that can be used as a cell substrate or cell encapsulation material isolated or synthesized, manufactured into desired shape and dimensions
- Cells seeded onto or into material, maintaining function, morphology
- Engineered structure placed into appropriate in vivo site

Cell-scaffold technology



Tissue engineering

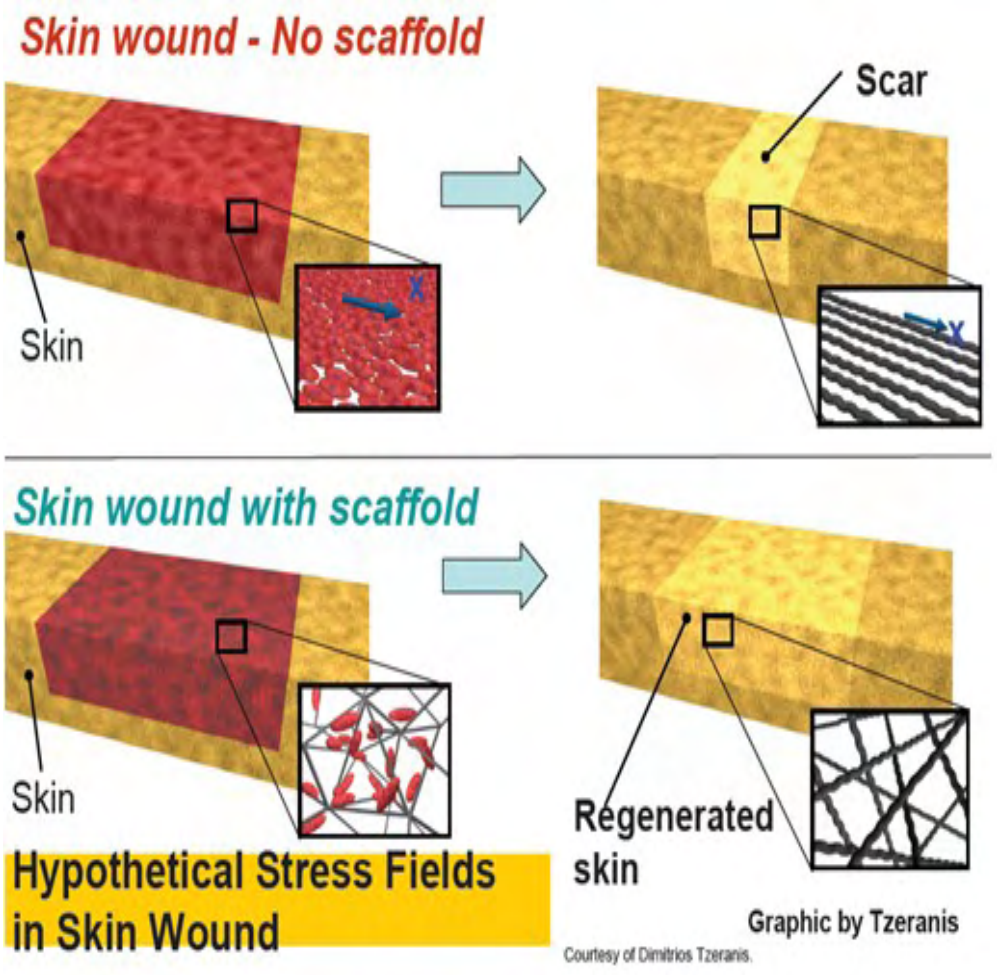
- Using well designed scaffolds and optimized cell growth, we can create tissues such as:
  - Skin
  - Bone
  - Cartilage
  - Intestine
- These have been successfully engineered to some extent

Tissue engineering

More complex organs

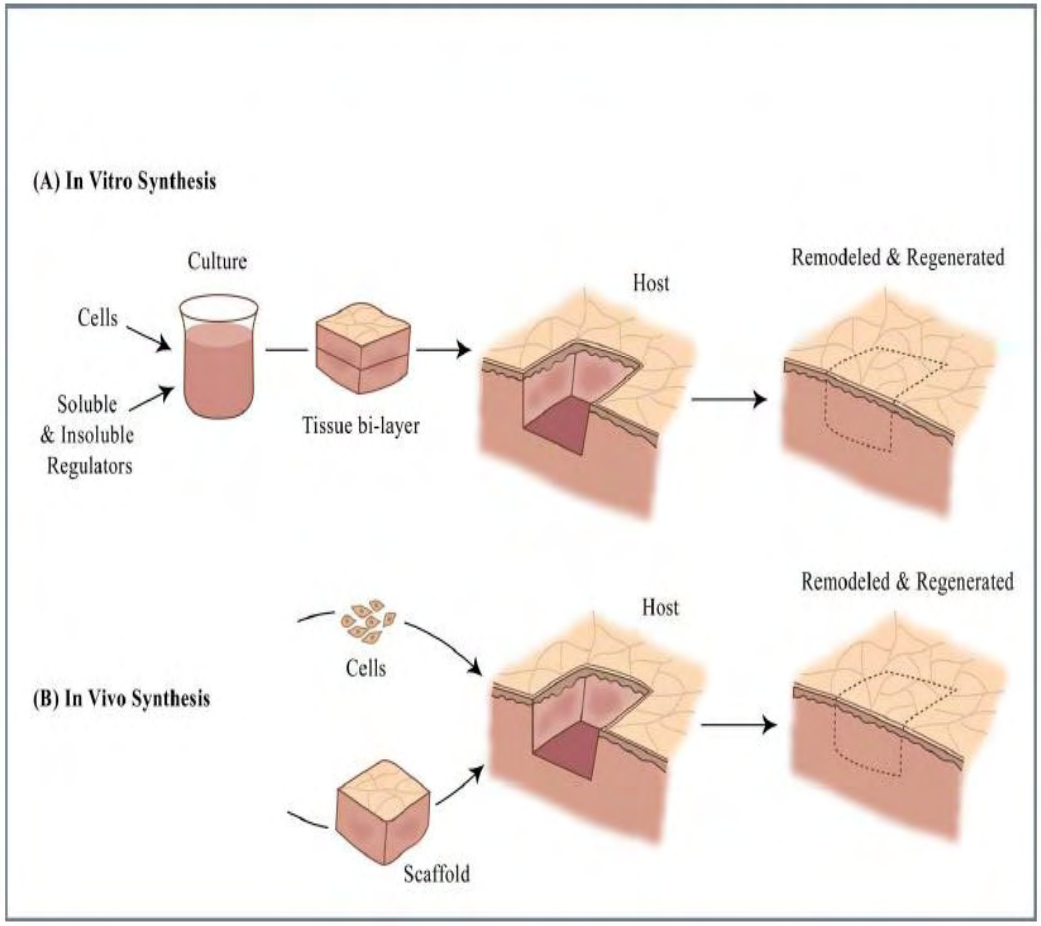
- Not very far in development
- Complex metabolic functions
- Require multiple types of cells and intricate scaffolds
  - Liver
  - Heart
  - Lung
  - Kidney

Skin tissue engineering

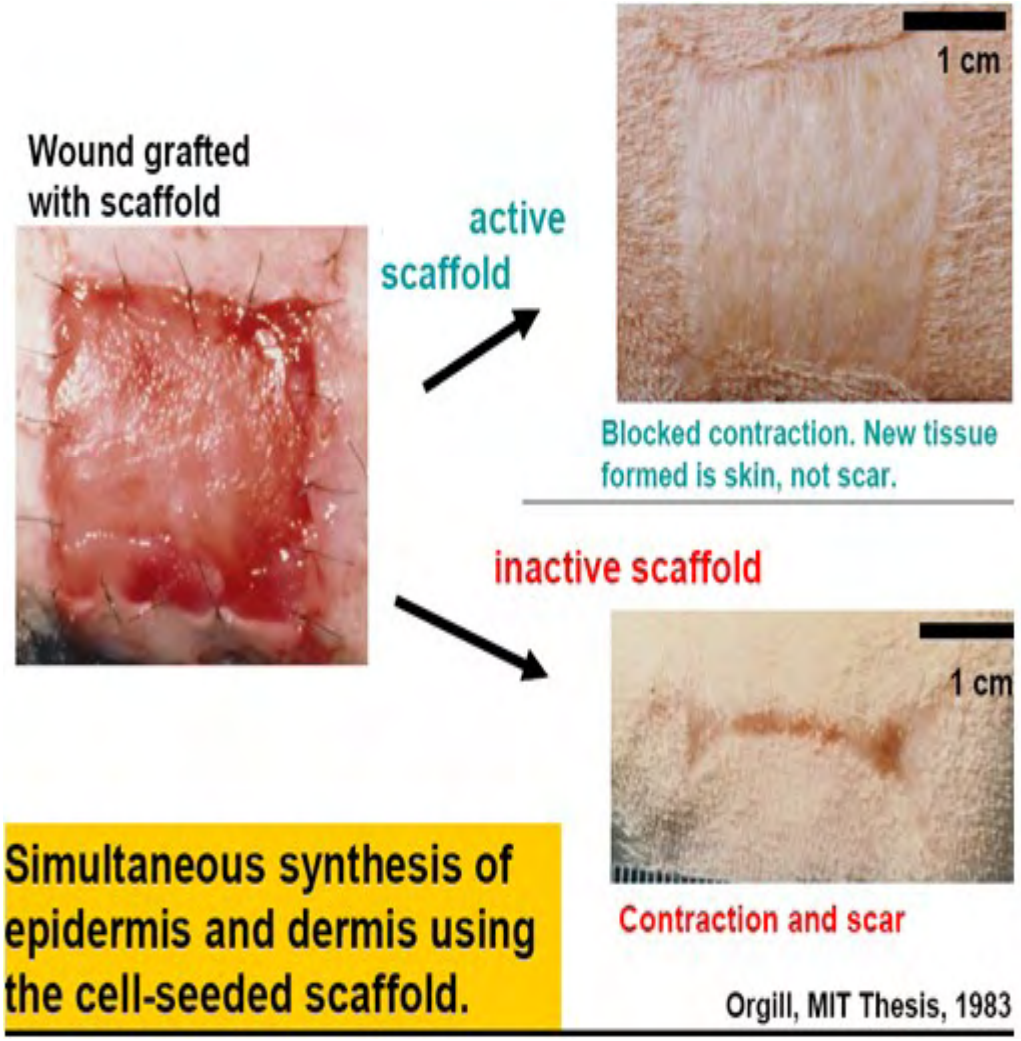


Skin repair

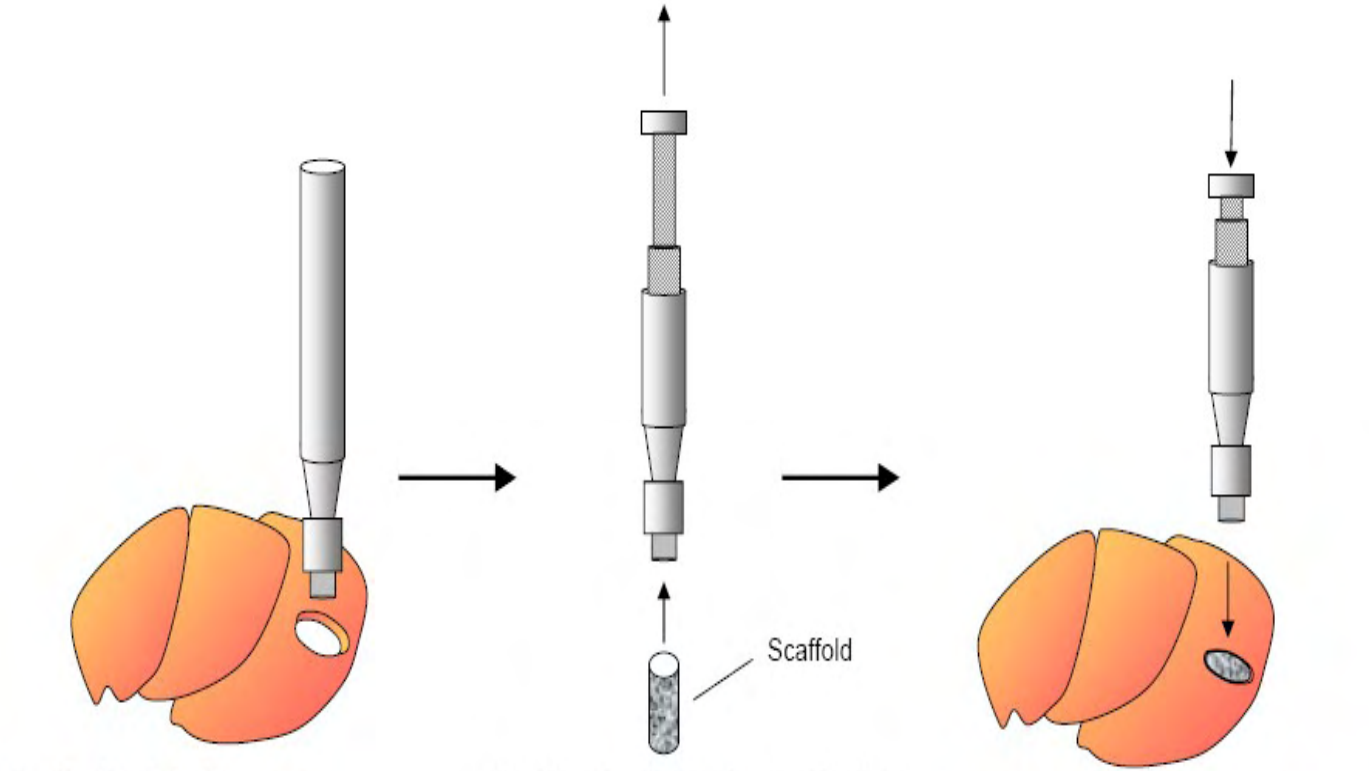
Skin: In vitro or in vivo synthesis?



Skin repair


Wound grafted with scaffold
active scaffold
1 cm
Blocked contraction. New tissue formed is skin, not scar.
inactive scaffold
1 cm
Contraction and scar
Simultaneous synthesis of epidermis and dermis using the cell-seeded scaffold.
Orgill, MIT Thesis, 1983

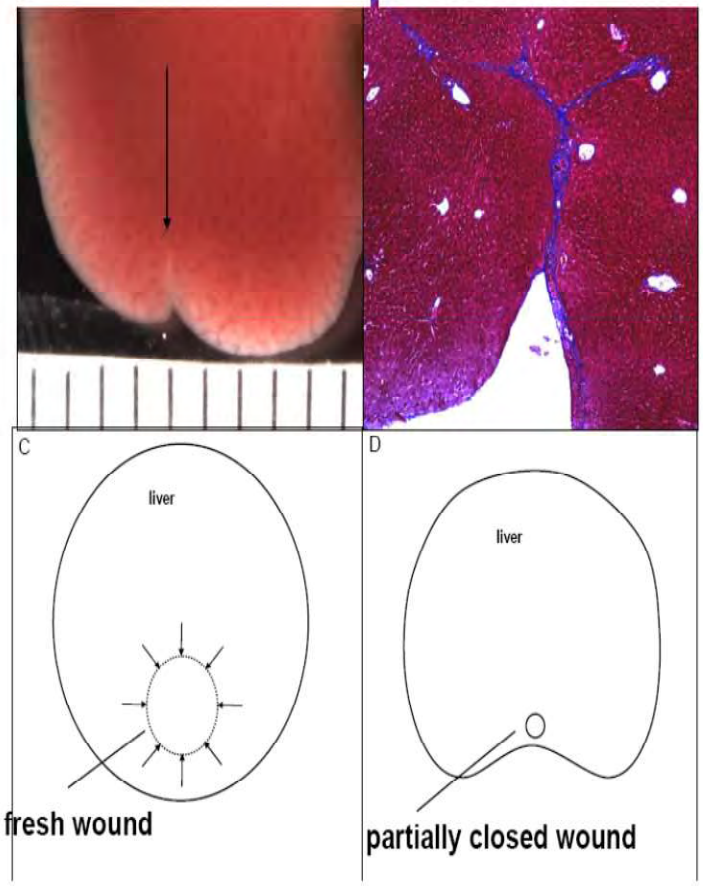
Schematic of wound model in adult mouse liver



Schematic of wound model in adult mouse liver

Spontaneously healed mouse liver
4 weeks following dissection of lobe.
Gross view.

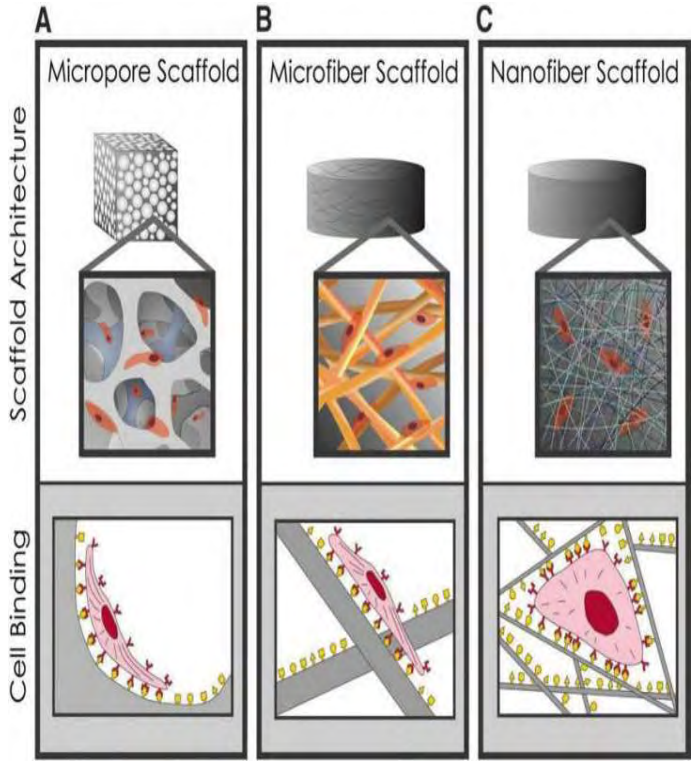
Histology (trichrome stain) shows
fibrotic tissue (blue) lining edges of
closed wound.



Why we apply nanotech in TE?

Why we apply nanotech in TE?

Tissue Engineering is a methodology
+
Nanotechnology is a tool



Cells on microfibrous scaffolds have a polarized relationship, with one side of the cell attached to the scaffold, the other exposed to physiological media. In comparison, it is likely that cells are more naturally constrained by nanofibrous scaffolds.

The Extracellular Matrix (ECM)

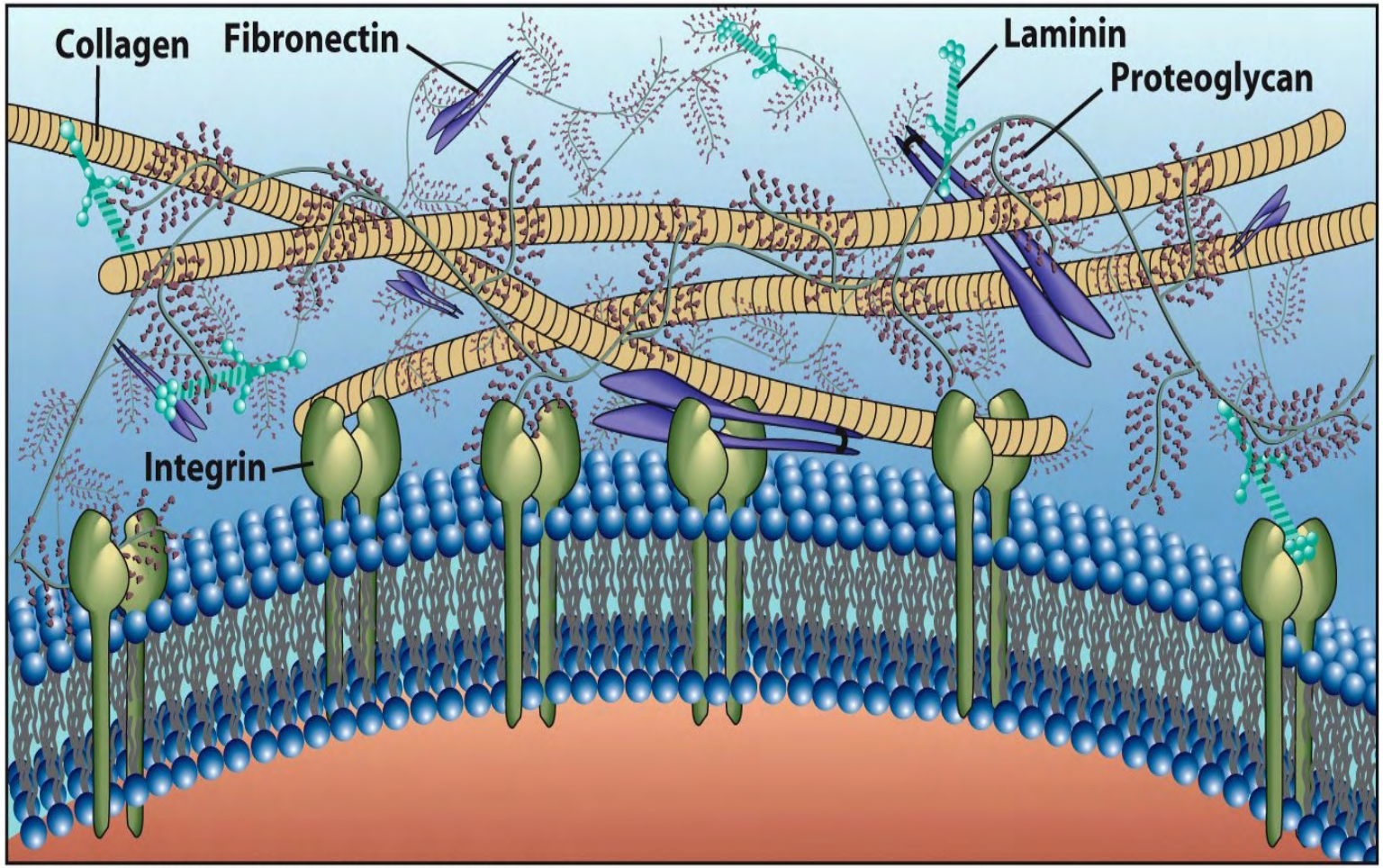
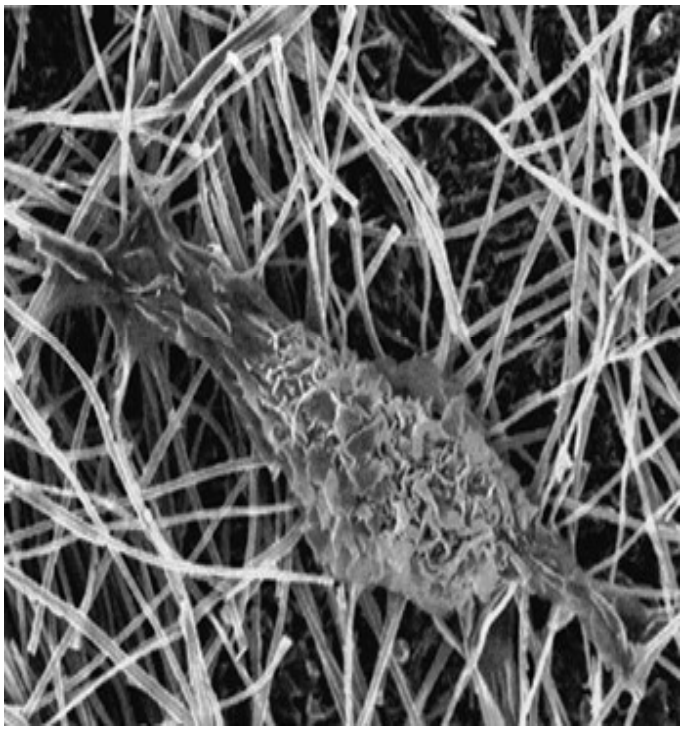


Figure 7-5 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Nanofibers - Introduction

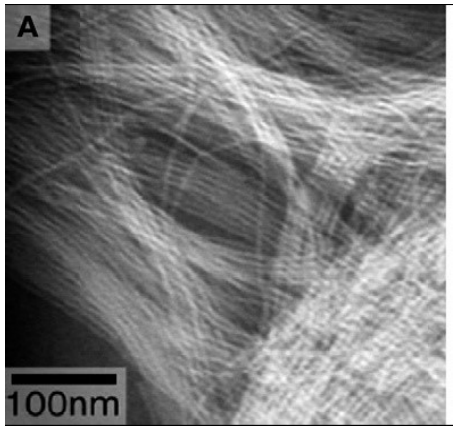
- ECM fibers ~ 50-500 nm in diameter
- Cell ~ several-10 um
- Fibers 1-2 orders of magnitude < cell

- Scale difference necessary
  - single cell contacts thousands of fibers
  - transmission of fine/subtle signals

