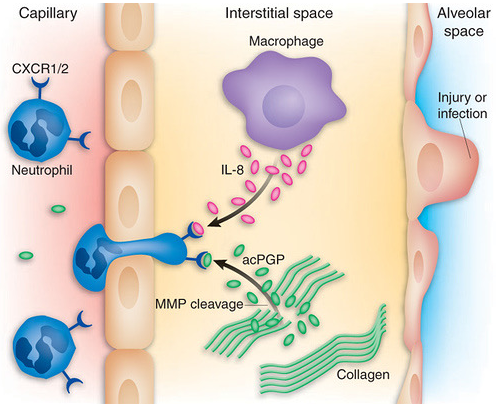
UNIVERSITÁ DEGLI STUDI DI TERAMO

Scaffolds Fabrication Techniques in Tissue Engineering

Why?

In-vitro

In-vivo



Because we can no longer 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.

SCAFFOLDS

Scaffold

To achieve the goal of tissue reconstruction, scaffolds must meet some **specific requirements.**

- A **high porosity** and an **adequate pore size** are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients.
- **Biodegradability** is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal.
- The rate at which degradation occurs has to coincide as much as possible with the rate of neo-tissue formation: This means that while cells are fabricating their own ECM around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the neotissue, newly formed tissue which will take over the mechanical load.

Scaffold

Characteristics of scaffolds:

1) Biocompatibility

- ✓ Cells must adhere, function normally, and migrate onto the surface and eventually through the scaffold and begin to proliferate before laying down new matrix.

- ✓ After implantation, the scaffold or tissue engineered construct must elicit a negligible immune reaction in order to prevent it causing such a severe inflammatory response that it might reduce healing or cause rejection by the body.

Scaffold

Characteristics of scaffolds:

2) Biodegradability