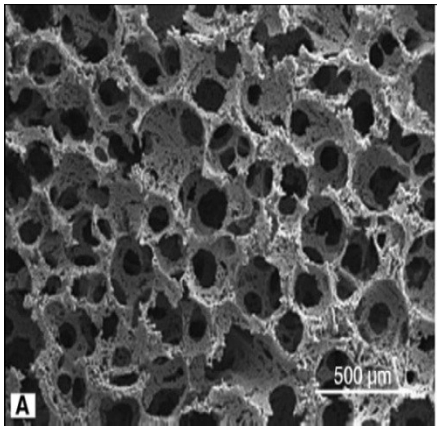


Techniques to achieve nanofibers for TE

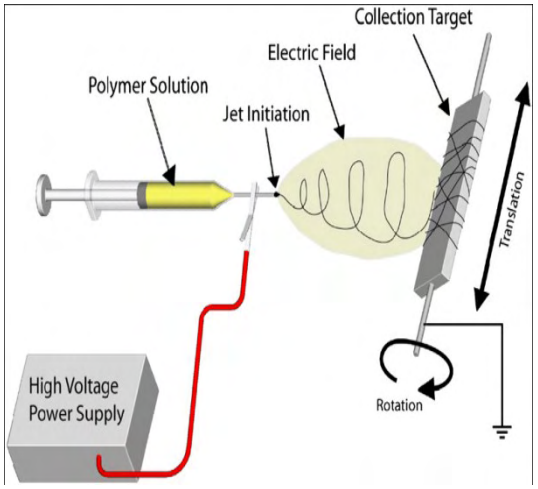
Self-assembly



Phase separation



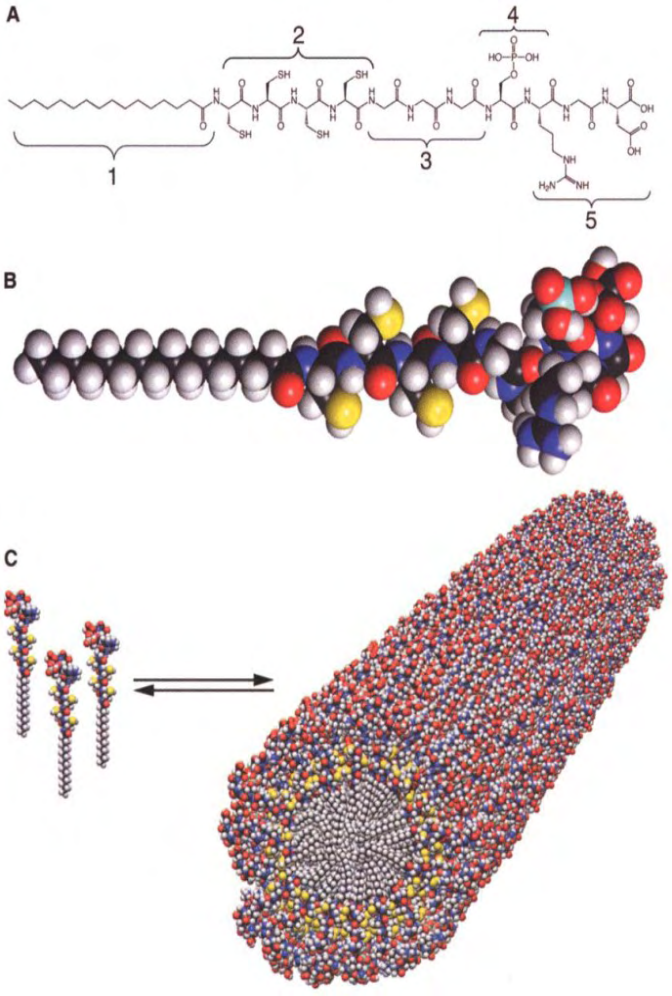
Electrospinning

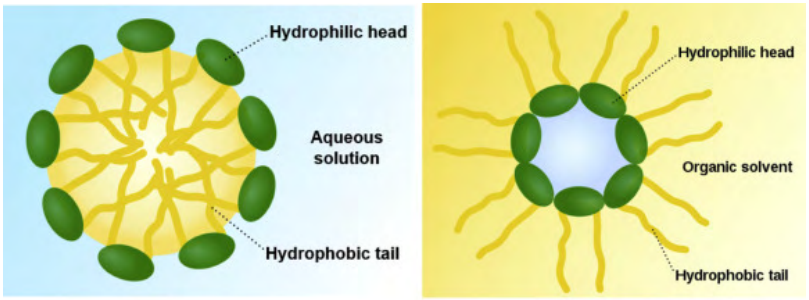


Self-assembly

- Relies on non-covalent interactions to achieve spontaneously assembled 3D structure.
- Biopolymers such as peptides and nucleic acids are used. Example is peptide-amphiphile (PA)

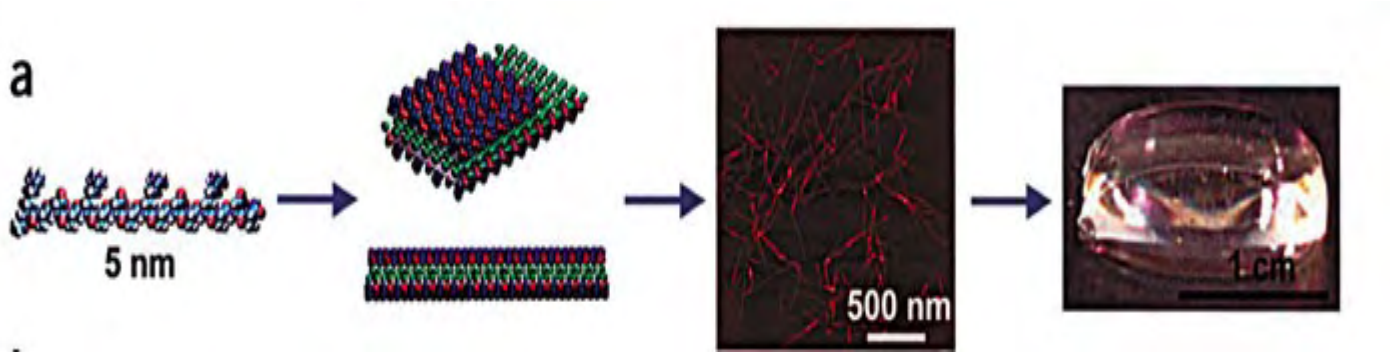
(A) Chemical structure of (PA)
(B) Molecular model of the PA showing the narrow hydrophobic tail to the bulkier peptide region
(C) Schematic of PA molecules into a cylindrical micelle.



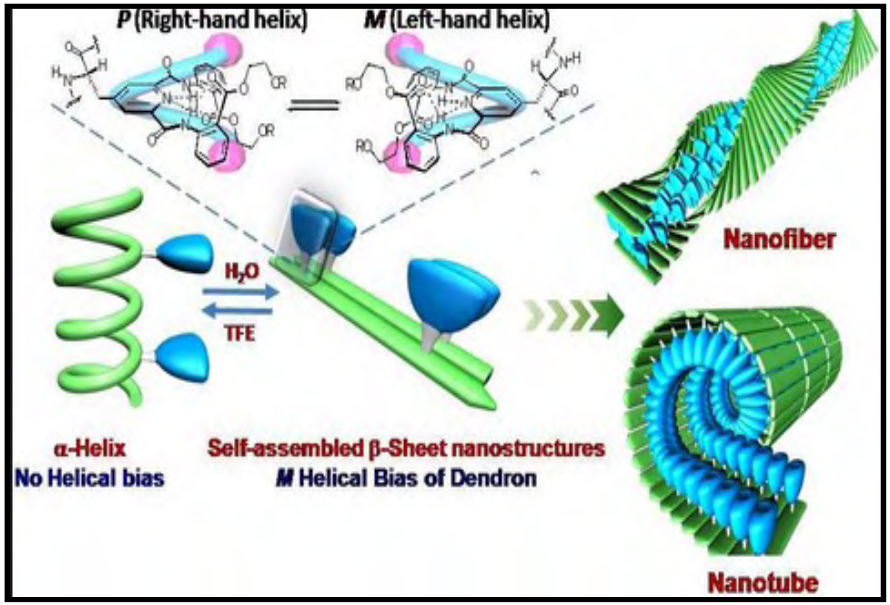


micelle reverse micelle

Self-assembly



- Peptide of 16 AA
- Alternating polar/nonpolar
- Form stable β-strands and β-sheets
- Form nanofibers by hydrophobicity
- Matrices with high H_2O content



Phase separation

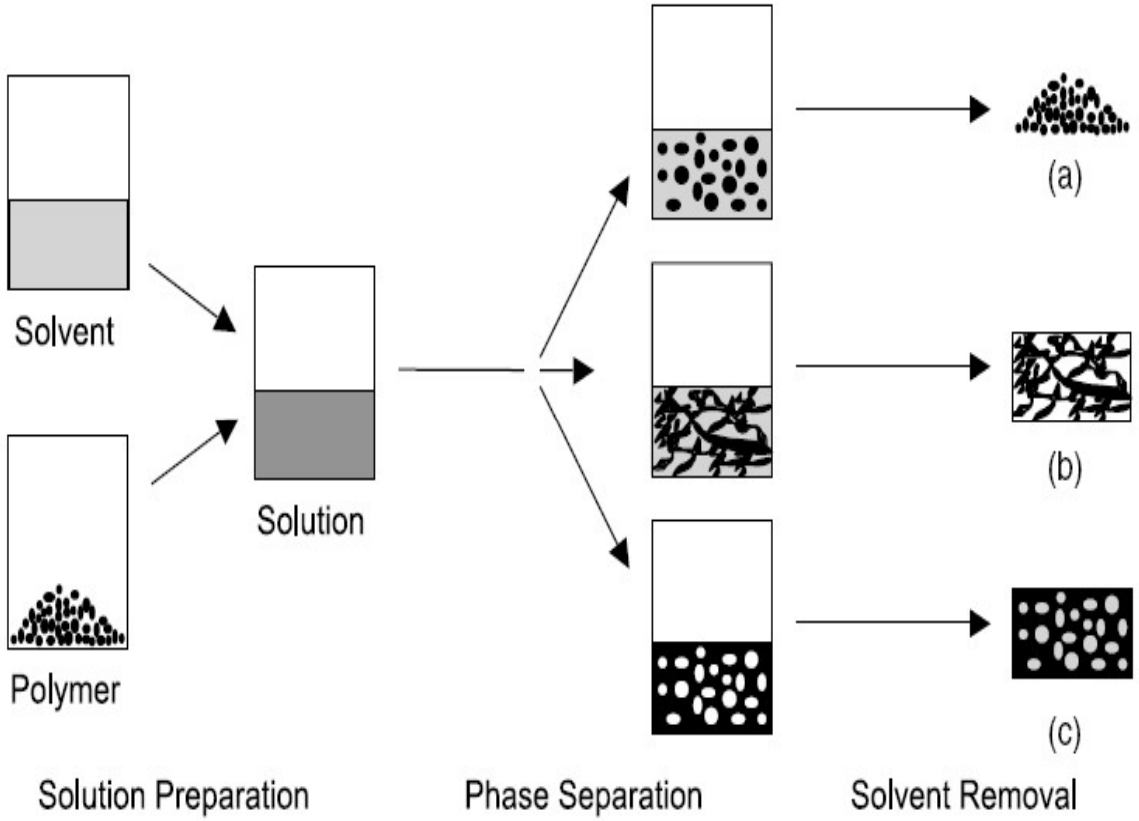
- This process involves dissolving of a polymer in a solvent at a high temperature followed by a liquid–liquid or solid–liquid phase separation induced by lowering the solution temperature.

- Capable of wide range of geometry, dimensions and irregular pore structures
- Simpler than self-assembly

Phase Separation

- Thermally induced phase separation (TIPS) is a technique that is particularly useful for generating scaffolds with a specific pore size.
- In this method, the temperature of the polymer solution is adjusted to a point at which a “polymer-rich” and a “polymer-poor” phase is generated.
- The solvent is removed, and the polymer-rich phase solidifies, forming a porous solid structure, which is then freeze dried.
- Nanofibrous scaffolds with varying fiber diameters and pore sizes can be generated by adjusting the polymer concentration, the type of solvent, and the phase separation temperature.
- Specifically, fibers ranging from 50 to 500 nm in diameter—similar to the size of native collagen—can be produced.

Phase separation

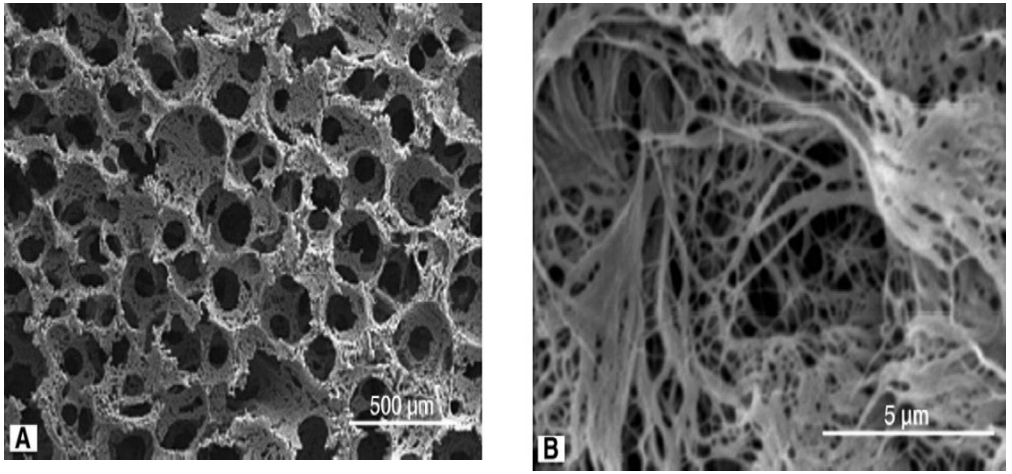


a) powder, b) scaffolds with continuous network, c) foam with closed pores

Phase separation

Drawbacks:

- limited to several polymers
- small production scale



SEM of nanofibrous scaffold with interconnected spherical macropores

Electrospinning basics

- Process by which high static voltages are used to produce fibers from a polymer solution
- Micron to submicron diameter
- Fibers have huge Surface area: Volume ratio

Making nanofibers

- “Melt” Fibers: some nanofibers can be made by melting polymers and spinning or shooting them through very small holes. As the fiber spins out it stretches smaller and smaller...

- Cotton candy is made by heating syrup to a high temperature and then the liquid is spun out through tiny holes. As the fiber spins it is pulled thinner and thinner. It cools, hardens and, presto! Cotton Candy!!

Electrospinning

- Electrospinning: A versatile method to produce fibers with diameters in the **nano range.**

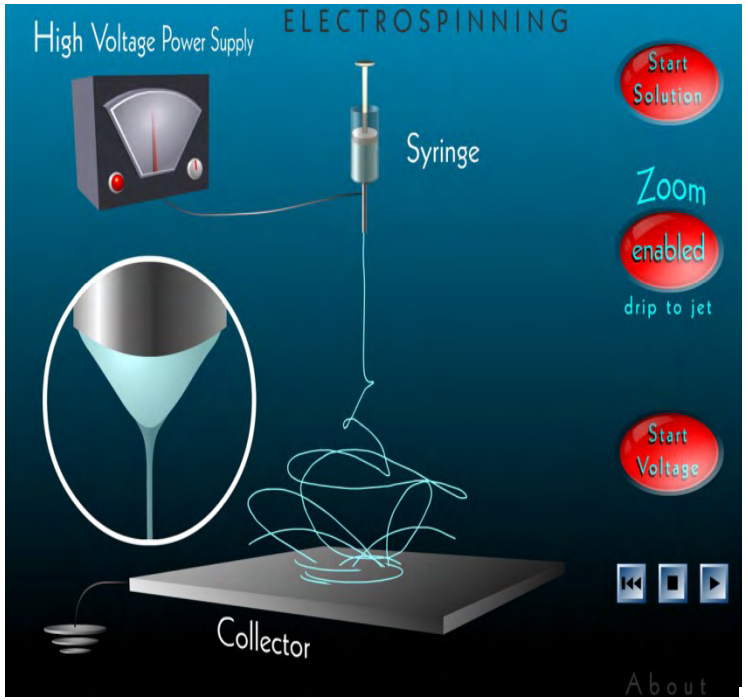
Electrospinning Procedure:

➢An **electrostatic potential** is applied between a spinneret and a collector.

➢A **polymer** fluid is slowly **pumped** through the spinneret.

➢The **droplet** is held by its own surface tension at the spinneret tip, until it gets **electrostatically charged**.

➢After threshold accumulation of charges polymer fluid assumes a **conical shape** and thin stream of **fiber elutes** from the droplet.

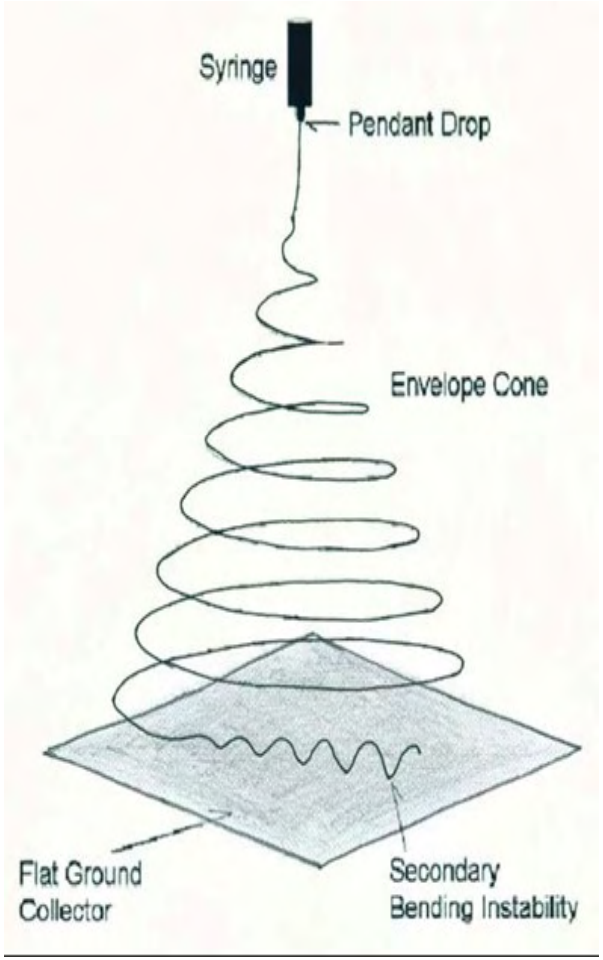


Electrospinning set up

Source: Burger, Christian, et. al. 2006.

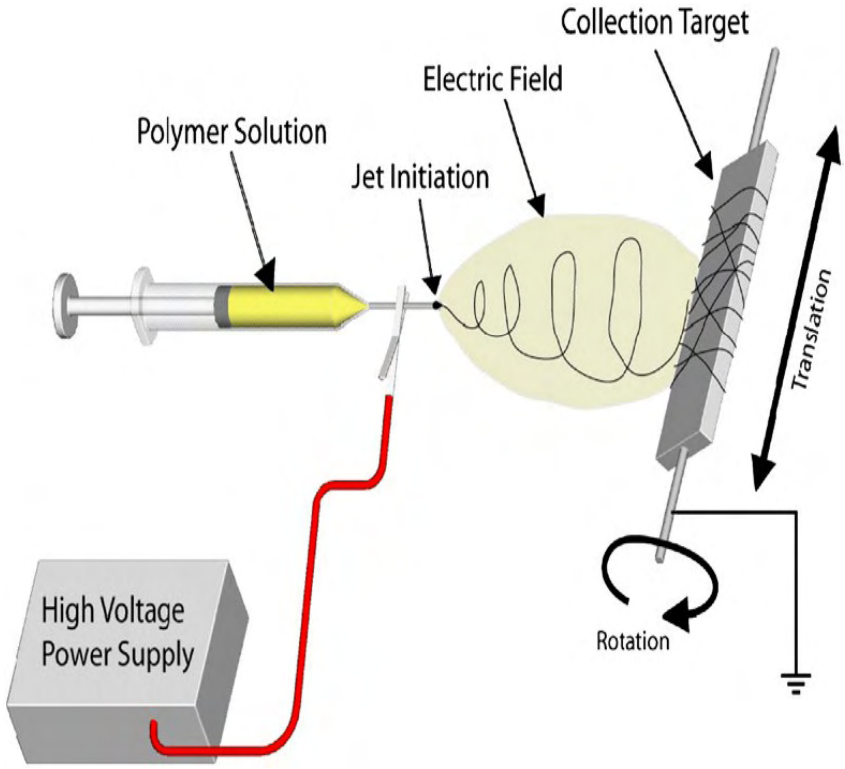
Electrospinning to make nanofibers

- An electric field pulls on a droplet of polymer solution at the tip of the syringe and pulls out a small liquid fiber. It is pulled thinner and thinner as it approaches the collection plate.

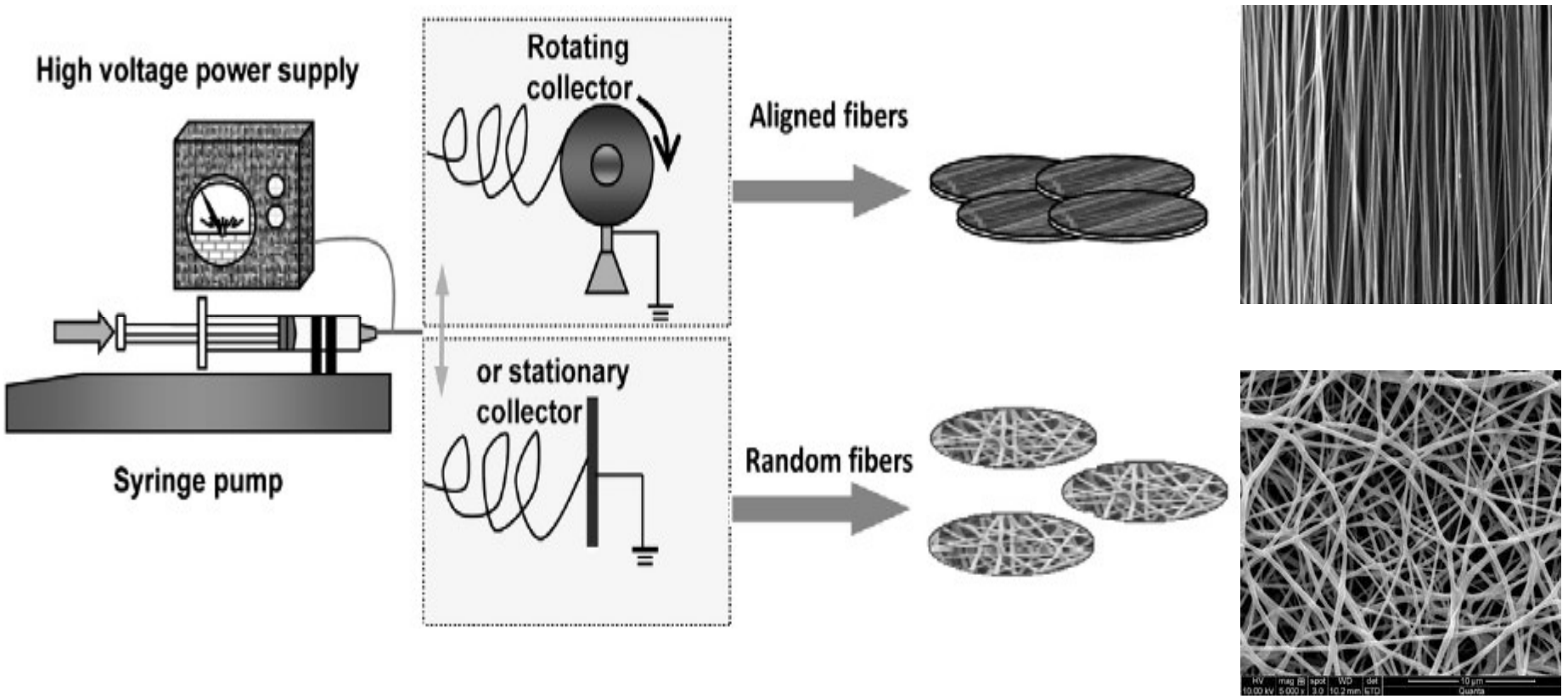


Electrospinning

- This process involves the ejection of a charged polymer fluid onto an oppositely charged surface.
- Multiple polymers can be combined
- Control over fiber diameter and scaffold architecture

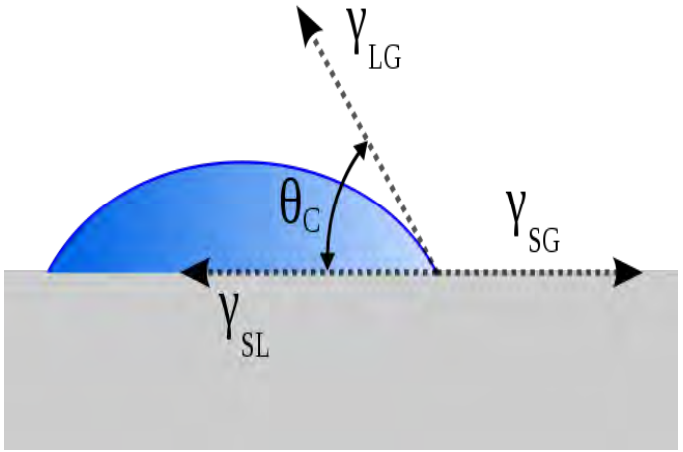


Aligned & Random fibers



S.H. Lim, H.-Q. Mao / Advanced Drug Delivery Reviews 61 (2009) 1084–1096

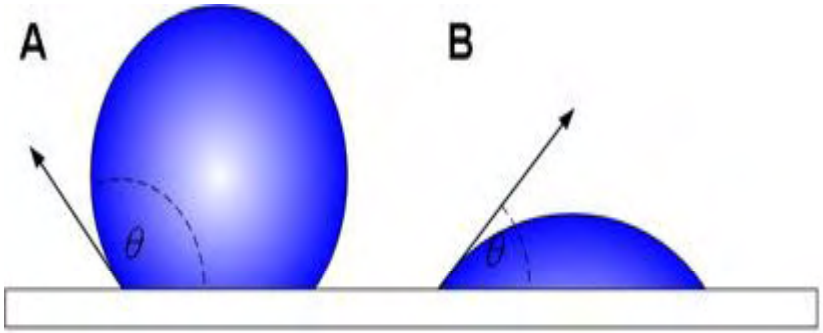
Contact angle

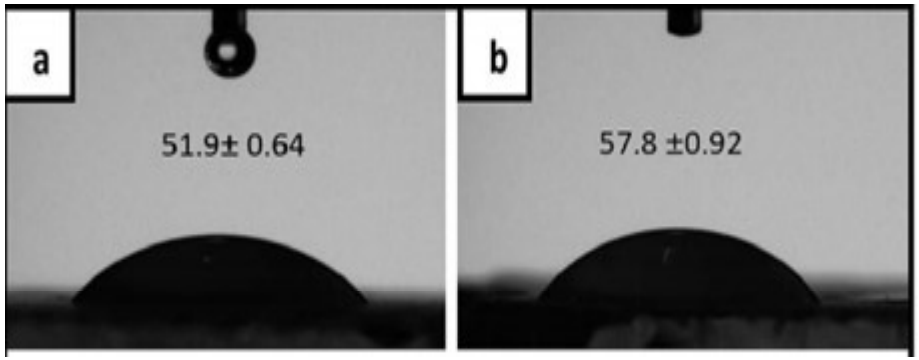


When we rest a small droplet of water on the solid surface, **tangential outline** of the droplet on the solid forms the **contact angle.**

Contact angle

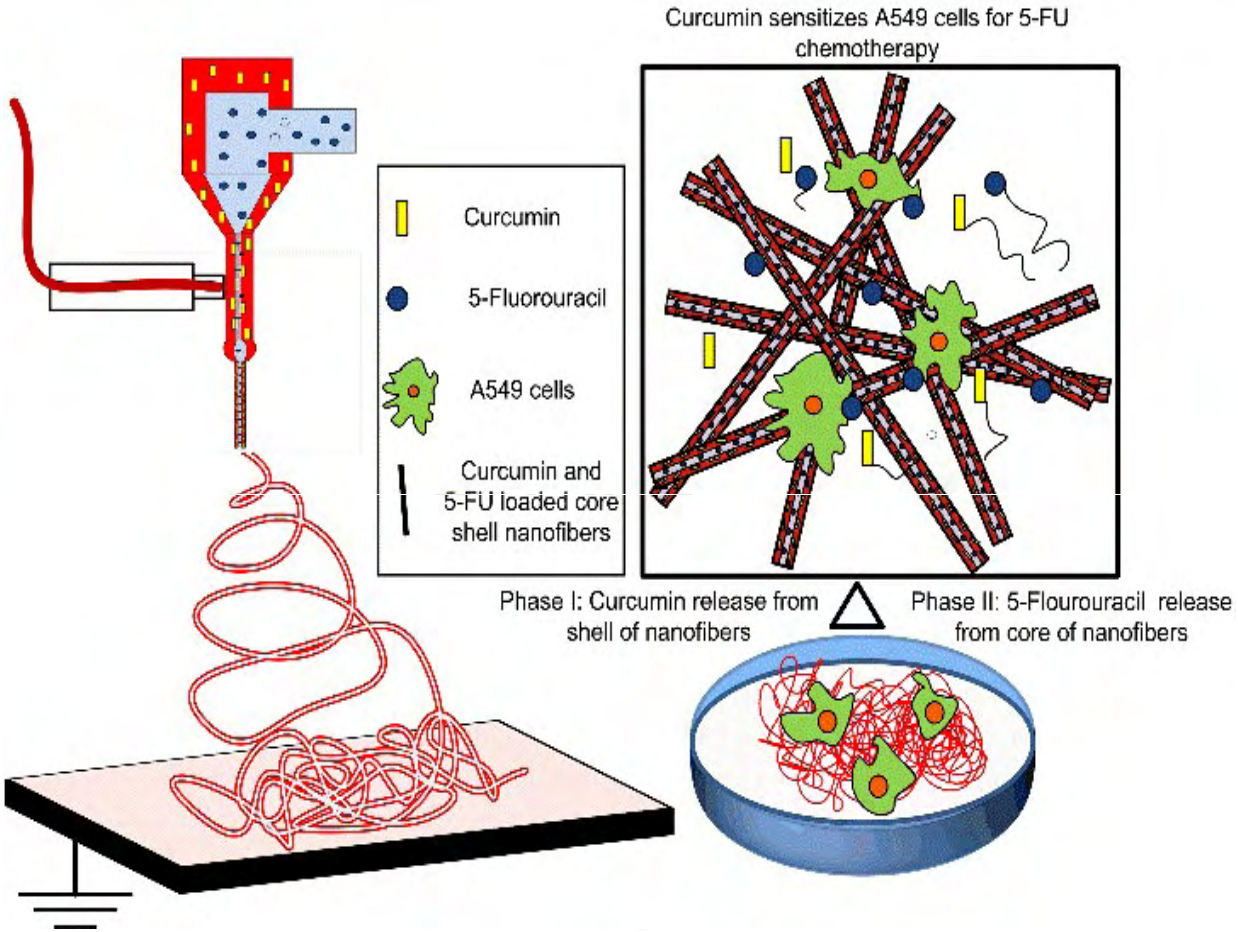
A – $\theta_c > 90^\circ$ - Hydrophobic surface **B – $\theta_c < 90^\circ$ – Hydrophilic surface**





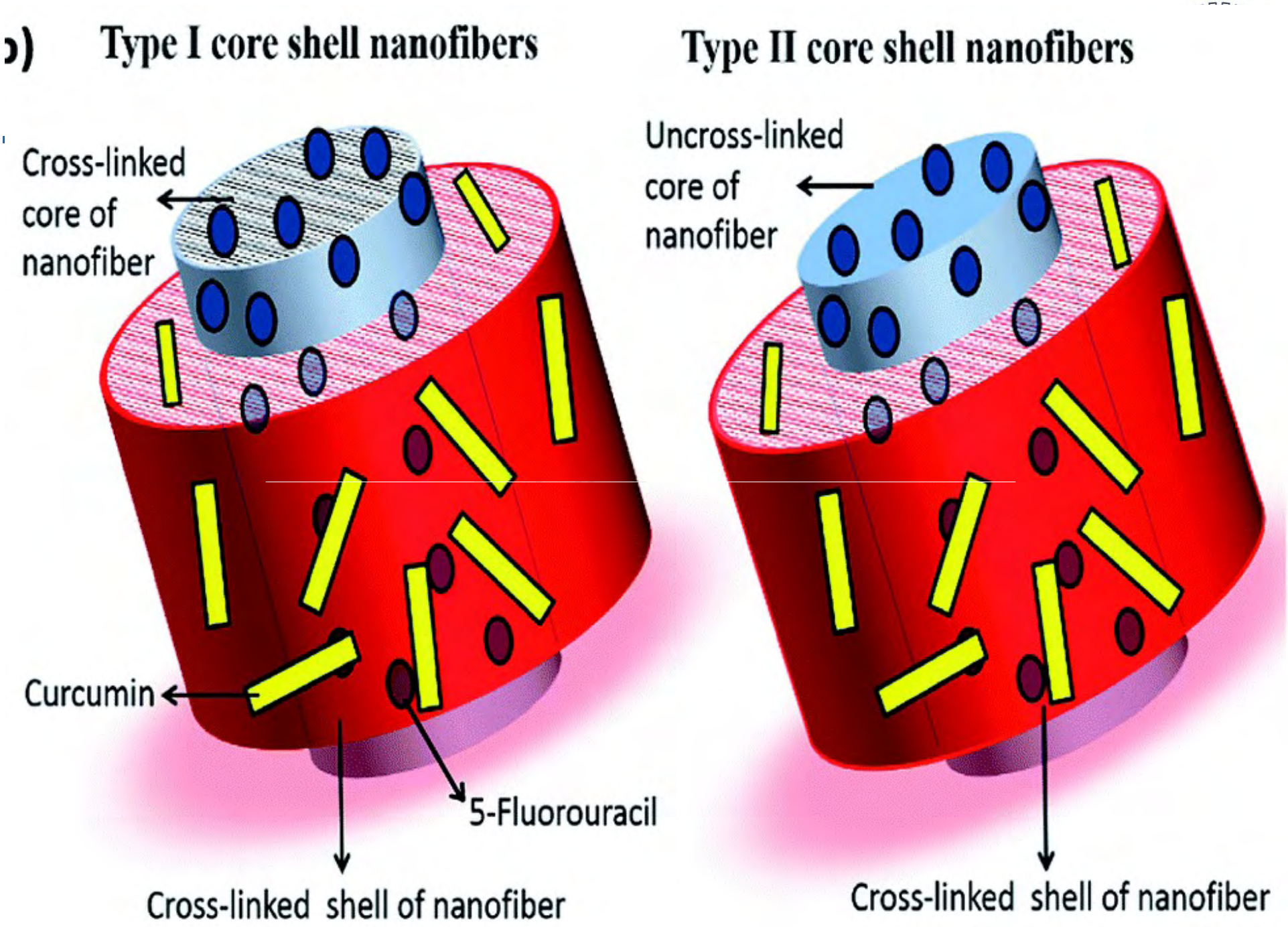
Condition	Nature of surface
$\theta_c < 90^\circ$	Hydrophilic
$\theta_c > 90^\circ$ $(90^\circ - 120^\circ)$	Hydrophobic
$\theta_c > 150^\circ$	Super-hydrophobic

Core-shell nanofibers for dual drug delivery



- In order to harness the **synergistic anticancer potential of 5-FU and curcumin** core-shell nanofibers have been fabricated in this work.

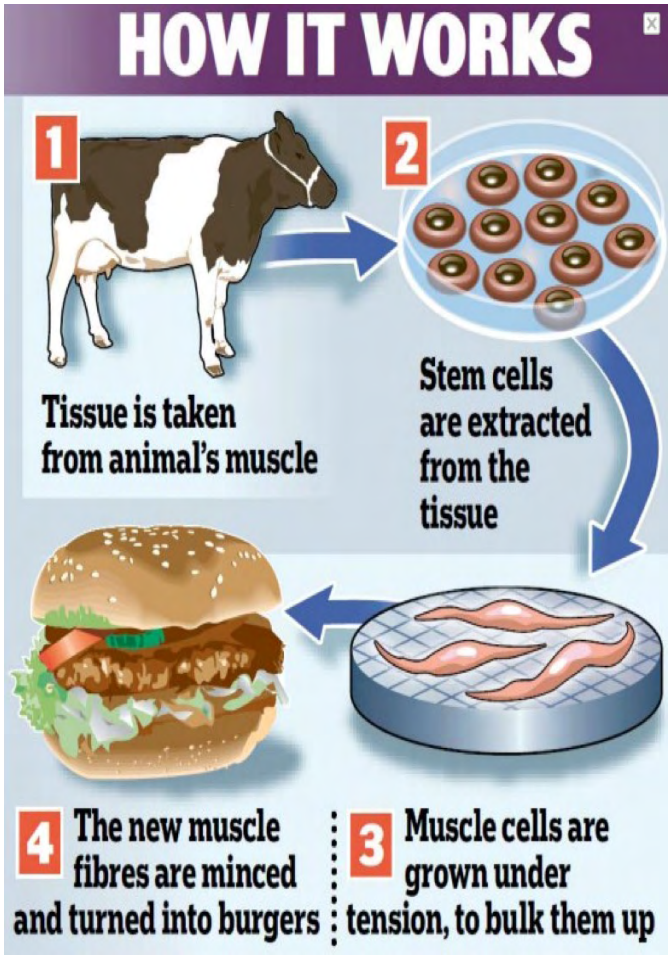
- **5-FU** is loaded in **nanofiber core** and **curcumin** is loaded in **nanofiber shell.**


Type I core shell nanofibers
Type II core shell nanofibers
Cross-linked
core of
nanofiber
Uncross-linked
core of
nanofiber
Curcumin
5-Fluorouracil
Cross-linked shell of nanofiber
Cross-linked shell of nanofiber

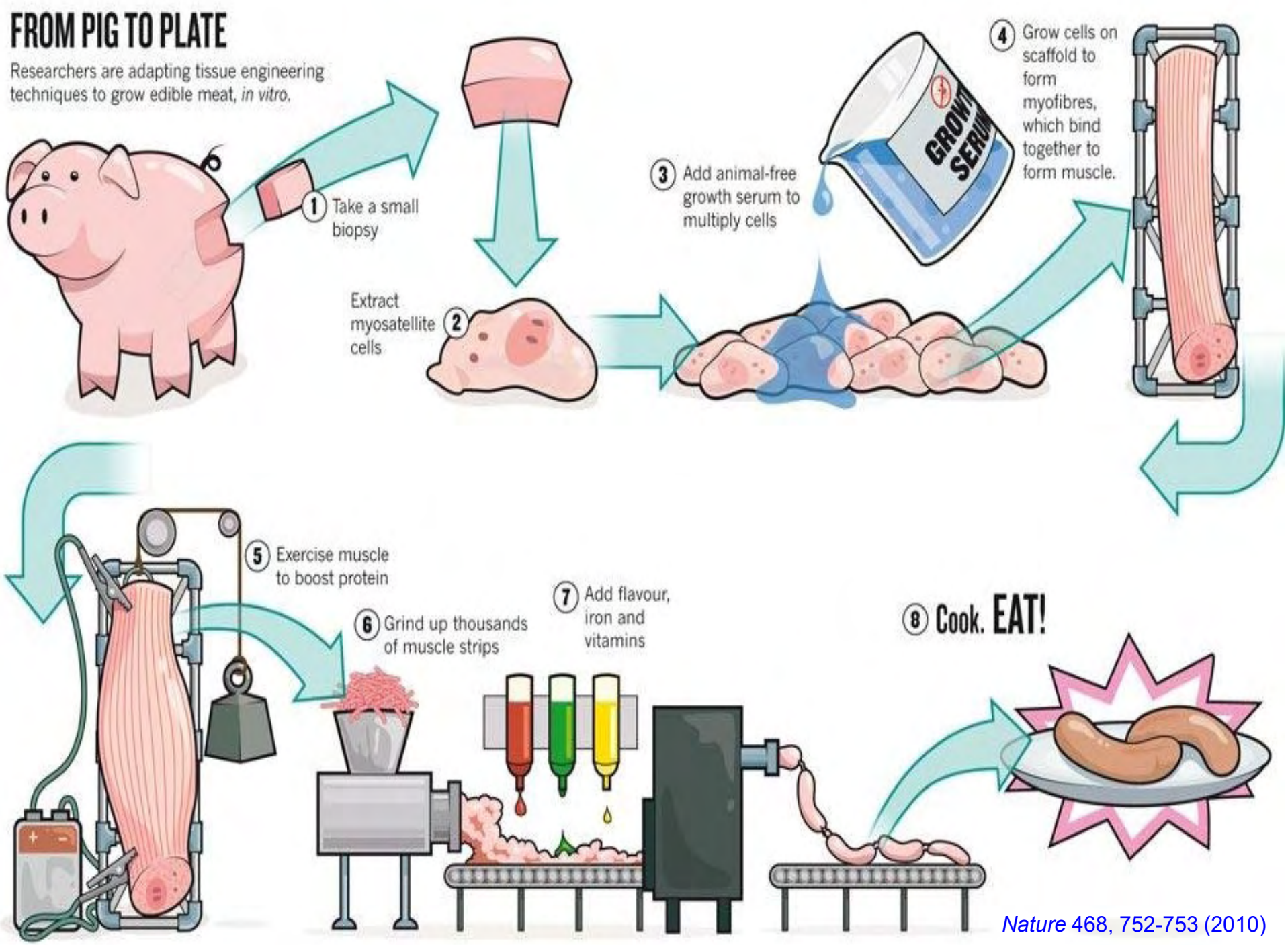
ANTICANCER DRUG LOADED NANOFIBERS FOR POTENTIAL POSTSURGICAL CANCER TREATMENT

Core-shell nanofibers provide a controlled and sustained release of anticancer drugs for preventing local tumor recurrence after surgery.

Test tube burger



https://www.geneticliteracyproject.org/2015/02/19/wheres-the-beef-and-fat-are-you-ready-for-a-juicy-test-tube-burger/



Nature 468, 752-753 (2010)

ARTIFICIAL CELLS

Contents

- Artificial cells
- Artificial RBC
- Applications of artificial cells

Introduction

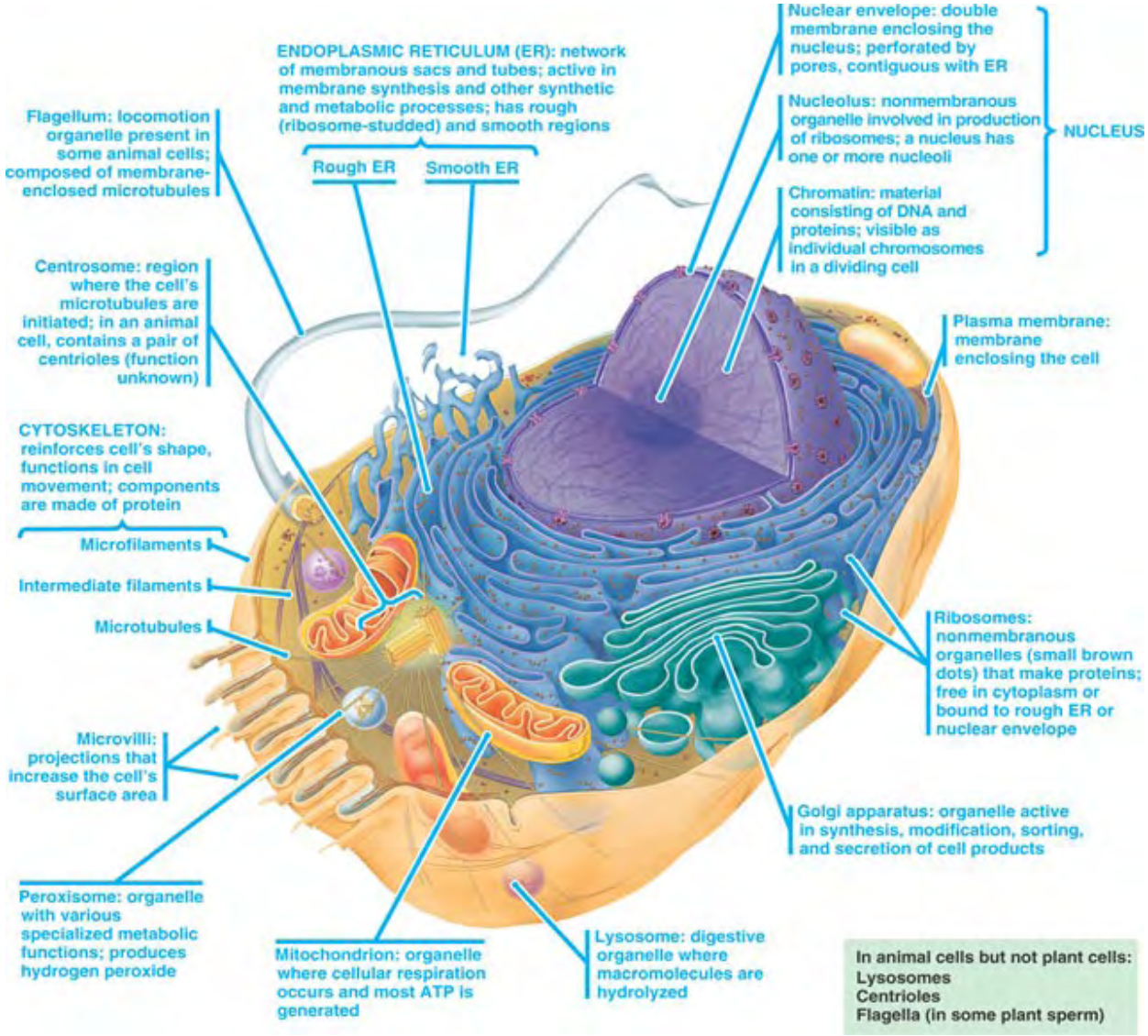
- Engineered artificial cells can be used to replace dysfunctional cells in the human body and may be used in the future to treat anemia, renal failure, bone defects, and many other health problems.

- The biggest advantage in using nano materials in the fabrication of artificial cells is their small size, ranging from 1to100 nm in diameter (Liang etal 2008).

Introduction

- Biological cells have a very high functional density and contain DNA coding for thousands of different proteins to carry out certain functions.
- Engineering artificial cells with the same magnitude of functional density as biological cells is a significant challenge and has only recently become feasible with advances in nanotechnology.
- The small size allows a larger and more beneficial surface area to volume ratio and contributes to their unique physical and chemical properties.

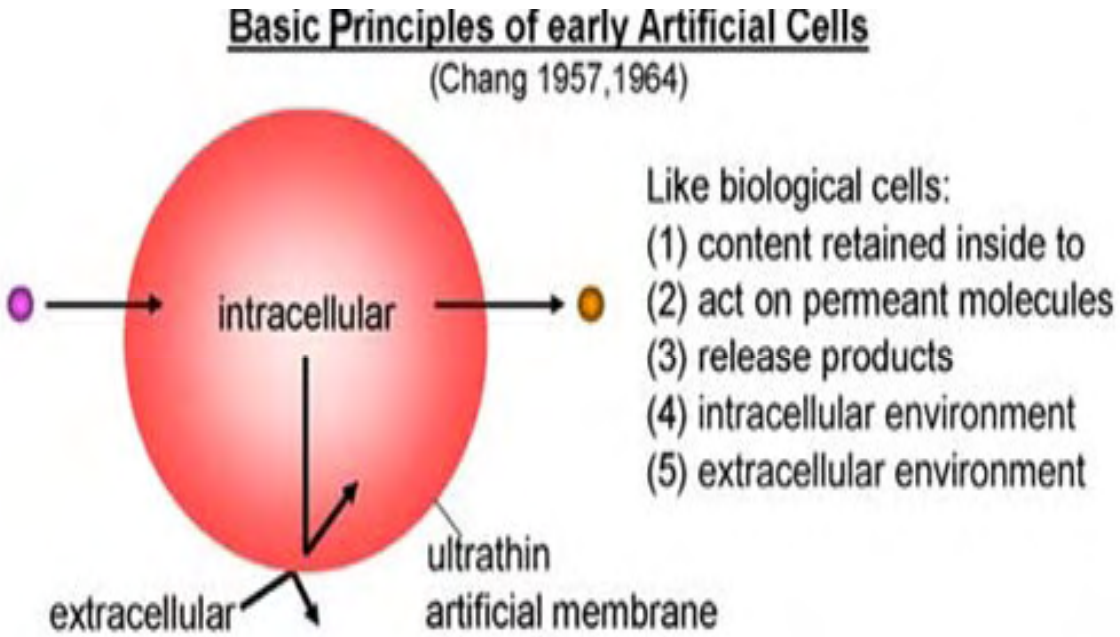
Overview of an animal cell

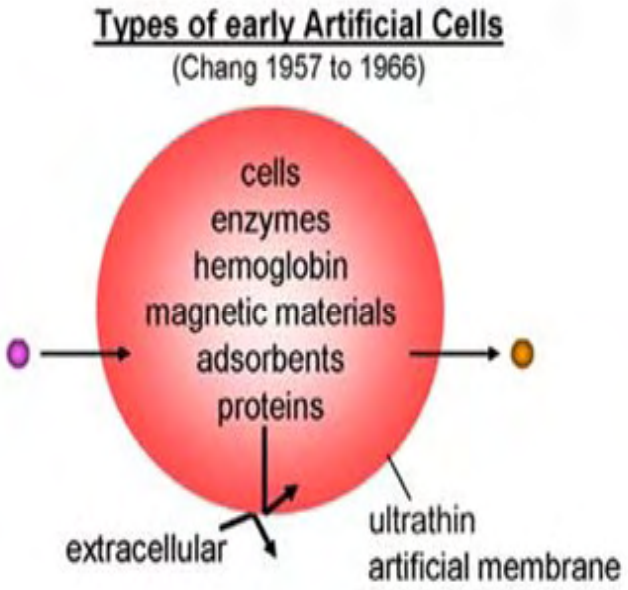


Dimensions of polymeric artificial cells

- Macro dimensions
  - For genetically engineered cells, stem cells, other cells, tissues, microorganisms, etc.
- Micron dimensions
  - For enzymes, genetically engineered microorganisms and other microorganisms, peptides, etc.
- Nano dimensions
  - For blood substitutes, enzymes, peptides, magnetic materials, drugs, etc.

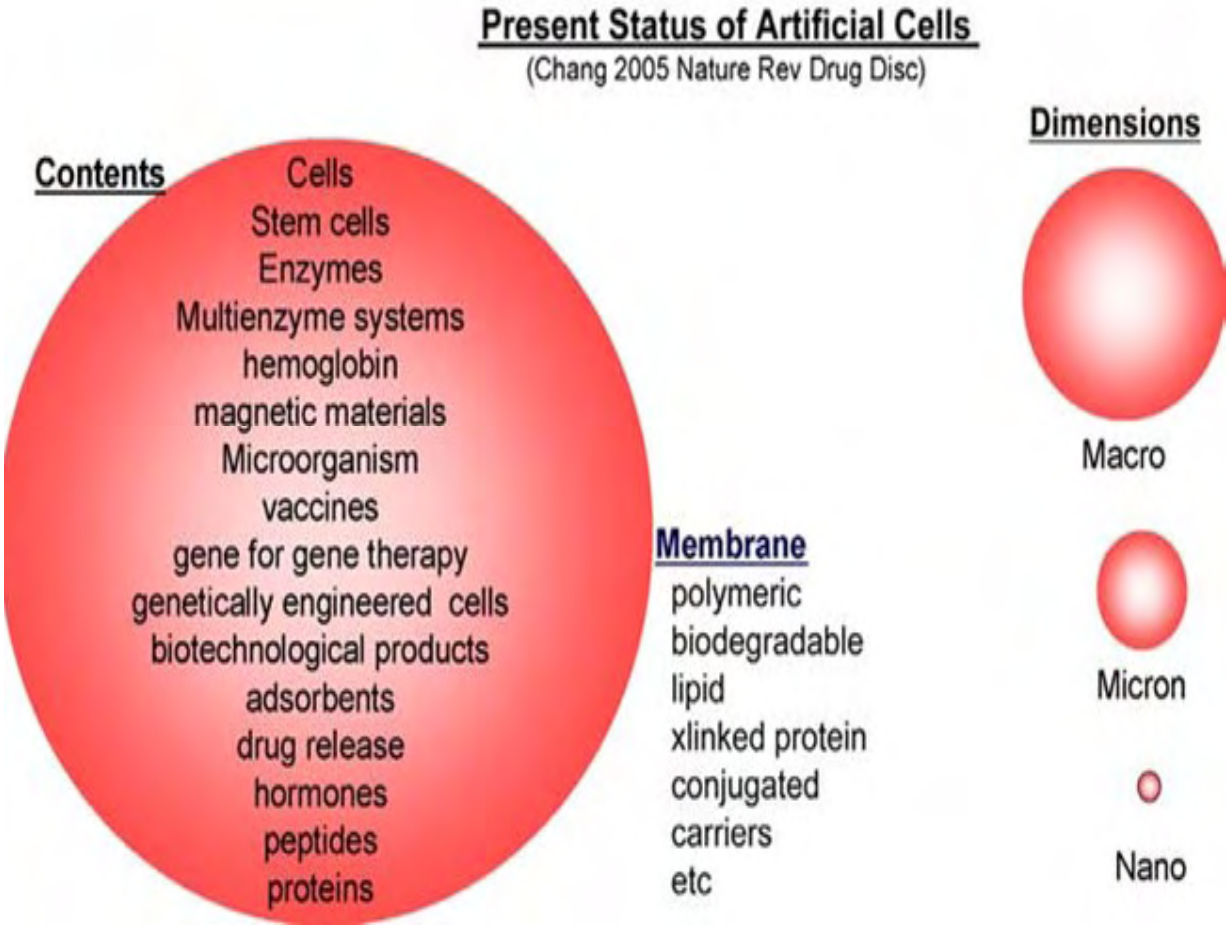
Artificial cells





http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

Artificial cells



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Artificial cell technology

- An artificial cell or cells are systems that biomimic native cellular function to recapitulate either function and/or structure.
- Artificial cell technology seeks to address the need for a more efficient temporary if not permanent replacement.
- Incontrast to tissue engineering, the field of artificial cells is concerned with singular cells and recapitulations of functions, instead of whole complex tissues.

Artificial cell technology

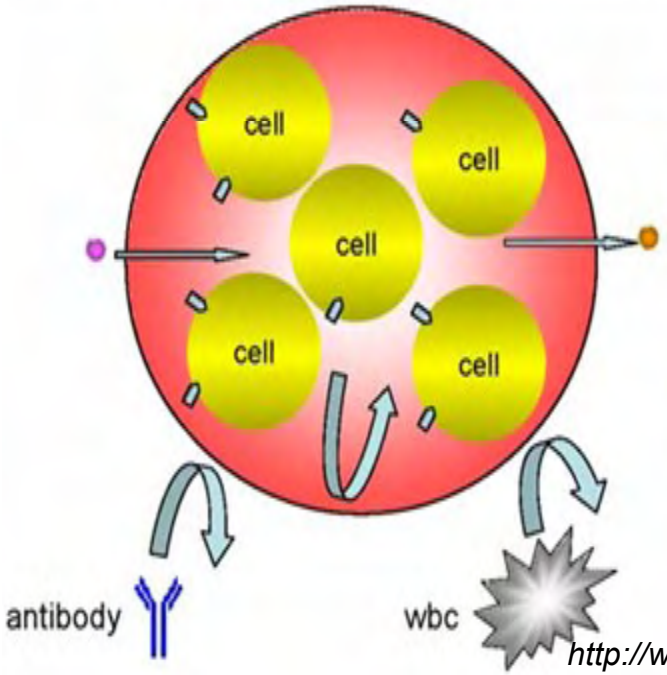
- The wide range of artificial cells can be glimpsed in the various applications that arose ranging from whole cell encapsulations of pancreatic islet cells and hepatocytes, liposome encapsulation of hemoglobin(Hb), and polymerized Hb.
- In the treatment of enzyme and single system defects, the application of whole cells may be detrimental, and replacing the enzyme or the single system may be more efficient as is the case with artificial red blood cells.
- The application of nanobiomaterials is necessary to both better biomimic cellular systems and construct a more efficient system than nature itself.

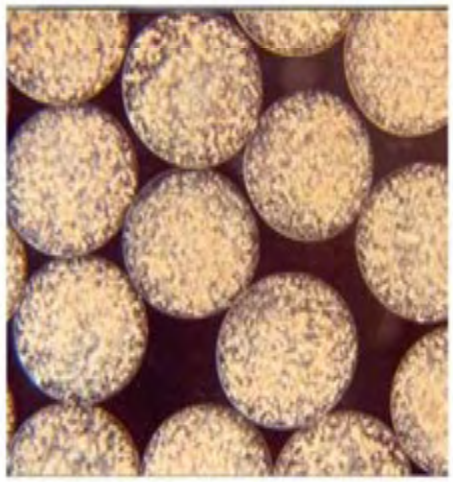
Cells in artificial cells

CELLS IN ARTIFICIAL CELLS (AC)

(Chang, 1964 *Science*; Chang, 1965; Chang *et al.*, 1966)

(1) Cells inside AC protected from immuno-rejection (antibody, wbc).
(2) Oxygen & nutrients equilibrate rapidly into artificial cells.
(3) Secretion (e.g. insulin) controllable by permeant material (e.g. glucose).
(4) Conversion of waste metabolites and toxins (e.g. hepatocytes).





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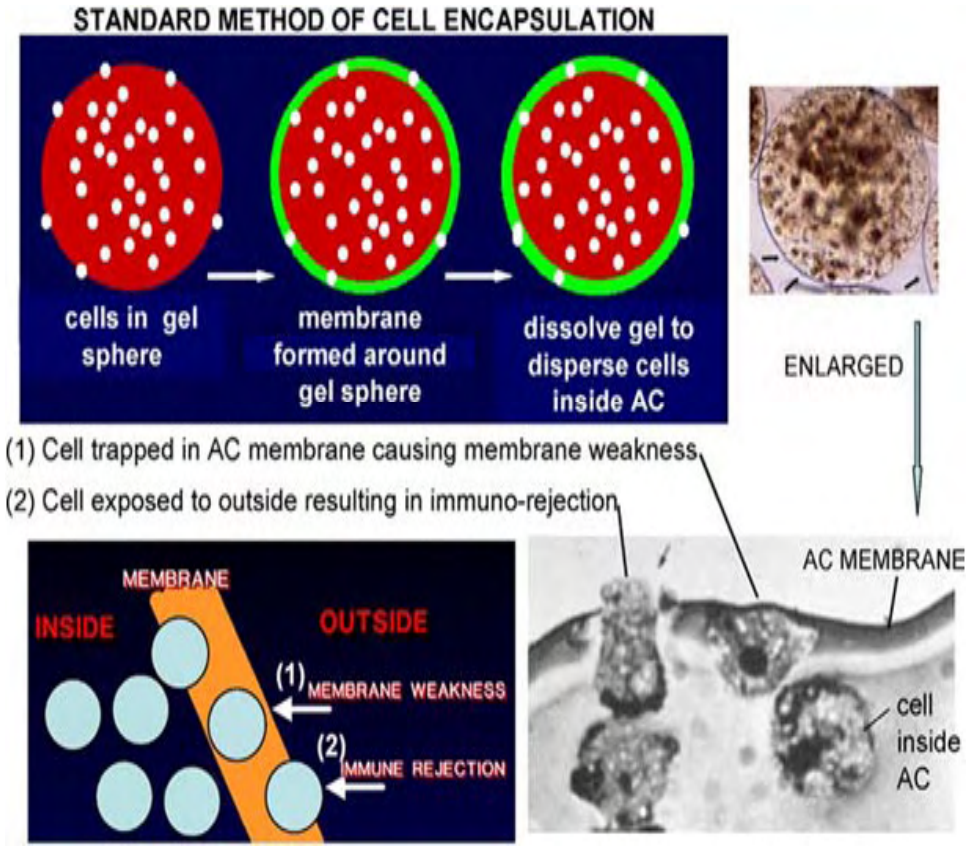
Artificial cells for cell encapsulation



- Standard drop method of preparing artificial cells to encapsulate cells results in weakening of the membrane and exposure of cells, resulting in immuno-rejection. New 2-step method solves this problem.
- This method involves first forming very small alginate gel microspheres containing cells. These small microspheres are then enclosed in larger gel microspheres.

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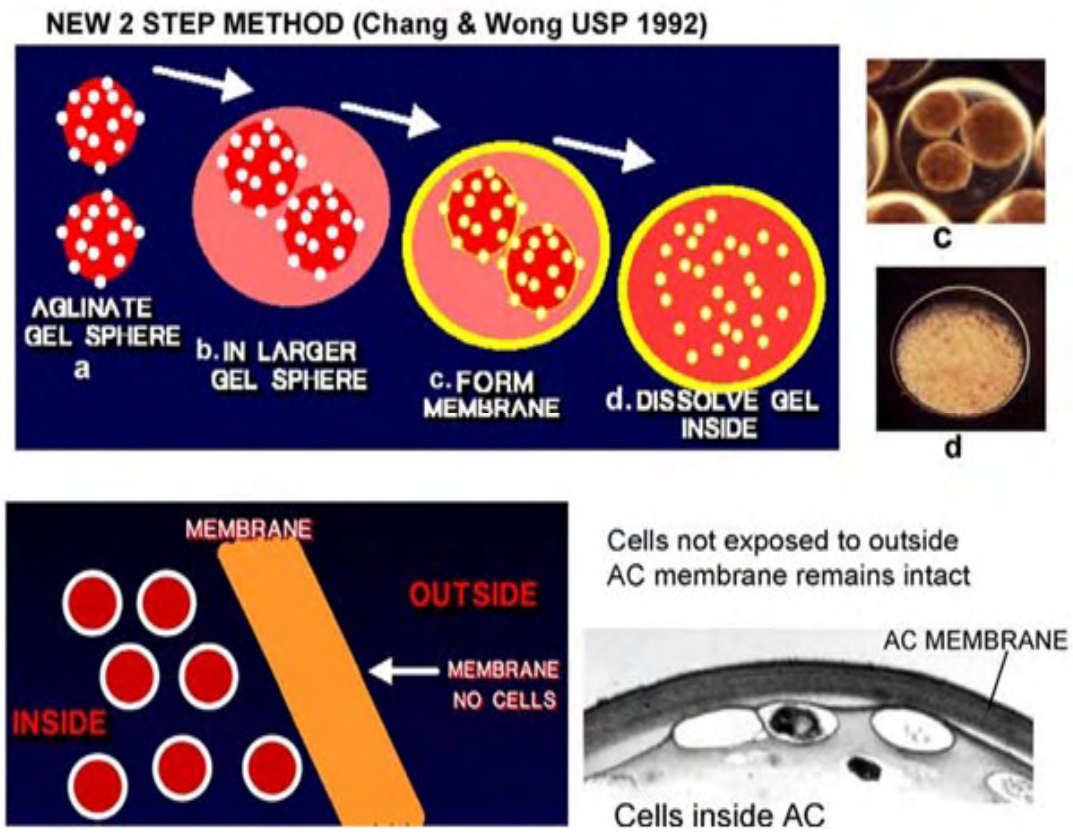
Artificial cells for cell encapsulation



In this way, there will be no cells on the surface of the larger gel spheres. As a result of this, no cells will be trapped in the alginate-polylysine-alginate membrane when it is formed around each larger gel microspheres.

The small alginate gel microspheres as well as the larger gel microspheres are then dissolved to release the cells, allowing them to be freely suspended in the artificial cells. Thus, the cells are dispersed freely inside the artificial cells.

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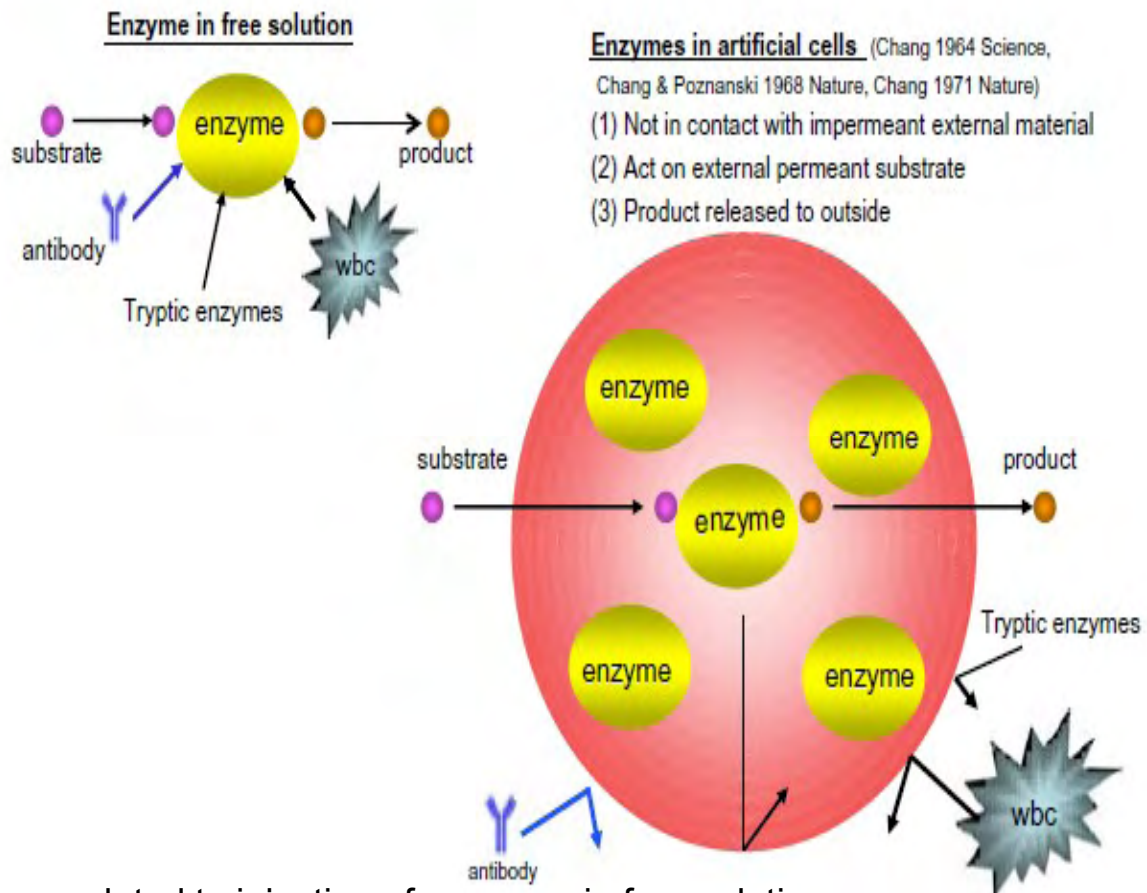
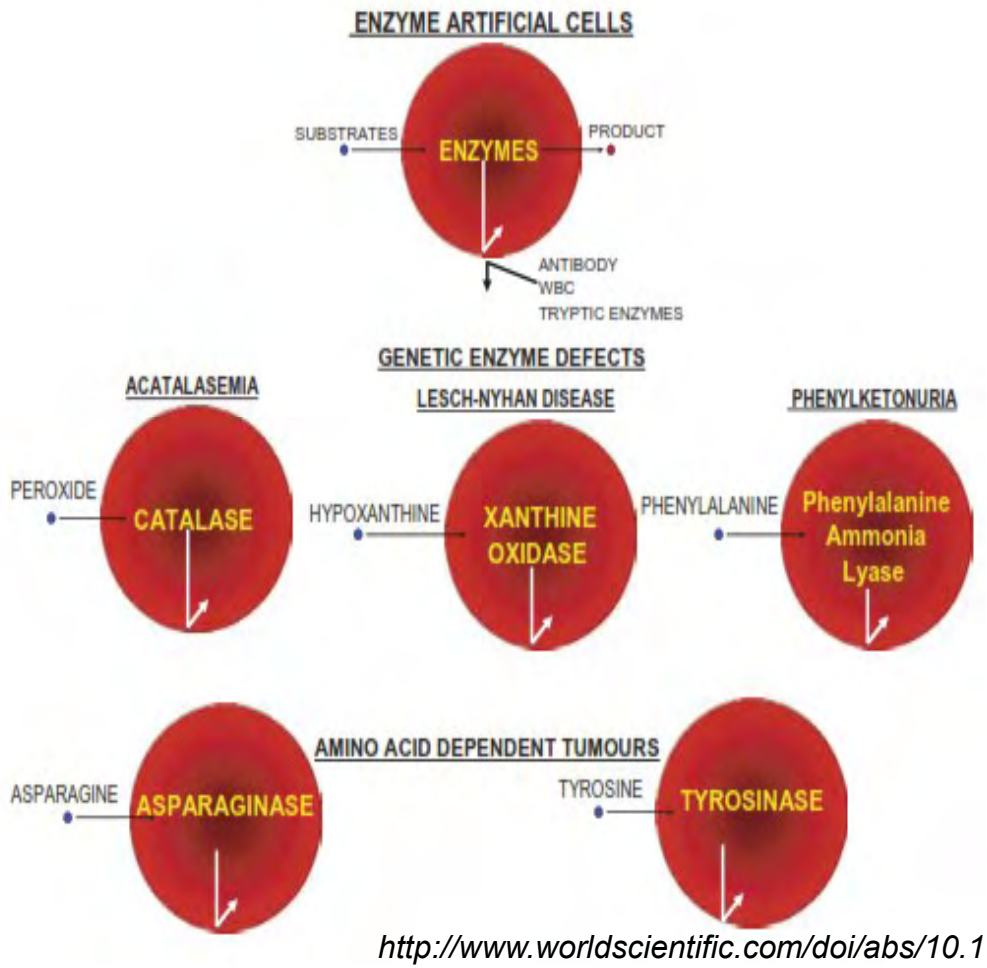
Enzyme artificial cells



(Left): Problems related to injection of enzymes in free solution.
(Right):Enzymes inside artificial cells no longer have these problems.

Enzyme artificial cells



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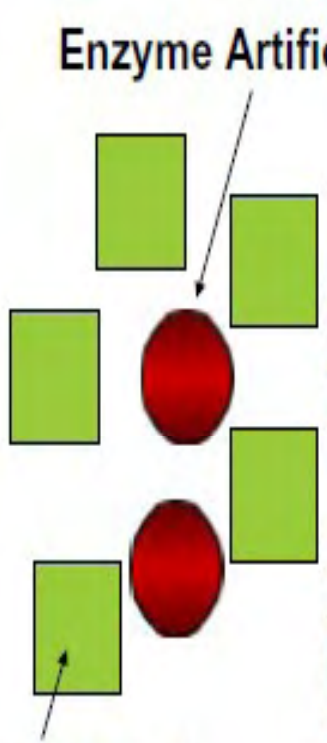
Enzyme artificial cells



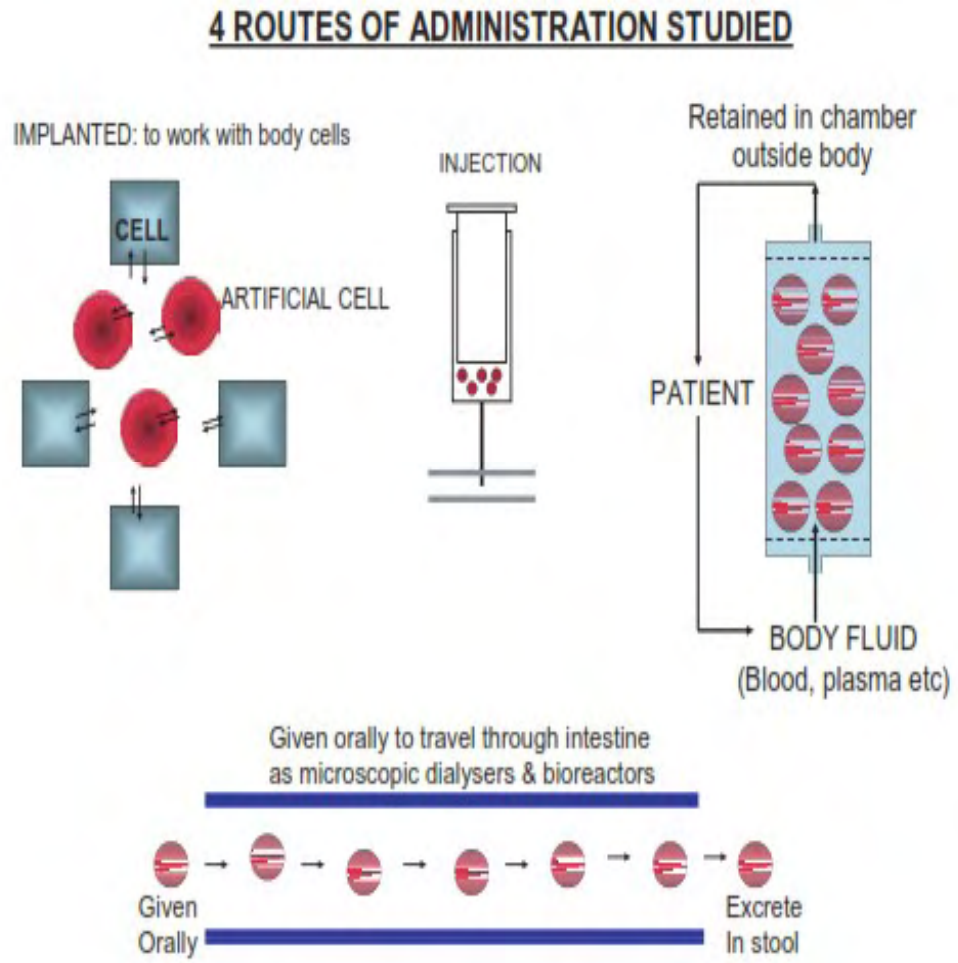
Inborn error of metabolism *(Chang&Poznansky,1968 Nature)*
Catalase Artificial Cells for Enzyme-gene therapy
in acatalasemic mice: congenital catalase defect

Suppress cancer growth *(Chang 1971 Nature)*
Asparaginase artificial cells suppress lymphosarcoma
in mice. Now extended by others as PEG-asparaginase for
clinical trials in leukemic patients

Suppress melanoma a fatal skin cancer
(Yu & Chang 2004 Melanoma Research J)

http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

Enzyme artificial cells



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Artificial cells containing microorganisms

- Microencapsulation of cholesterol removing microorganisms-
  - *Pseudomonas pictorum*

Nano biomaterials for artificial red blood cells

- One major finding in the development of artificial cells is their possible use as oxygen carriers in the bloodstream for the treatment of anemia
- Blood substitutes, unlike blood itself, serve solely to carry oxygen and carbon dioxide throughout the body.
- A lot of the research being done focuses on hemoglobin-based products, which include PEG modified liposome-encapsulated hemoglobin, nanoparticle and polymersome encapsulated hemoglobin, and polymerized hemoglobin solutions (Sarkar 2008)
- One example of an oxygen carrier encapsulates a solution of concentrated hemoglobin and is called a hemoglobin vesicle (HbV).
- HbV was found to have oxygen-carrying capacity comparable to that of normal red blood cells (Bucci2009)

Red blood cell substitutes

- Hemoglobin molecules extracted from red blood cells are modified by microencapsulation or cross-linkage to produce red blood cell substitutes.
- The encapsulation and linkage processes stabilize the hemoglobin molecules and also allow sterilization of the products to remove human immunodeficiency virus (HIV) and other microorganisms.
- The membranes of artificial cells allow permeant molecules such as oxygen and substrates to enter and allow metabolic products, peptides, and other material to leave.

Red blood cell substitutes

- In this way the enclosed materials are protected from immunological rejection and from other materials in the external environment.

- There are many situations wherein modified hemoglobin has the potential to substitute for red blood cells, including surgery and in emergency treatment for severe traumatic injuries resulting from traffic accidents and other accidents that cause severe bleeding and hemorrhagic shock.

- Modified hemoglobin does not contain red blood cell membrane and therefore no blood group antigens, it can be used without the need for cross-matching or typing.

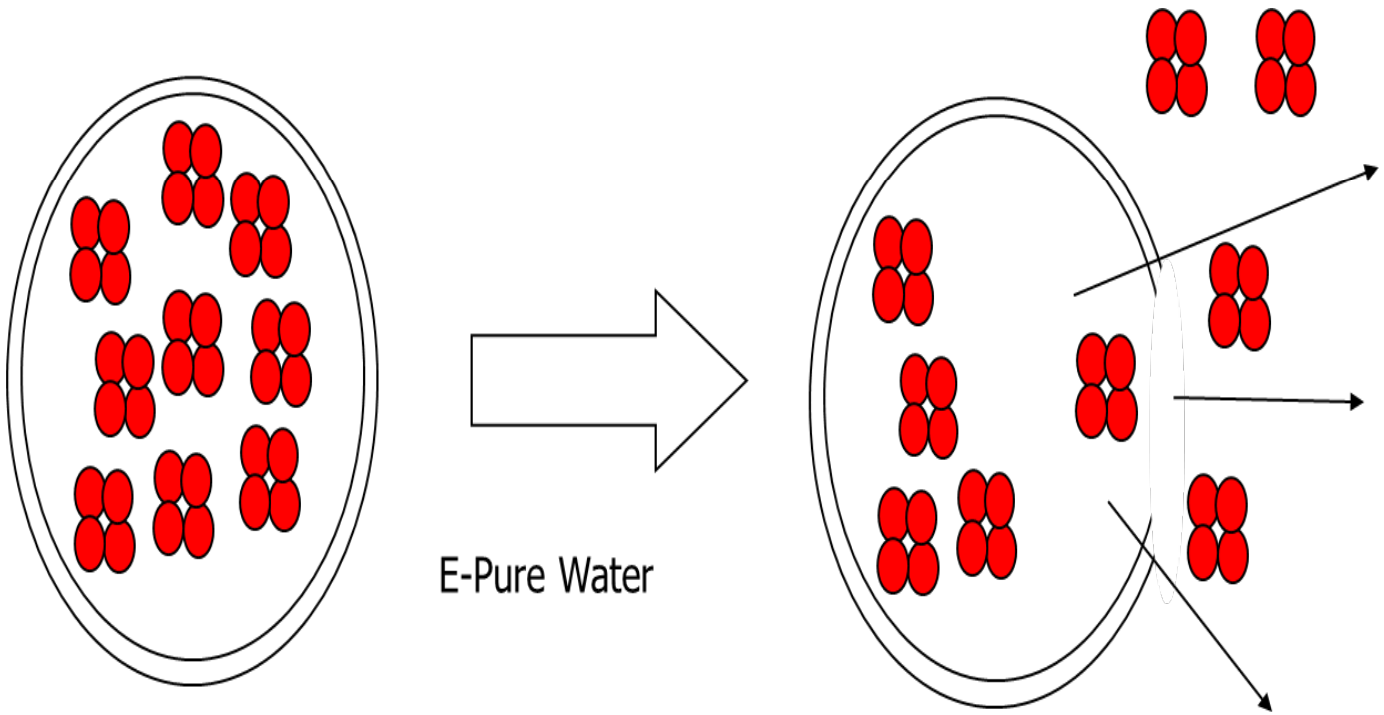
- Modified hemoglobin can be lyophilized and stored as a stable dried powder that can be reconstituted with the appropriate salt solution just before use.

Artificial red blood cells

- Modified hemoglobin
  - high oxygen carrying capacity
  - do not have blood group antigens
  - longer half life
  - non toxic

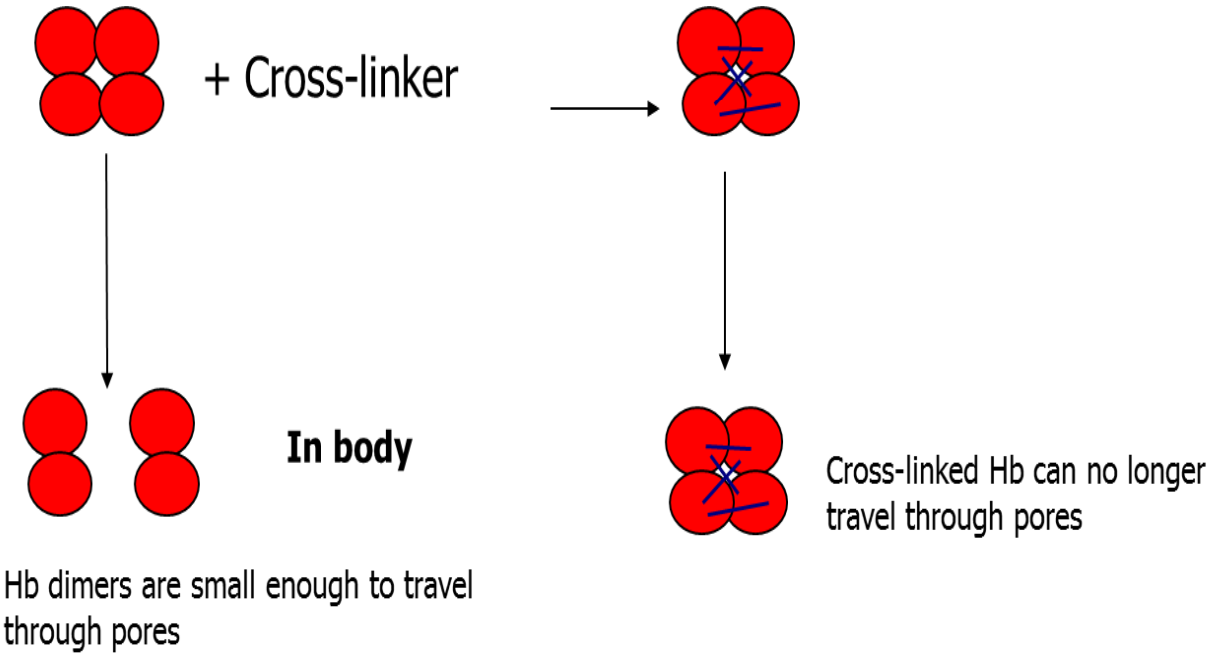
Extracting Hb from RBC's

- RBC's contain Hb which transport O_2 through body
- RBC's are lysed with E-Pure water to extract Hb

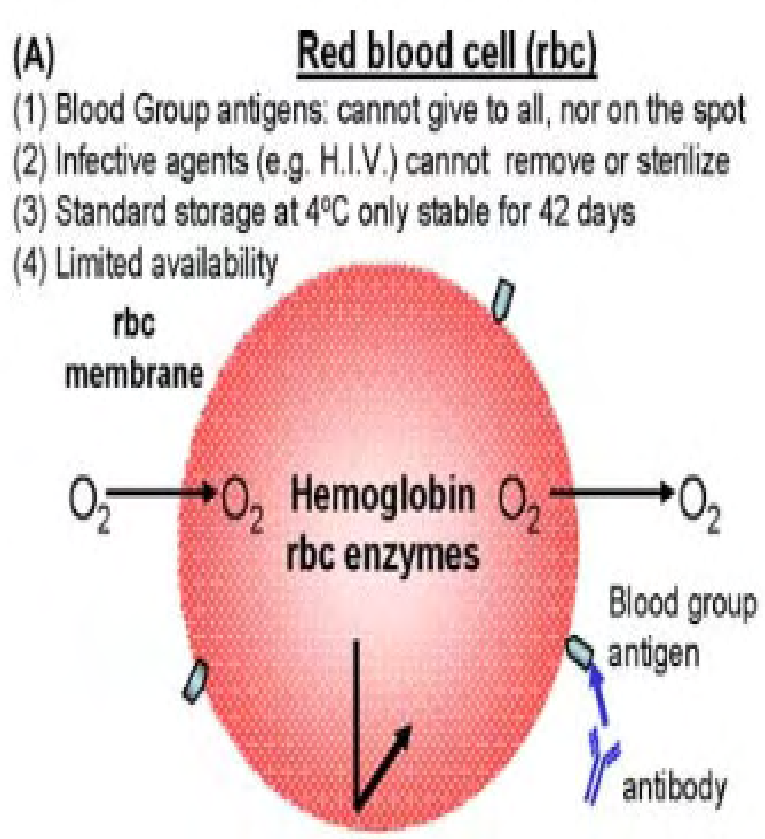


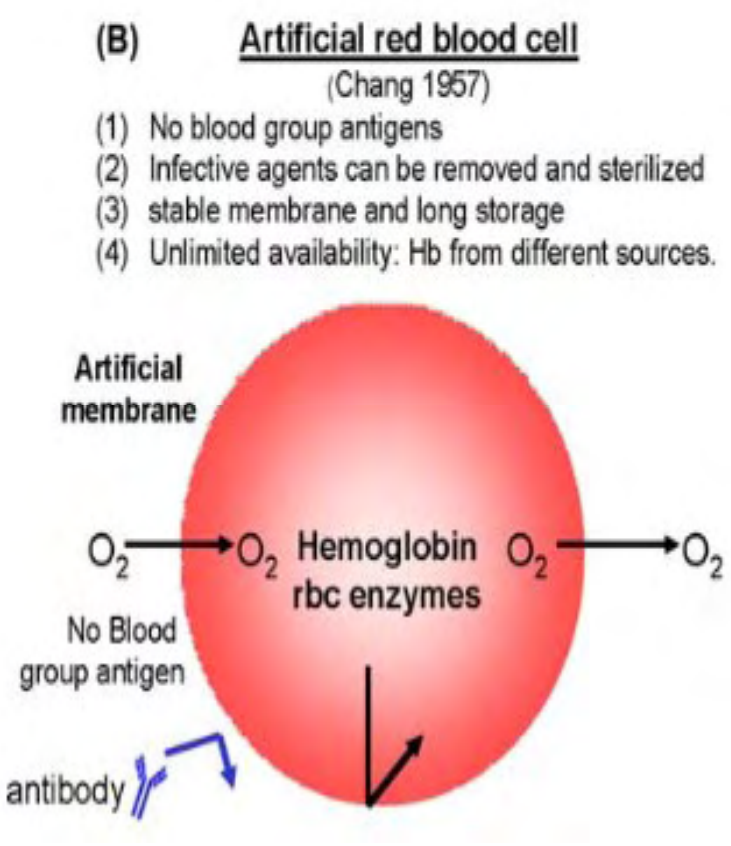
Why cross-link Hb?

- Hemoglobin must be cross-linked when placed in the blood stream.
  - Hb breaks into dimers which can travel through capillary pores (holes) and cause death.



Red blood cell vs Artificial blood cells





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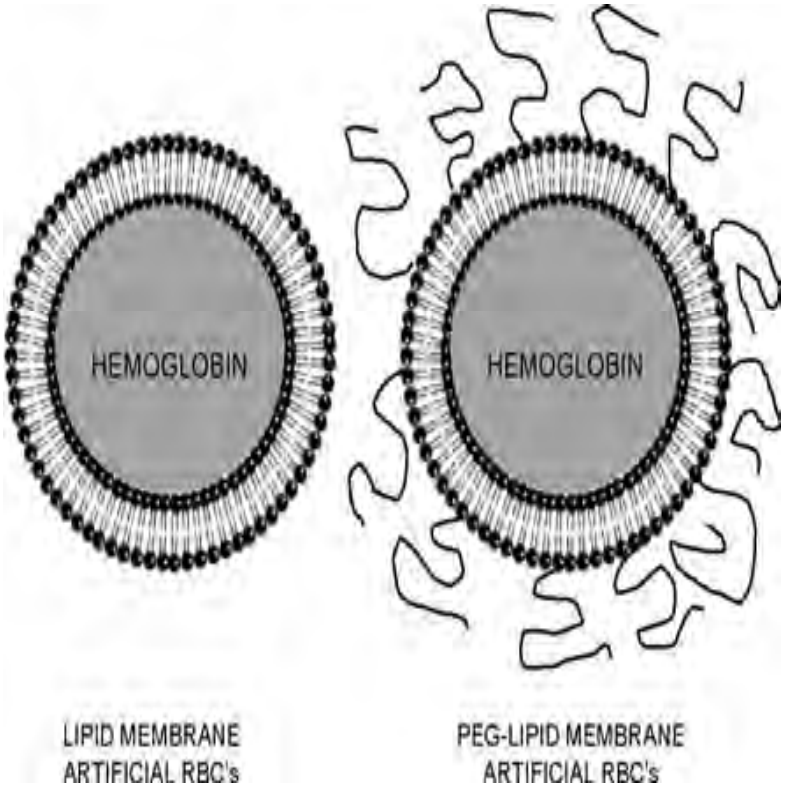
Size of artificial red blood cells

- Several investigations have been conducted in order to find the “ideal” size requirements for nanoparticles developed as oxygen carrying artificial cells.
- Theoretically, the nanoparticles must be able to circulate freely through even the smallest of capillaries, which can be as small as 4–7 μm in diameter.
- However, nanoparticles above approximately 200 nm will be removed by the spleen through mechanical filtration and consequently accumulate in the spleen.
- On the other hand, nanoparticles with diameters below approximately 70 nm will be removed by the liver.
- As a result, it has been suggested that the 70–200 nm range is optimal for intravenous delivery and circulation.

PEGylation

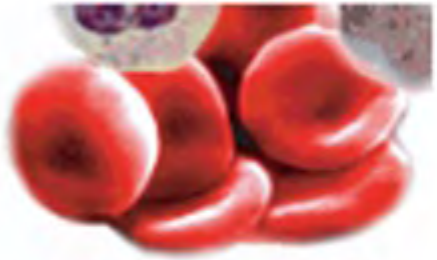
- Artificial cells modified with PEG have longer circulation times

- Incorporation of polyethylene-glycol (PEG) to the lipid membrane resulted in marked improvement in the circulation time of hemoglobin PEG-lipid vesicles (PEG-lipid membrane artificial rbc's).



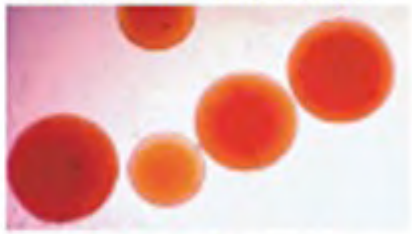
Artificial RBCs

RBC MEMBRANE



RED BLOOD CELLS (RBC's)
7-8 μ

ARTIFICIAL MEMBRANE



ARTIFICIAL RBC's
(Chang 1957,1964)
1μ or larger

↓

LIPID MEMBRANE NANO RBC's
(Djordjevich & Miller 1980)
0.2-0.4 μ (200-400 nm)

↓

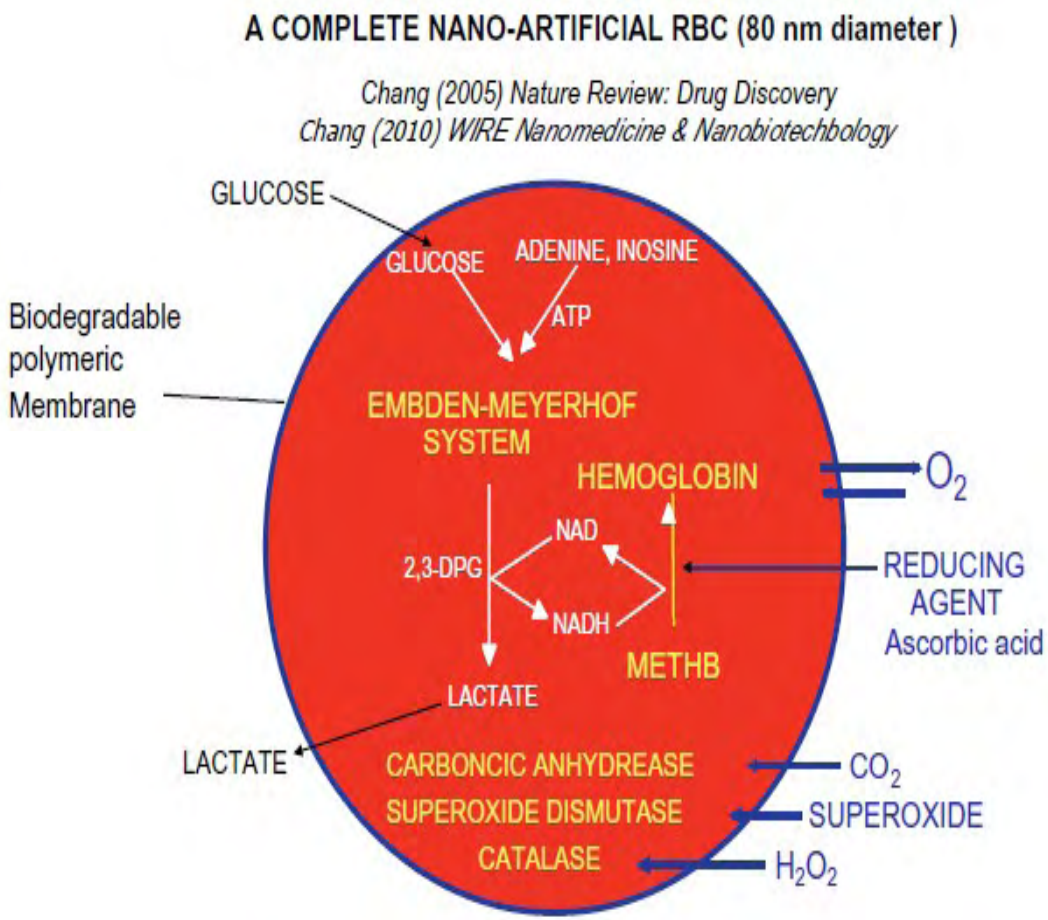
BIODEGRADABLE POLYMERIC MEMBRANE NANO RBC's
(Chang & WPYu, 1994)
80-200 nm

Left : Red blood cells.

Right : First artificial rbc's of 1 micron or larger diameters; first lipid membrane nanodimension artificial rbc's, first nano dieamension biodegradable polymeric membrane artificial rbc's.

http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

Artificial RBCs



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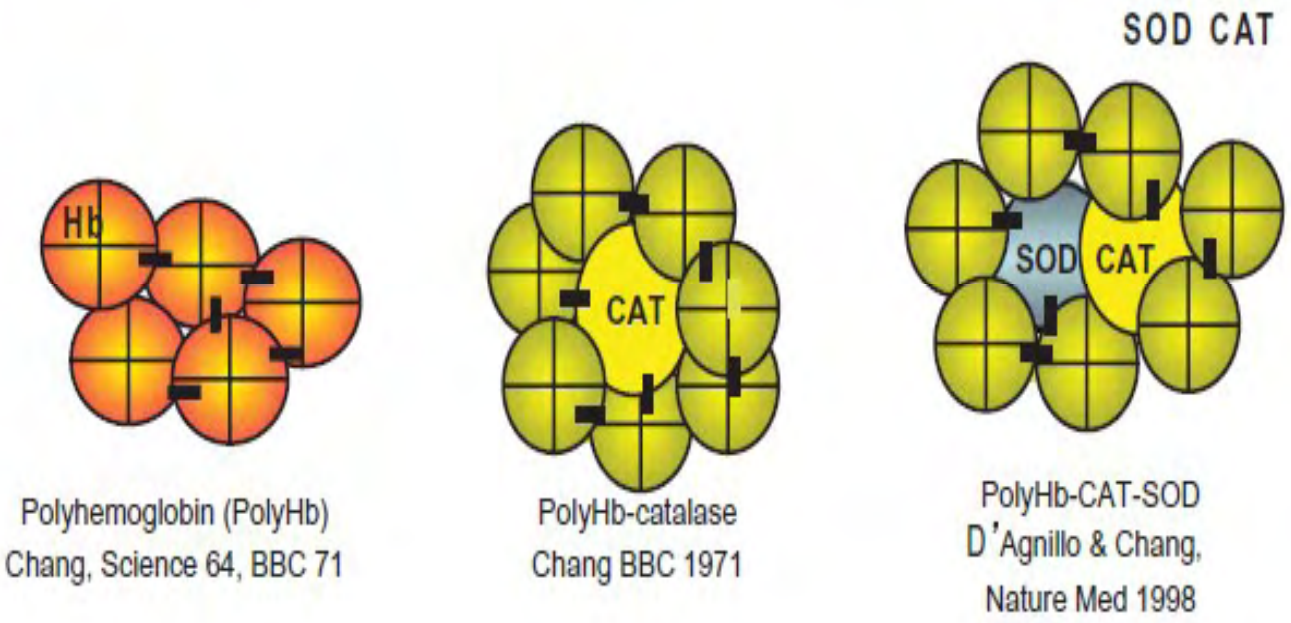
Artificial cells in Molecular Dimensions

- **As Oxygen Carrier**



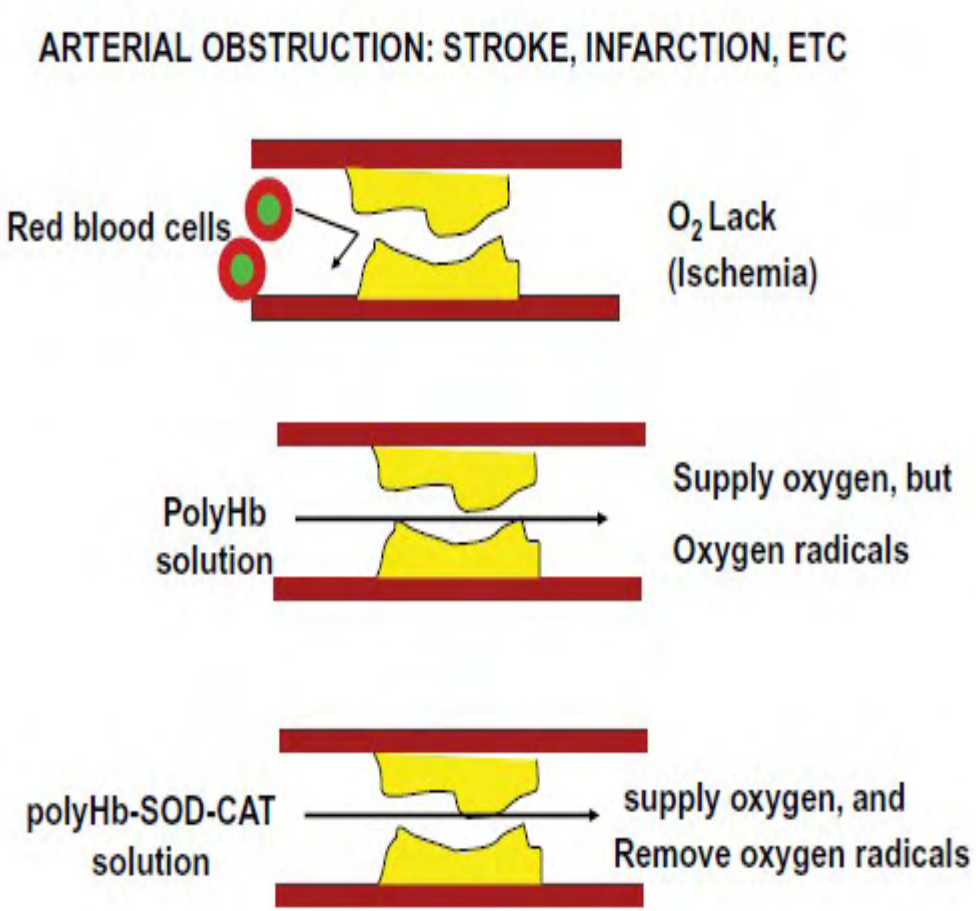
Polyhemoglobin (PolyHb)
Chang, Science 64, BBC 71

PolyHb-catalase
Chang BBC 1971

PolyHb-CAT-SOD
D'Agnillo & Chang,
Nature Med 1998

http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

Artificial cells in Molecular Dimensions



Arterial obstruction from the narrowing of the artery can result in stroke and heart attack.

Red blood cells, being 7 to 8 micron in diameter, have difficulty flowing through obstructed vessels to supply the needed oxygen.

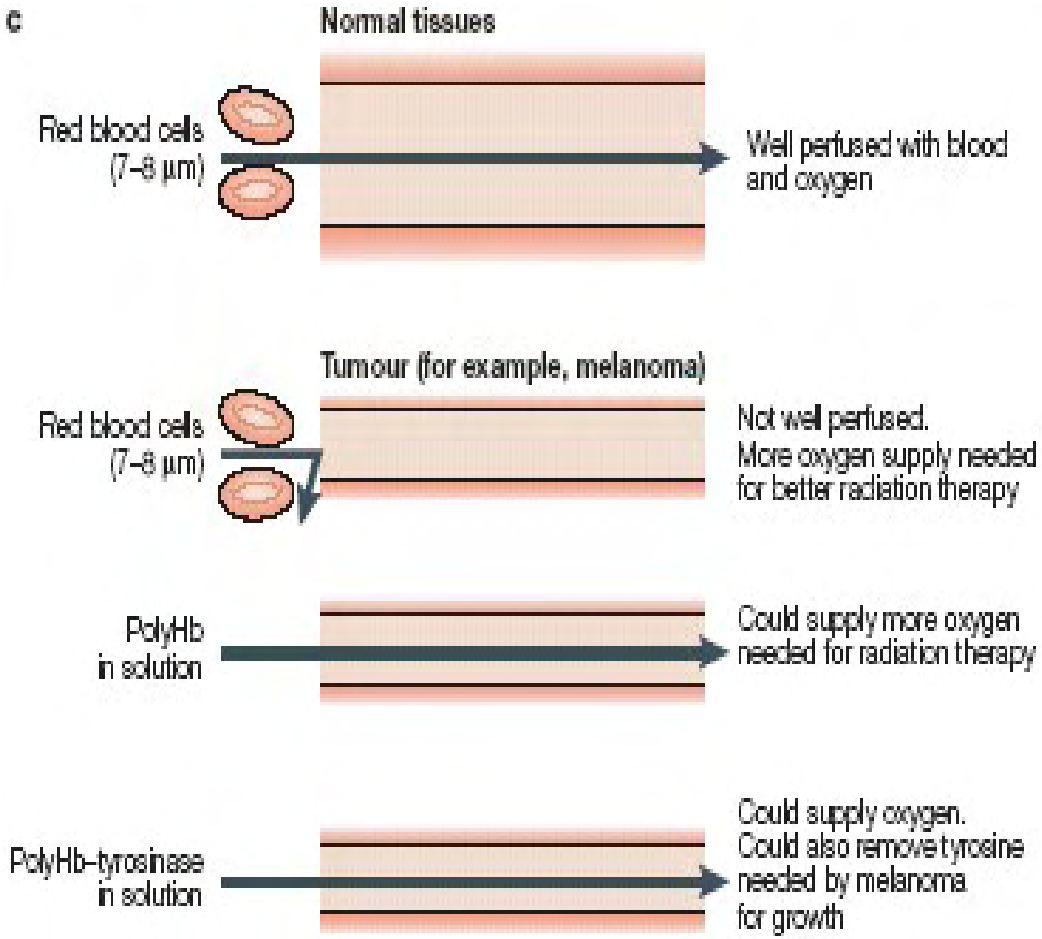
PolyHb, being a solution, can perfuse through to supply the needed oxygen.

However, if oxygen lack is prolonged, reperfusion with an oxygen carrier can release damaging oxygen radicals.

One possible solution is to use PolyHb-SOD-CAT that has the dual function of being an oxygen carrier and having the ability to remove oxygen radicals.

http://www.worldscientific.com/doi/abs/10.1142/9789814472869_0001

Aid in cancer therapy



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Hemoperfusion

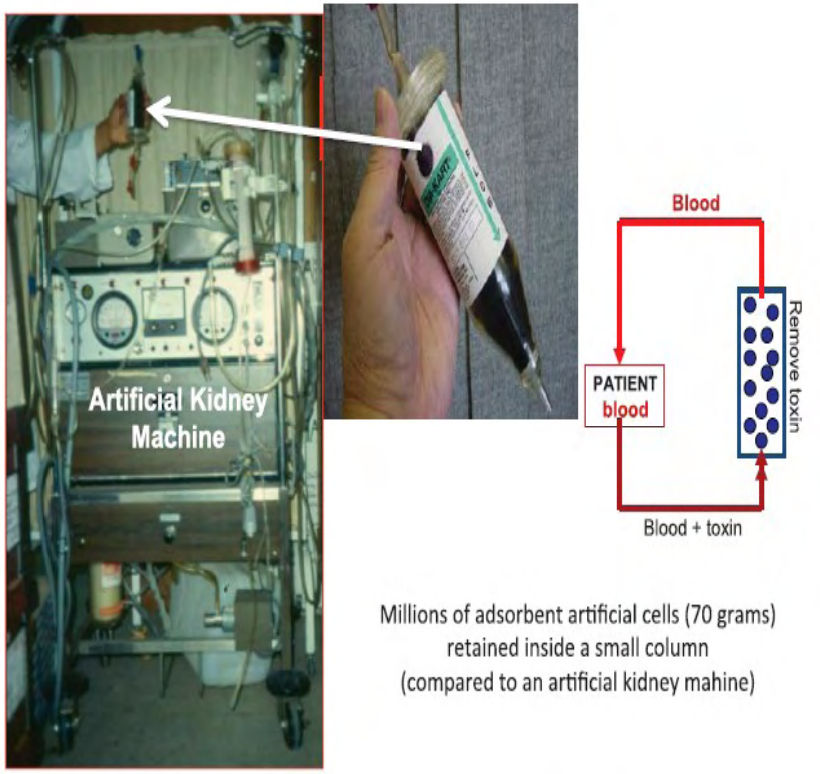
- Artificial cells are also used for **hemoperfusion**, i.e. removal of toxic substances from the blood of a patient.
- These ‘cells’ contain adsorbent materials which retain the contaminants in the blood that diffuse through the membrane.
- The artificial cells are a cheaper and more effective option compared to the available methods of blood detoxification.

Hemoperfusion

- Since they restrict the movement of the encased adsorbents into the patient's blood, they are also considered to be safe.

- Recent researches have been conducted with artificial cells composed of nanosponges encapsulated within natural red blood cell membranes which can be used for removal of toxins from blood.

Hemoperfusion



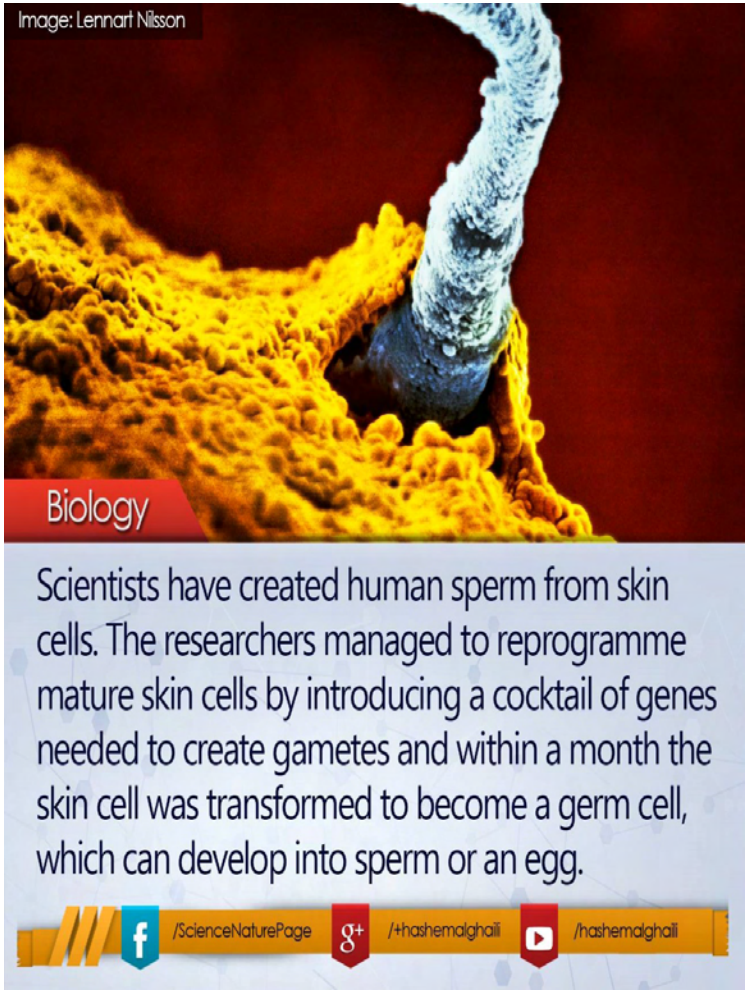
Millions of adsorbent artificial cells (70 grams) retained inside a small column (compared to an artificial kidney mahine)

http://www.worldscientific.com/doi/suppl/10.1142/8776/suppl_file/8776_chap01.pdf

- A hemoperfusion device containing 70 grams of ultrathin membrane artificial cells containing activated charcoal (on the right side) is compared to the artificial kidney machine.

Artificial Sperm

- Dr. Orly Lacham Kalpan succeeded in fertilizing a normal egg with an artificial sperm.
- Embryo Developed normally in Lab
- Artificial Human Eggs Possible In 5 years



ORGAN PRINTING

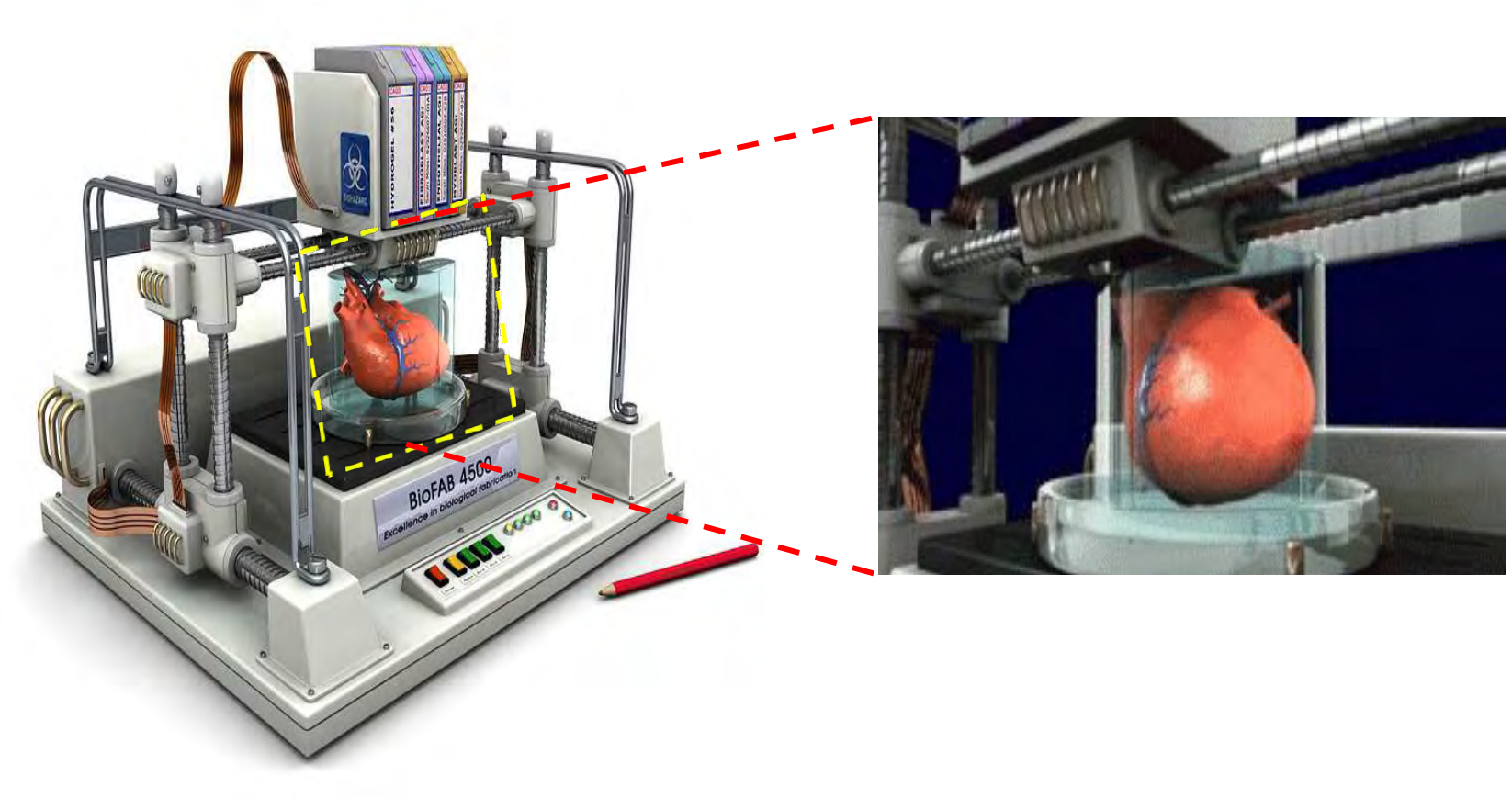
What is organ printing?

- Integrating biology and 3-D printing technology.
- A process where an artificial organ can be created using a 3-D printer/bioprinter.
- Organ printing is a rapid prototyping computer-aided 3D printing technology, based on using layer by layer deposition of cell and/or cell aggregates into a 3D gel with sequential maturation of the printed construct into perfused and vascularized living tissue or organ.

Organ printing

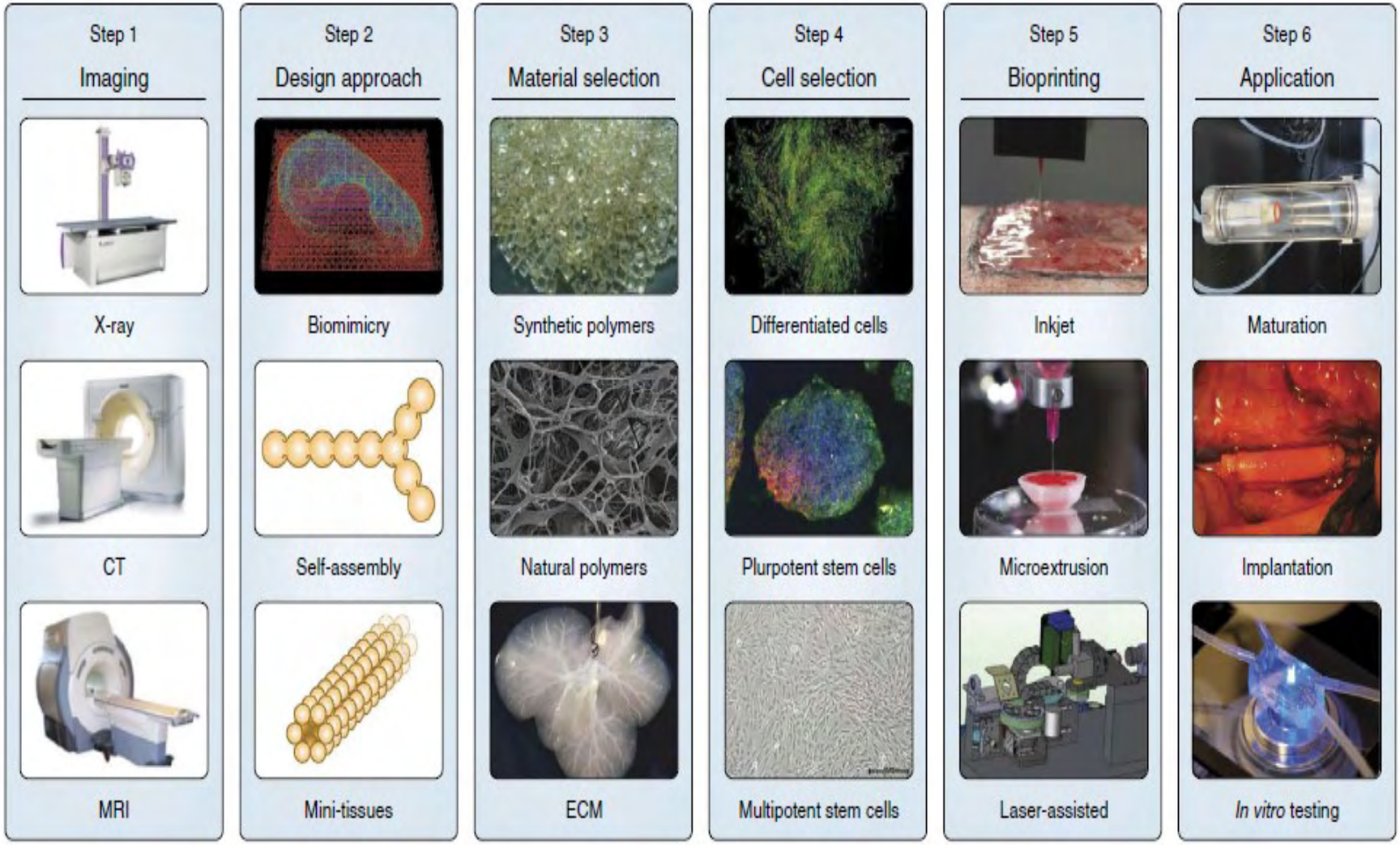
- An organ printer incorporates 2 technologies, tissue engineering and a 3D printer.
- Instead of paper, Petri dishes are used.
- Instead of ink, cells and chemical called a “crosslinker” are used.
- The cells are individually made from the patient.

Conceptual Bioprinter



http://explainingthefuture.com/bioprinting.html

A typical process for bioprinting 3D tissues



A typical process for bioprinting 3D tissues

1) create a blueprint of an organ with its vascular architecture;

2) generate a bioprinting process plan;

3) isolate stem cells;

4) differentiate the stem cells into organ specific cells;

5)prepare bioink reservoirs with organ specific cells, blood vessel cells, and support medium and load them into the printer;

6) bioprint; and

7)place the bioprinted organ in a bioreactor prior to transplantation.

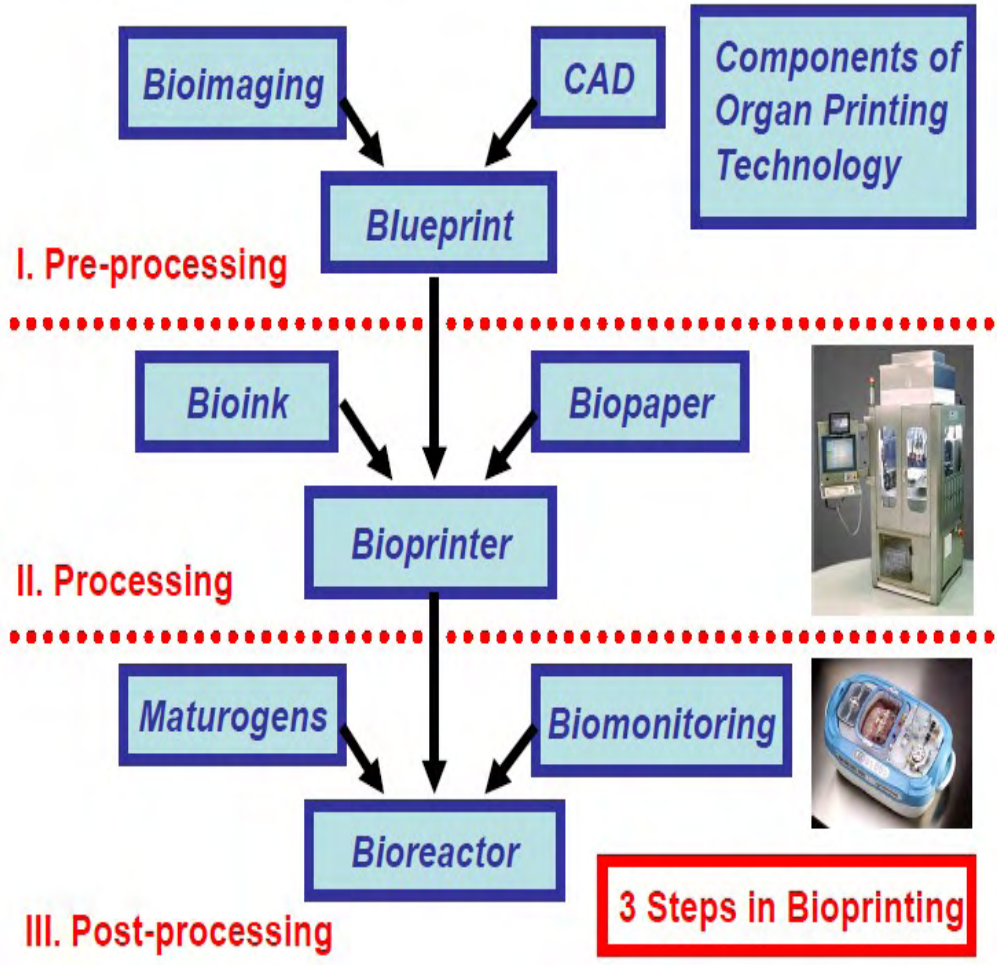
How organ printing works

The procedure of organ printing can be subdivided into three sequential steps:

1. Pre-processing
2. Processing
3. Post processing

http://www.sciencedirect.com/science/article/pii/S0167779903000337

Components of organ printing technology

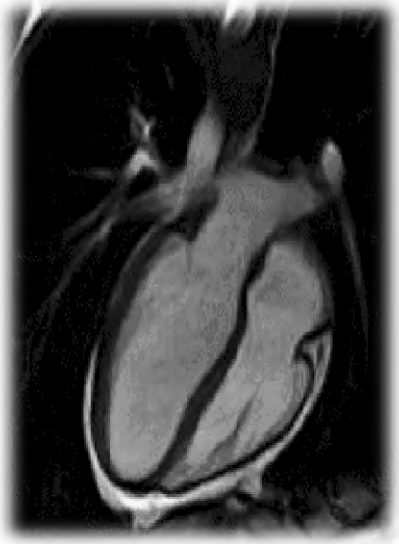


http://downloads.deusm.com/designnews/25419-Organ_Printing_How_to_Print_a_Human_Organ.pdf

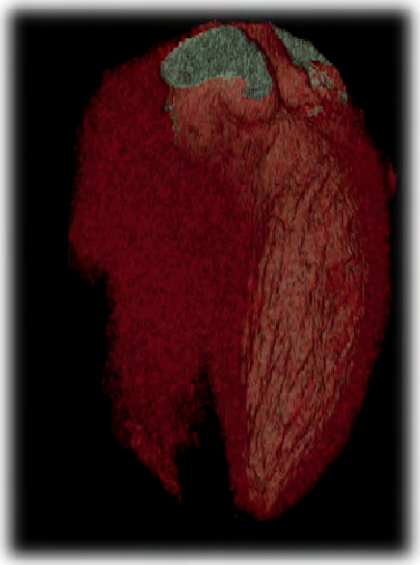
Step1: Pre-processing

- Pre-processing primarily deals with the development of a computer-aided design (CAD) or blueprint of a specific organ.
- The design can be derived from digitized image reconstruction of a natural organ or tissue.
- Imaging data can be derived from various modalities including non-invasive scanning of the human body (e.g. MRI or computerized tomography) or a detailed 3D reconstruction of serial sections of specific organs

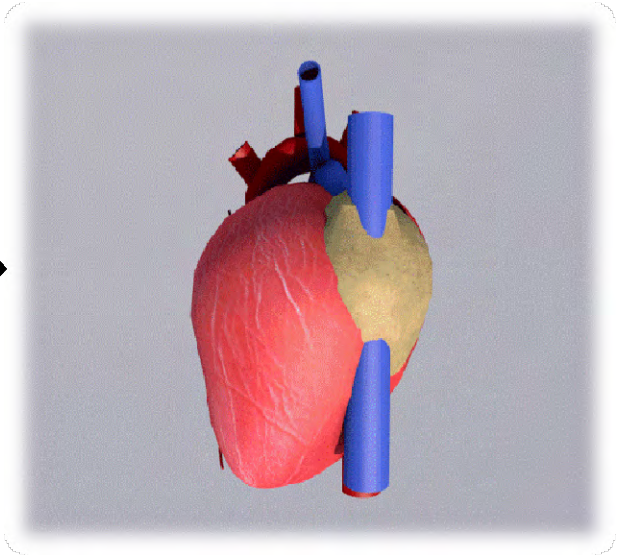
Component of pre-processing



Bio imaging



CAD
Computer Aided Design



Blueprint

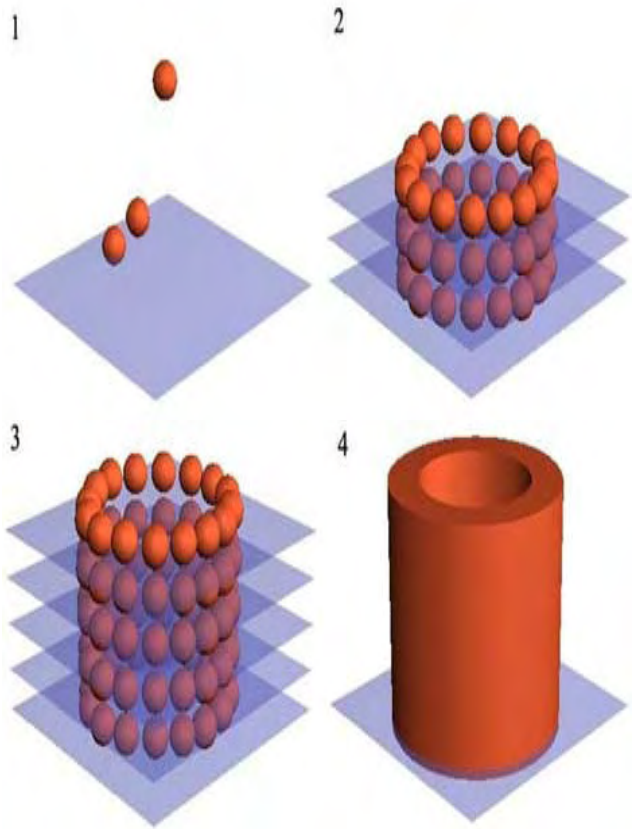
http://www.sciencedirect.com/science/article/pii/S0167779903000337

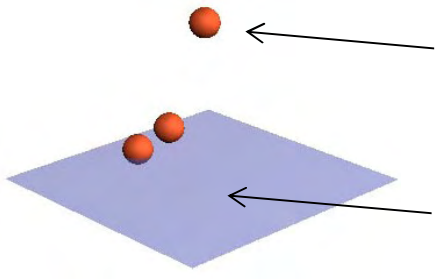
Step 2: Processing

- Processing usually refers to actual computer-aided printing or layer- by- layer placement of cells or cell aggregates into a 3D environment using CAD or blueprints.
- The petri dish is filled with water.
- When the printer “prints” the cross linker transforms the water into a Jell-O like substance which allows the cells to be put in.
- Once one dish is filled, a new one is placed on top of it.

Processing

- This method is repeated until the organ that you want is created.
- Once the organ is constructed, the petri dishes are removed and all that is left is the organ.

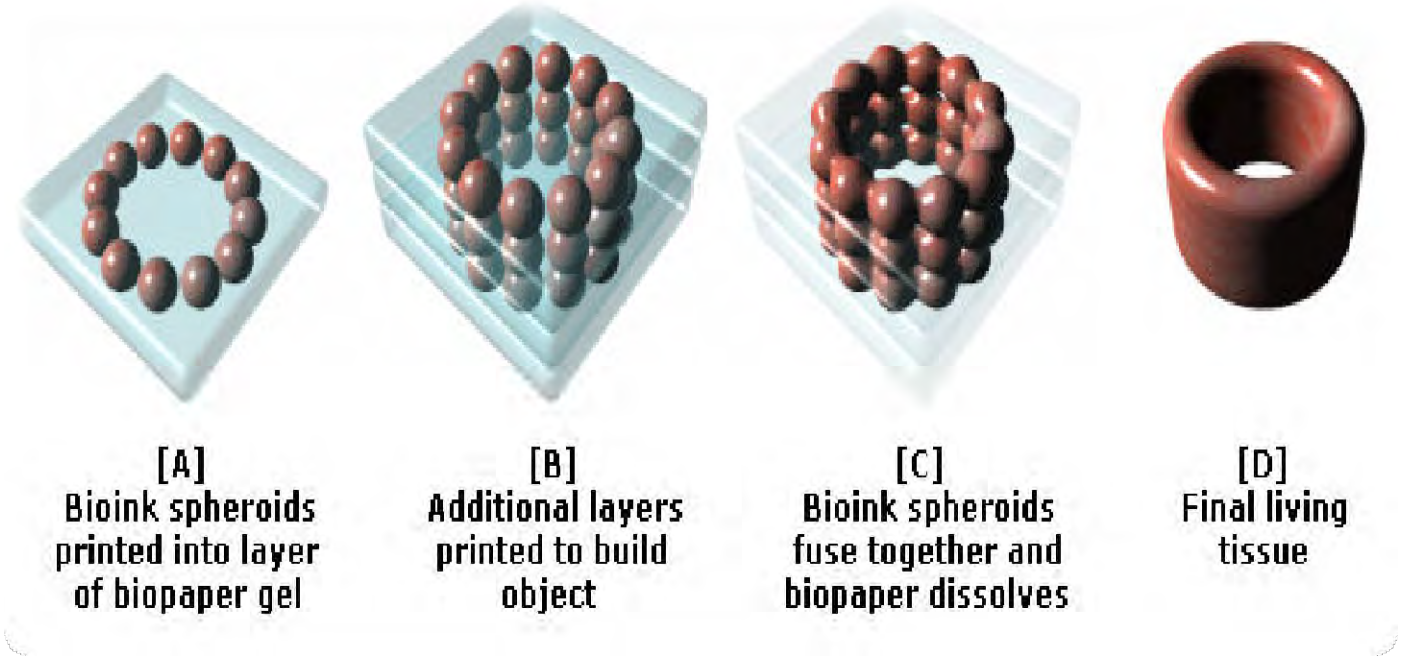




Spherical cell aggregate: the bioink

Supporting biocompatible gel: the biopaper

Components of processing

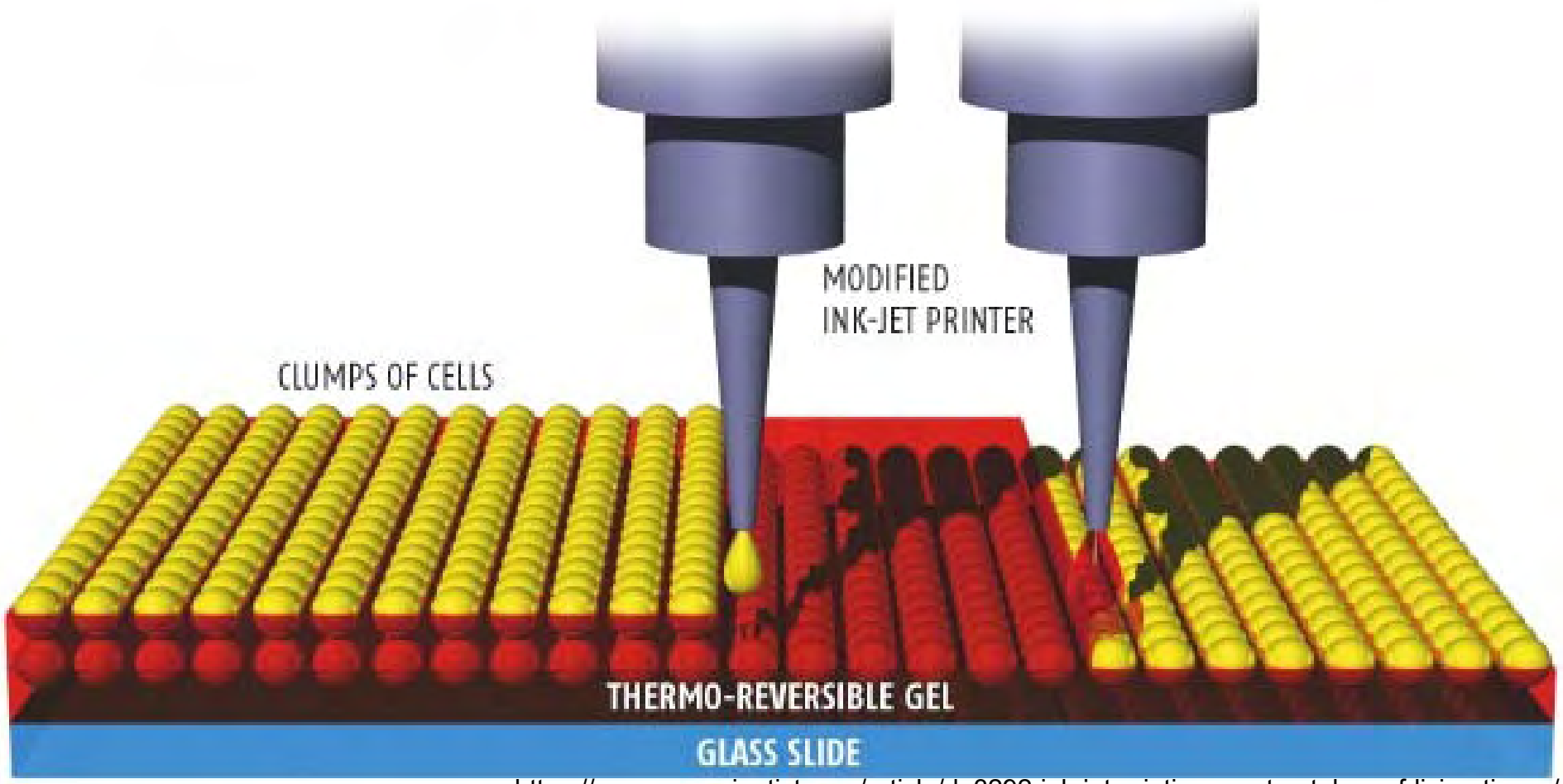


Bioink + Biopaper → Bioprinter

https://www.3dprinter.net/organovo-the-bioprinting-pioneer-to-watch

PRINTING ORGANS

Organs could be built up layer by layer by printing clumps of cells onto a gel that turns solid when warmed. Once the cells have fused the gel can be removed simply by cooling it



https://www.newscientist.com/article/dn3292-ink-jet-printing-creates-tubes-of-living-tissue/

Bioprinters

- Bio printers have three major components. These are the hardware used, the type of bio-ink, and the material it is printed on (biomaterials).
- "Bio-ink is a material made from living cells that behaves much like a liquid, allowing people to "print" it in order to create a desired shape.
- To make bio-ink, scientists create a slurry of cells that can be loaded into a cartridge and inserted into a specially designed printer, along with another cartridge containing a gel known as bio-paper.“
- Potential uses for bio-ink include creating sheets of skin for skin grafts and vascular tissues to replace veins and arteries.

Bioink

- Prepared by mixing cells with biocompatible materials (Hydrogels).
- Suitable hydrogels are chosen based on the Organ to be printed.

(Ex. Collagen is widely used for bone printing)

Bio-ink materials

- Collagen
- Alginate
- Fibrin
- Polycaprolactone
- Thorbin

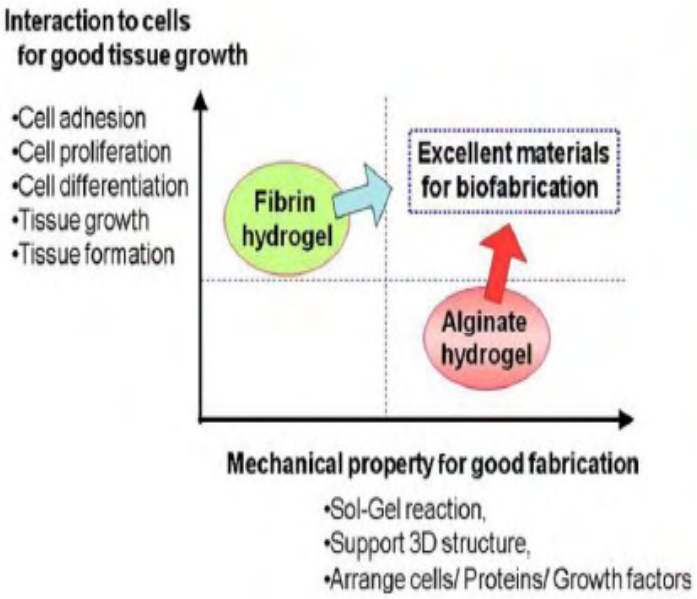
Widely used →

Collagen
Fibrin
Alginate

reason ↓

- Excellent Biocompatibility.
- Homogenously incorporate cells, growth factors.
- Processed under mild conditions.
- Easy chemical modification.
- Sol-gel transition.

Biopaper



Type	Hydrogel
Thermosensitive	Agaros Collagen Gelatin Matrigel PNiPAAm Pluronics PEG triblocks chitosan
pH sensitive	HPMC
Ionic crosslinking	Alginate
Photo polymerizable	PEG-based PVA Hyaluronic acid
Enzymatic	Fibrin

Source: Nakamura et al. Biofabrication 2 (2010) 014110

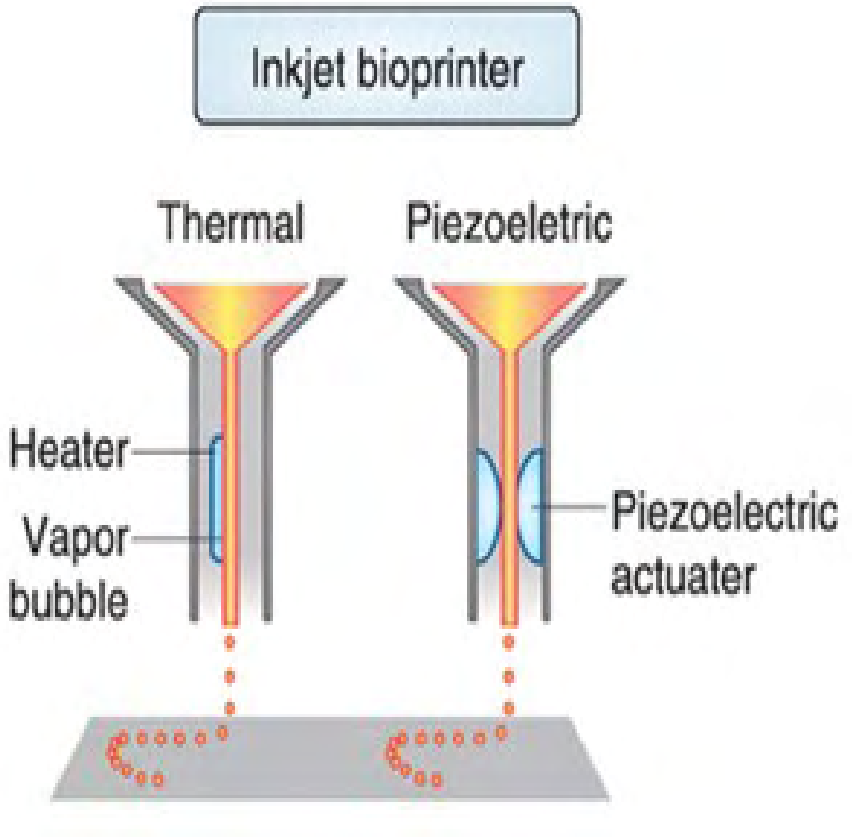
Types of bioprinters

- In bio printing, there are three major types of printers that have been used.
- These are
  - Inkjet
  - Extrusion printers
  - Laser-assisted

Materials 2016, 9, 802; doi:10.3390/ma9100802

Inkjet printers

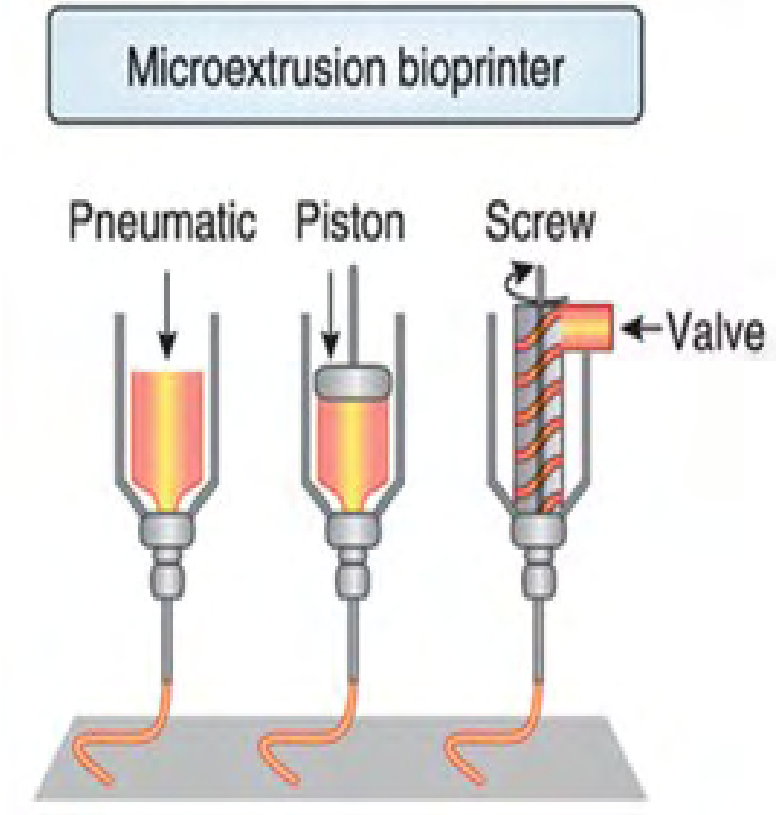
- Inkjet printers are mainly used in bio printing for fast and large-scale products. One type of inkjet printer, called drop-on-demand inkjet printer, prints materials in exact amounts, minimizing cost and waste.
- Thermal inkjet printers electrically heat the print head to produce air-pressure pulses that force droplets from the nozzle, whereas acoustic printers use pulses formed by piezoelectric or ultrasound pressure.



http://www.nature.com/nbt/journal/v32/n8/full/nbt.2958.html

Extrusion printers

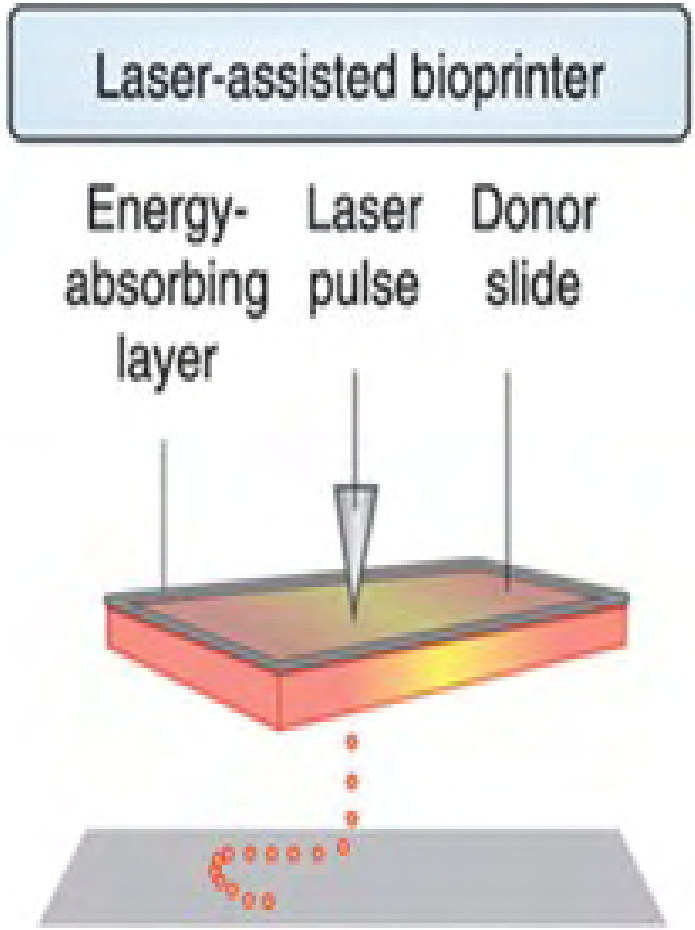
- Extrusion printers print cells layer-by-layer, just like 3D printing to create 3D constructs. In addition to just cells, extrusion printers may also use hydrogels infused with cells.
- Micro extrusion printers use pneumatic or mechanical (piston or screw) dispensing systems to extrude continuous beads of material and/or cells.



http://www.nature.com/nbt/journal/v32/n8/full/nbt.2958.html

Laser-assisted bioprinters

- Printers that utilize lasers provide high-resolution printing; however, these printers are often expensive.
- Laser-assisted printers use lasers focused on an absorbing substrate to generate pressures that propel cell-containing materials onto a collector substrate.



http://www.nature.com/nbt/journal/v32/n8/full/nbt.2958.html

Comparison of bioprinters types

Table 1 Comparison of bioprinter types

	Bioprinter type			
	Inkjet	Microextrusion	Laser assisted	Refs.
Material viscosities	3.5–12 mPa/s	30 mPa/s to >6 x 10^7 mPa/s	1–300 mPa/s	48,63,78,107
Gelation methods	Chemical, photo-crosslinking	Chemical, photo-crosslinking, sheer thinning, temperature	Chemical, photo-crosslinking	64,85,106,110
Preparation time	Low	Low to medium	Medium to high	38,64,94,107
Print speed	Fast (1–10,000 droplets per second)	Slow (10–50 μm/s)	Medium-fast (200–1,600 mm/s)	49,58,76,90
Resolution or droplet size	<1 pl to >300 pl droplets, 50 μm wide	5 μm to millimeters wide	Microscale resolution	49,68,69,76
Cell viability	>85%	40–80%	>95%	42,54,80,104
Cell densities	Low, <10^6 cells/ml	High, cell spheroids	Medium, 10^8 cells/ml	42,49,88,89
Printer cost	Low	Medium	High	77

http://www.nature.com/nbt/journal/v32/n8/full/nbt.2958.html

Step 3: Post Processing

- Post processing is concerned with the perfusion of printed organs and their biomechanical conditioning to both direct and accelerate organ maturation.

- To maintain the object, both mechanical and chemical stimulations are needed. These stimulations send signals to the cells to control the remodelling and growth of tissues.
- In addition, in recent development, bioreactor technologies have allowed the rapid maturation of tissues, vascularization of tissues and the ability to survive transplants.

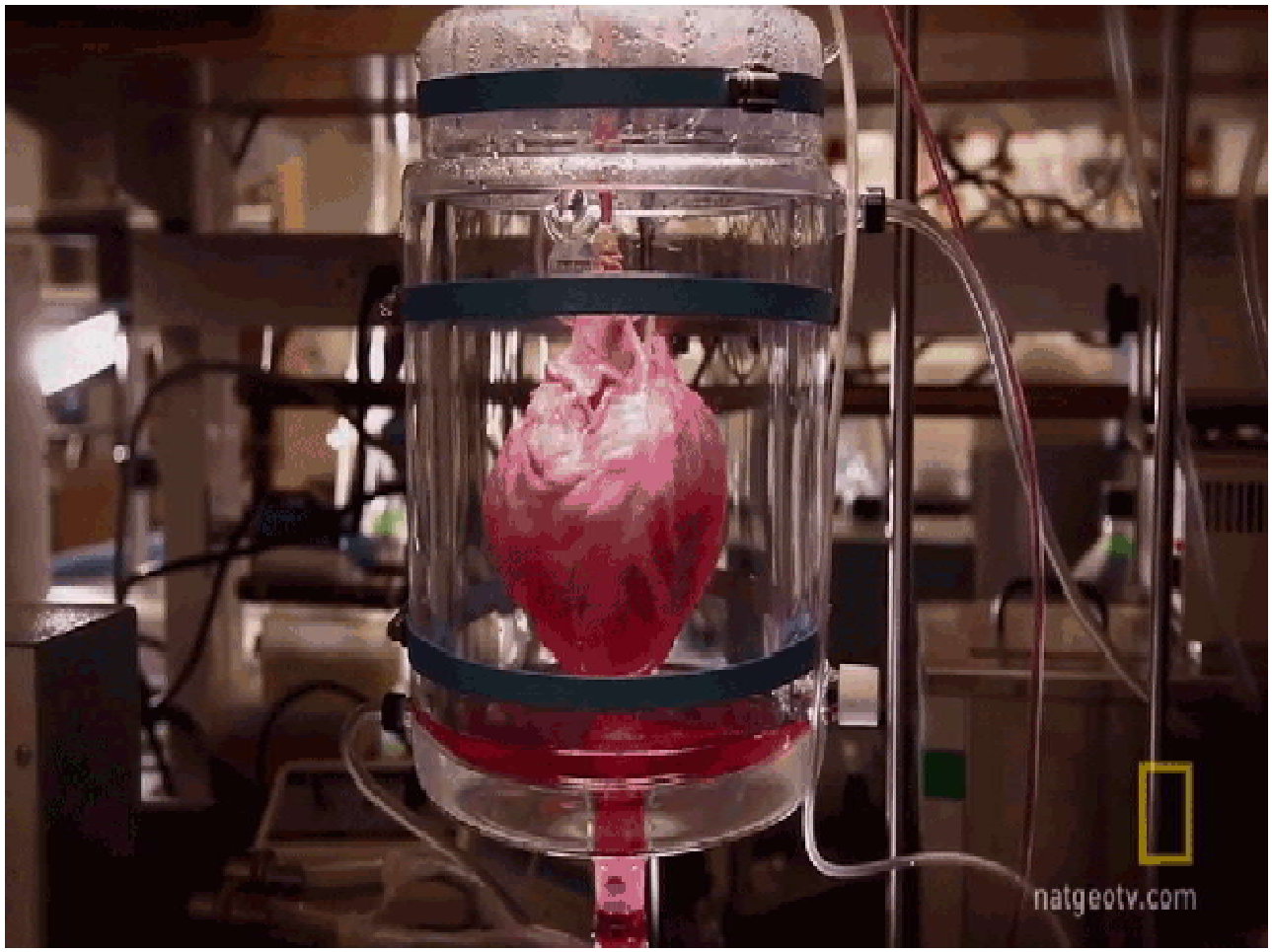
http://www.sciencedirect.com/science/article/pii/S0167779903000337

Post Processing

- Bioreactors work in either providing convective nutrient transport, creating microgravity environments, changing the pressure causing solution to flow through the cells, or add compression for dynamic or static loading.

- Each type of bioreactor is ideal for different types of tissue, for example compression bioreactors are ideal for cartilage tissue.

Post Processing



https://www.gizmodo.com.au/2014/01/the-biggest-science-stories-of-2013/

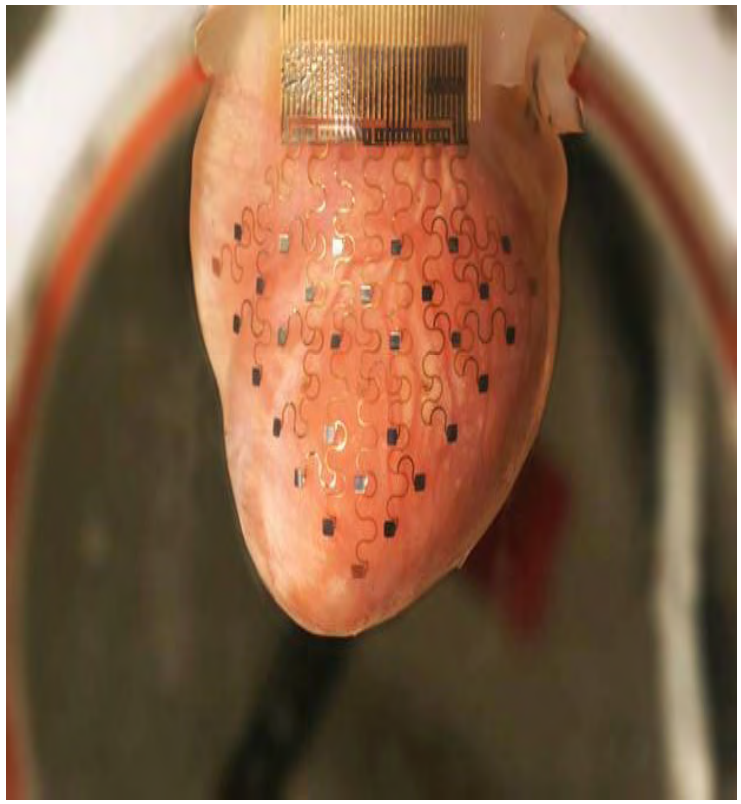
Maturogens + Bio monitoring → Bioreactor

Electronic membrane that could replace pacemakers

Scientists have created a revolutionary new electronic membrane that could replace pacemakers, fitting over a heart to keep it beating regularly over an indefinite period of time.

The device uses a "spider-web-like network of sensors and electrodes" to continuously monitor the heart's electrical activity and could, in the future, deliver electrical shocks to maintain a healthy heart-rate.

Researchers used computer modelling technology and a 3D-printer to create a prototype membrane and fit it to a rabbit's heart, keeping the organ operating perfectly "outside of the body in a nutrient and oxygen-rich solution".

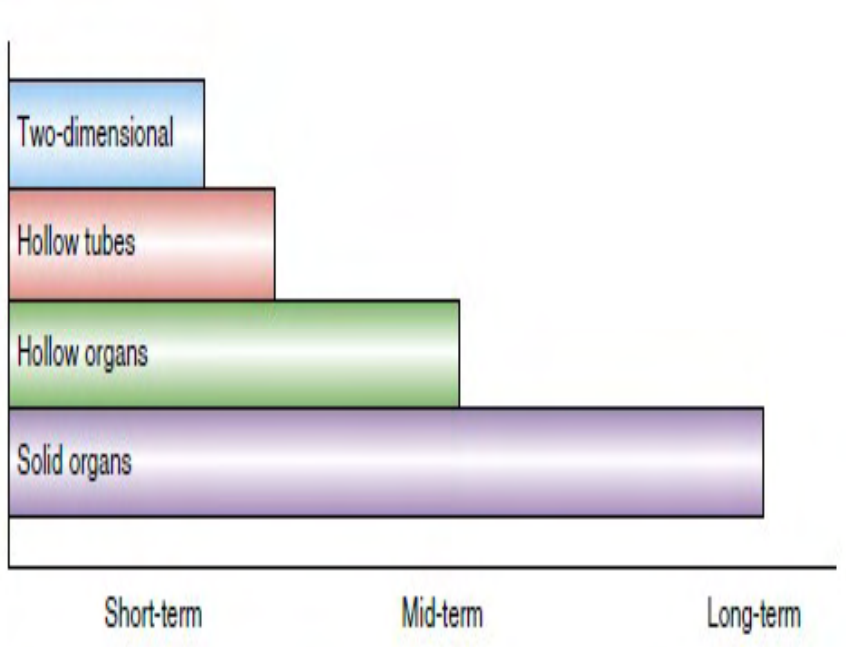


http://www.independent.co.uk/news/science/3d-printed-electronic-glove-could-help-keep-your-heart-beating-for-ever-9166004.html

Examples

- Human scale bioprinted tissues

1. 2Dimensional tissue
   - Skin and Cartilage
2. Hollow tubes
   - Trachea, Heart valve and Vasculature
3. Solid organs
   - Kidney



http://www.nature.com/nbt/journal/v32/n8/pdf/nbt.2958.pdf

Challenges

- -In 2011, successfully printed a kidney from human cells in seven hours (**Doctor Anthony Atala,** at Wake Forest Institute of Regenerative Medicine)
- -Not functional in humans yet but his research is still in progress

Why Doesn't it Work?

- -Difficult to create blood vessels between tissue layers
- -Organs have many specialized functions difficult to replicate

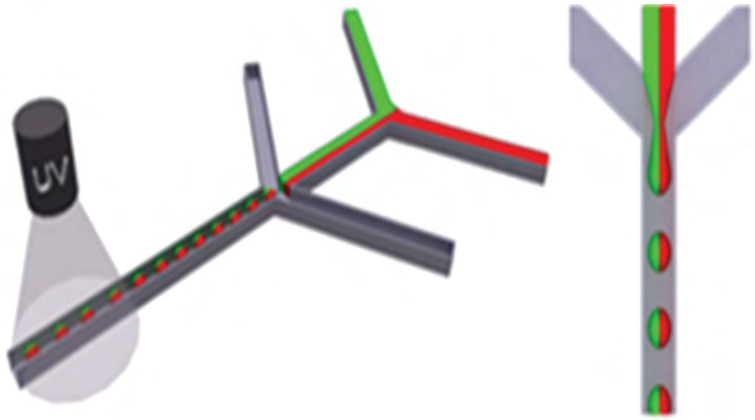
-Team: Dr. Jordan Miller, Dr. Christopher Chen, and Dr. Sangeeta Bhatia

- -Created a sugar template that can helps shape development of a vascular network for artificial organs
- -After network is printed, cells are inserted and network then grows
- -Sugar template is dissolved after completion of development

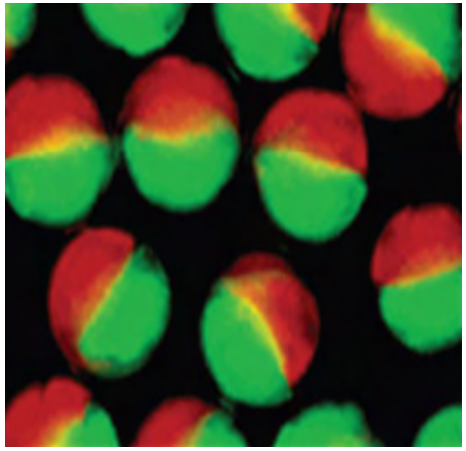
Nanotechnology in organ printing

Fabrication of janus-like self-assembling tissue spheroids with magnetic nanoparticles

Janus particles are special types of nanoparticles whose surfaces have two or more distinct physical properties.



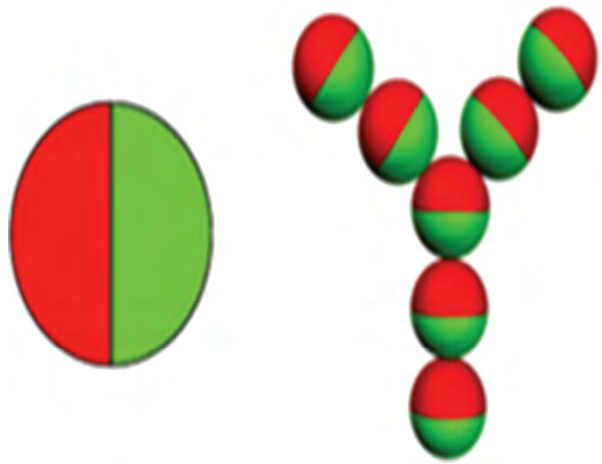
Biofabrication of janus-like tissue spheroids using microfluidics



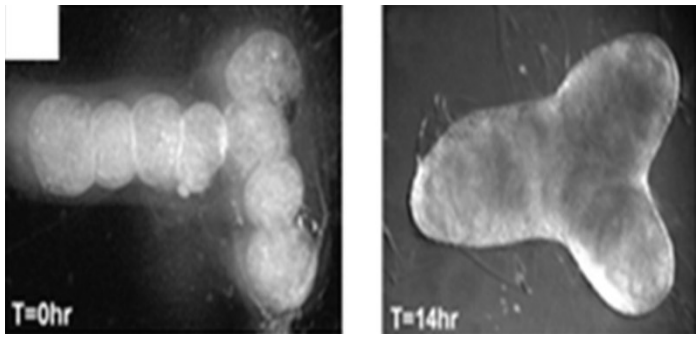
Janus-like spheroids fabricated by microfluidics devices

Journal of Nanotechnology, Volume 2012, Article ID 149264

Fabrication of janus-like self-assembling tissue spheroids with magnetic nanoparticles



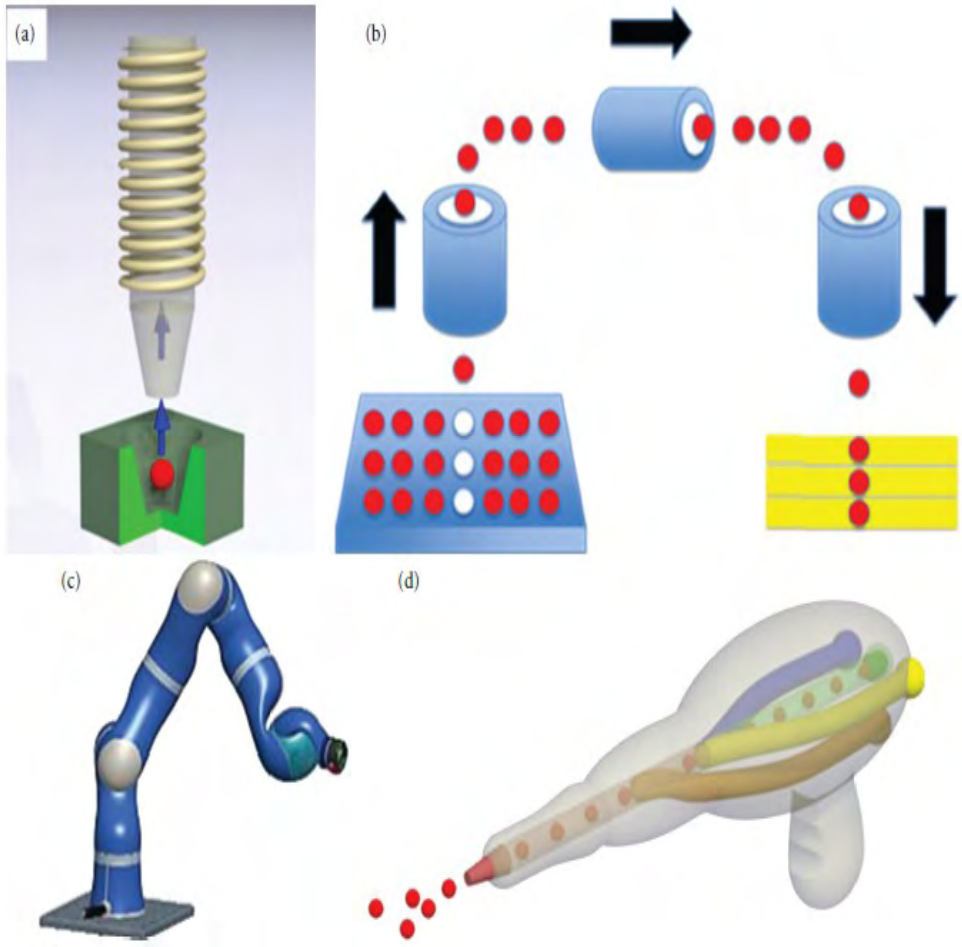
Scheme demonstrating magnetic-forces-driven self-directed self-assembly of closely placed janus-like magnetic tissue spheroids



Branched structure formed as a result of fusion of closely placed tissue spheroids

Journal of Nanotechnology, Volume 2012, Article ID 149264

Design elements of a clinical robotic bioprinter



Journal of Nanotechnology, Volume 2012, Article ID 149264

(a) Tissue spheroids harvester based on magnetic levitation of tissue spheroids;

(b) Principal scheme of clinical robotic bioprinter demonstrating how tissue spheroids can be harvested from multiwells, translocated and dispensed in living tissue using magnetic levitation;

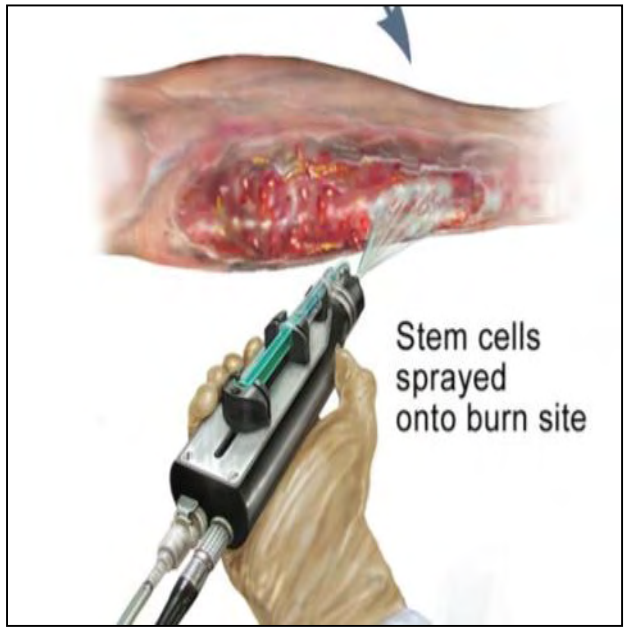
(c) Elegant robotic hand developed by group of robotics and mechanotronics at German Aerospace Institute(http://www.dlr.de/rm/en/desktopdefault.aspx/tabid-3803/6175 read-8961/) which can be employed as an essential component in robotic clinical bioprinter for automated nozzle positioning;

(d) Computer-aided design of nozzle of clinical robotic

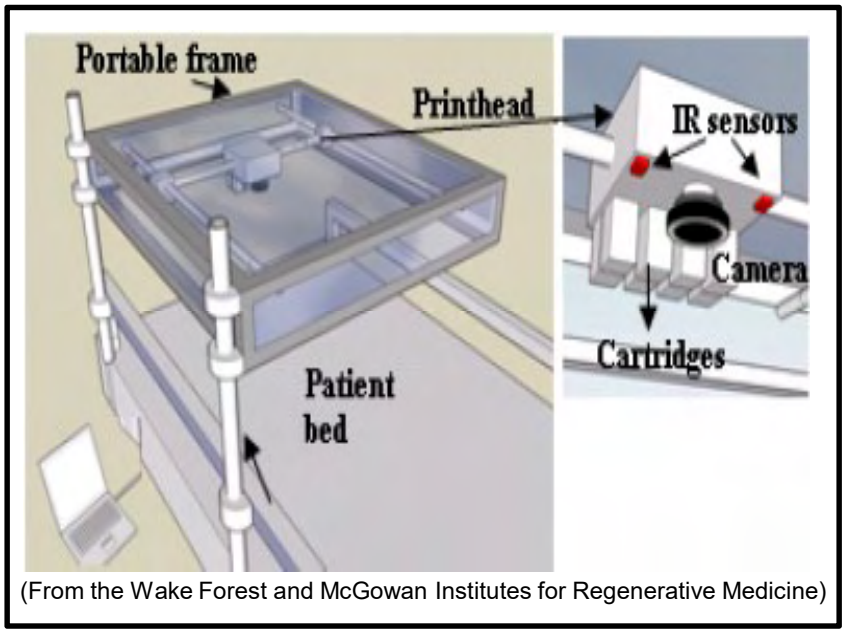
bioprinter containing several channels: two channels for fibrinogen and thrombin, one channel for tissue spheroids and additional channel for pressurized air for enabling fibrin hydrogel spraying.

New methods for burn treatment

Skin Cell Spray Gun



Portable Printer for Skin



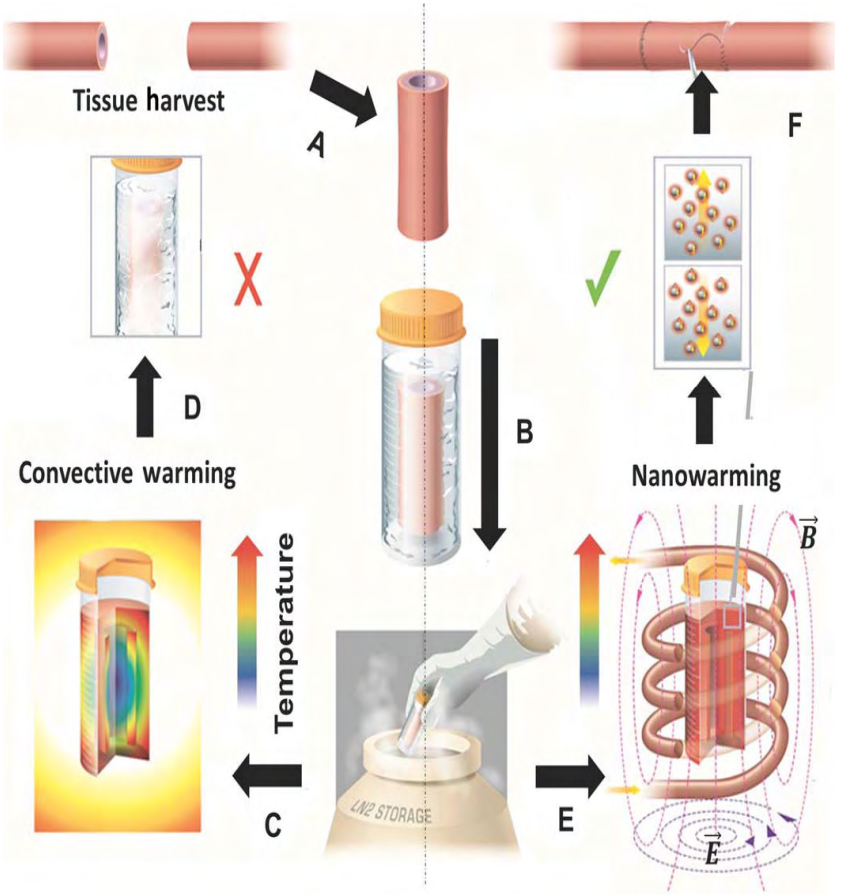
(From the Wake Forest and McGowan Institutes for Regenerative Medicine)

An adapted ink-jet printer to provide on-site "printing" of skin for soldiers with life-threatening burns. Skin cells are placed in the sterilized ink cartridge, along with a material to support them, and are printed directly on the wound.

Nanotechnology to evenly reheat cryogenically preserved tissue

We know how to cool organs to cryogenic temperatures, which is usually below 320 degrees Fahrenheit. But the organs can't be stored for long — sometimes only four hours for heart and lungs — because they get damaged when you try to warm them up. As a result, more than 60 percent of donor hearts and lungs aren't transplanted.

With the new method, tissue can be re-warmed with no sign of damage, and without contamination.



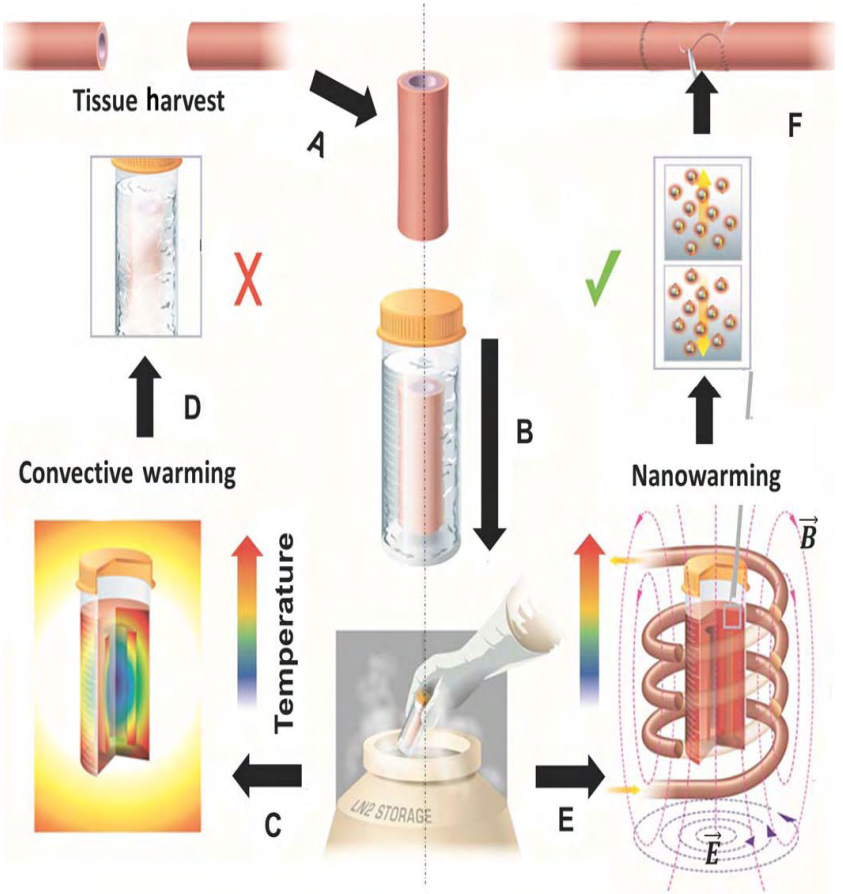
Manuchehrabadi et al., Sci. Transl. Med. 9, eaah4586 (2017)

Nanotechnology to evenly reheat cryogenically preserved tissue

Silica-coated iron oxide, were mixed into a solution before being applied to the tissue. The external magnetic field was then activated, causing the nanoparticles to warm up and provide even heating throughout the tissue.

None of the nanowarmed tissue showed any sign of damage, unlike the control tissue which was slowly reheated over ice. This is because in the new method all cells are heated at the same rate, avoiding the damage caused by uneven temperature changes.

The nanoparticles were also successfully washed away after the process, preventing any contamination-associated issues, and ensuring that the method is viable for tissue preservation.



Manuchehrabadi et al., Sci. Transl. Med. 9, eaah4586 (2017)

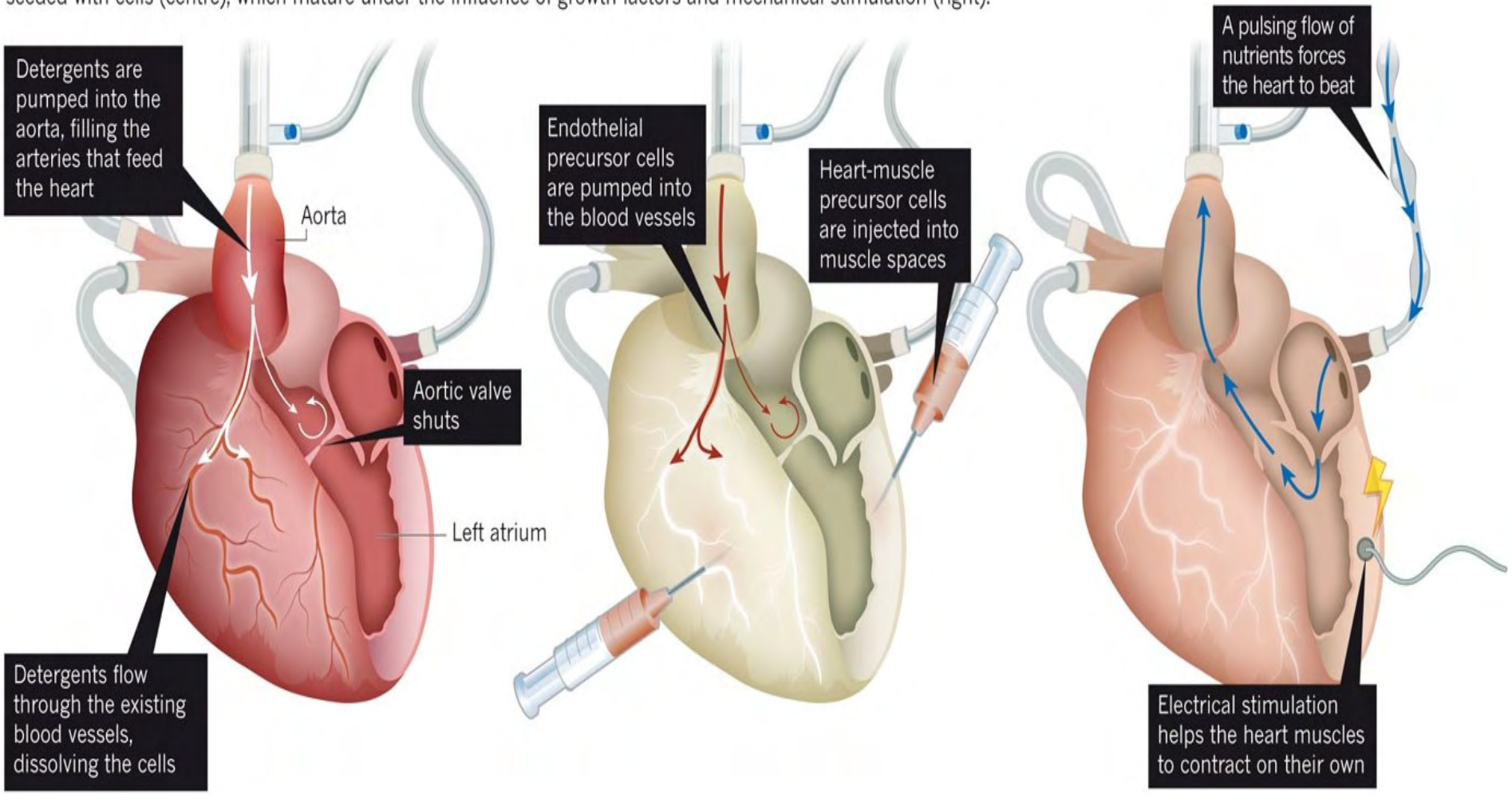
Organ decellularization approach

Dr. Harald Ott

http://www.youtube.com/watch?v=5wfdhB_VyJw

CUSTOMIZED ORGANS

To construct a new heart, researchers first remove all cells from a donor organ (left), leaving a protein scaffold. That is seeded with cells (centre), which mature under the influence of growth factors and mechanical stimulation (right).



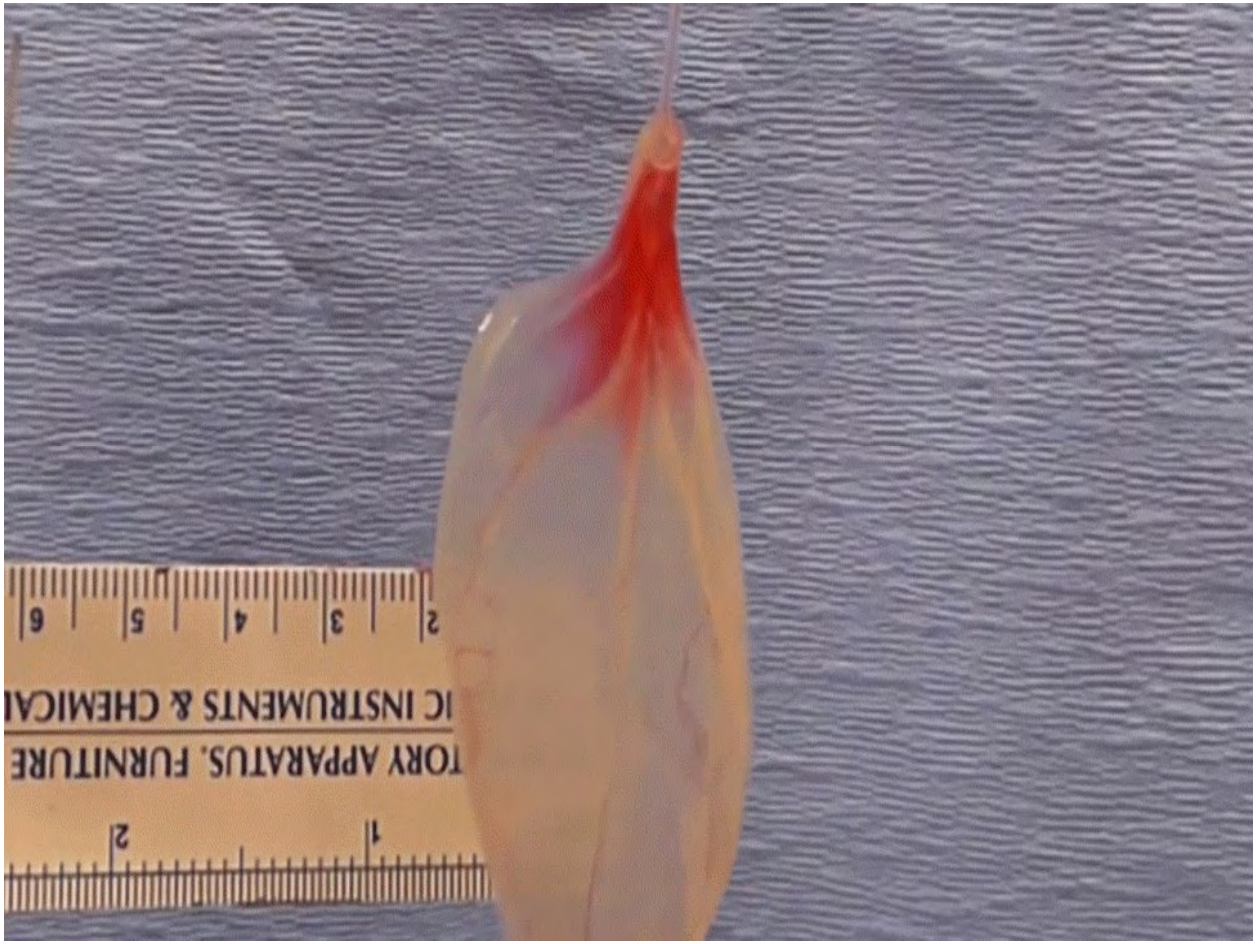
http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327

Heart tissue grown on spinach leaves

In this sequence, a spinach leaf is stripped of its plant cells, a process called decellularization, using a detergent. The process leaves behind the leaf's vasculature. Researchers at Worcester Polytechnic Institute (WPI) were able to culture beating human heart cells on such decelluralized leaves.

Biomaterials, Volume 125, May 2017, Pages 13–22
https://www.sciencedaily.com/releases/2017/03/170322152753.htm

Heart tissue grown on spinach leaves



http://www.popularmechanics.com/science/health/a25829/spinach-blood-vessels/

Benefits

- One major benefit this has is that the cells used to print the organ are samples of the patient's own stem cells, virtually eliminating the possibility of rejection.
- The organ will not wear out or need occasional maintenance like a fully mechanical organ transplant.
- 3D printing eliminates the need for a scaffold (a basic structure) to grow the cells on, which most artificially grown organs require.
- Can by pass the organ donor list.
- Another benefit is that the organ can be printed from a 3D computer model of an actual organ, and be sized up or down on the computer before printing- the organ can be customized to better suit the patient.

Difficulties and Limitations

- It's difficult to print vascularization in an organ, so effective blood flow in the organs has been a major roadblock.

- The lifespan of the organs themselves is very limited, ranging from a few minutes to days, thus longevity of the organ needs to be worked on before they can be transplanted into a patient.

- Some organs have advanced functions beyond movement, storage, and filtration (such as the liver's ability to regenerate) which have not been replicated in this particular lab setting.

Future Goals

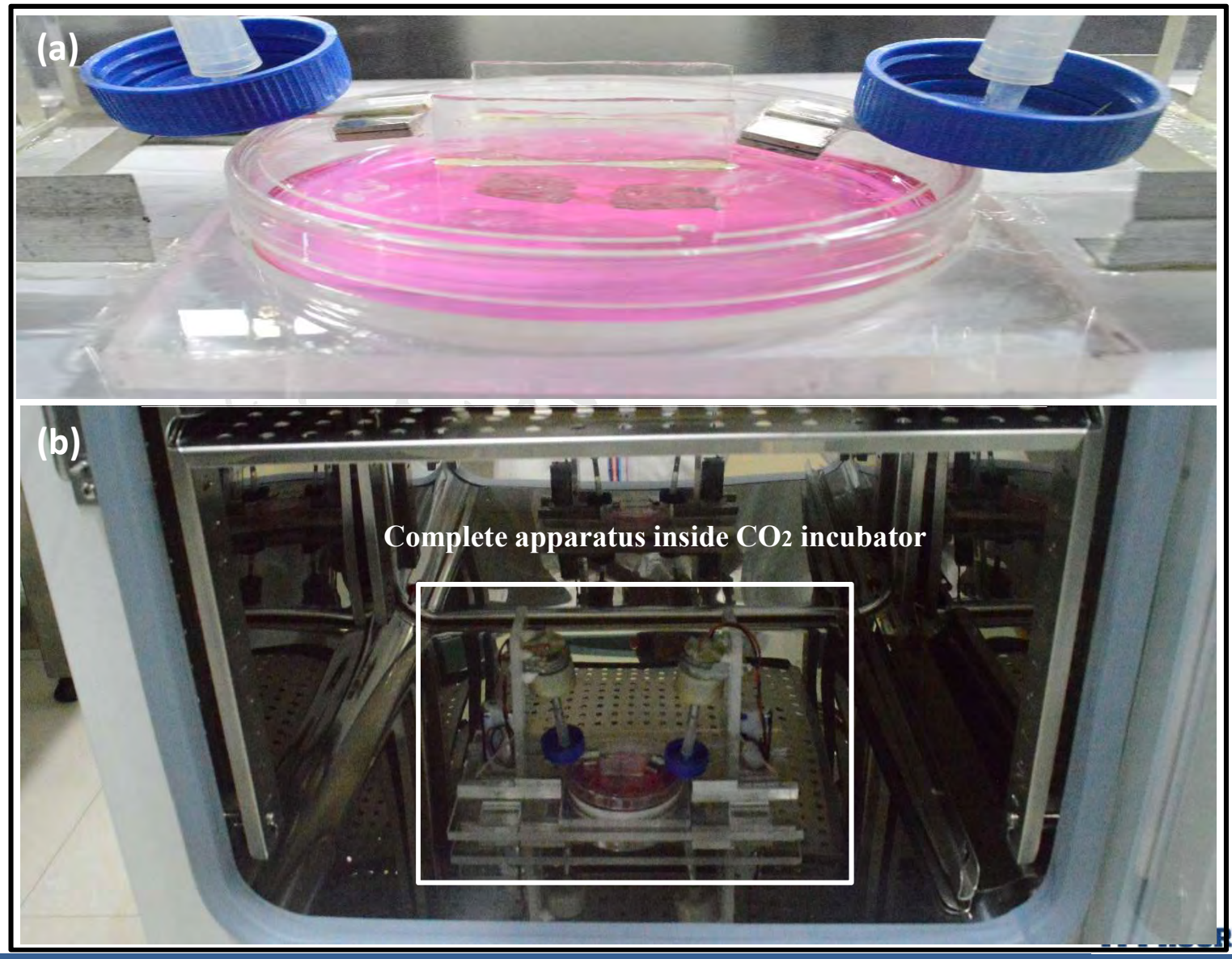
- Developing more refined printers which can print smaller details, thus eliminating the need to make a separate vascular system for the organs.

- Using this technology to print bones which are strong enough for implantation (bone-like replicas have been in progress since the 1990's out of artificial powders).

- Longer lifespans and better conditioning of the organs themselves, to be able to actually use these in the medical field.

- Reduction of costs to make this technology available to more people.

Patent and Technology transfer (4 D cell culture device)



Biomedical nanotechnology

https://onlinecourses.nptel.ac.in/noc18_bt18/preview

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Biomedical nanotechnology

ABOUT THE COURSE

Biomedical nanotechnology is a rapidly developing field, which includes a diverse collection of disciplines. The applications of nanotechnology are gaining overwhelming response in almost all the fields. Especially in healthcare sector, tremendous developments have been achieved. For example, cancer diagnosis and therapy, medical implants, tissue engineering etc. In the coming years, the developments in this field are expected to fluorish and lead to several life saving medical technologies and treatment methods. Thus, the main objective of this course is to impart knowledge on biomedical applications of nanotechnology.

Important For Certification/Credit Transfer:
Weekly Assignments and Discussion Forum can be accessed ONLY by enrolling here
Scroll down to Enroll

Note: Content is Free!
All content including discussion forum and assignments, is free

Final Exam (in-person, invigilated, currently conducted in India) is mandatory for Certification and has INR Rs. 1100 as exam fee

INTENDED AUDIENCE

UG/PG students of Biotechnology/ Nanotechnology
It is an elective course for UG/PG

PRE-REQUISITES

Basic knowledge in biology

INDUSTRIES THAT WILL RECOGNIZE THIS COURSE

Nil

4967 students have enrolled already!!

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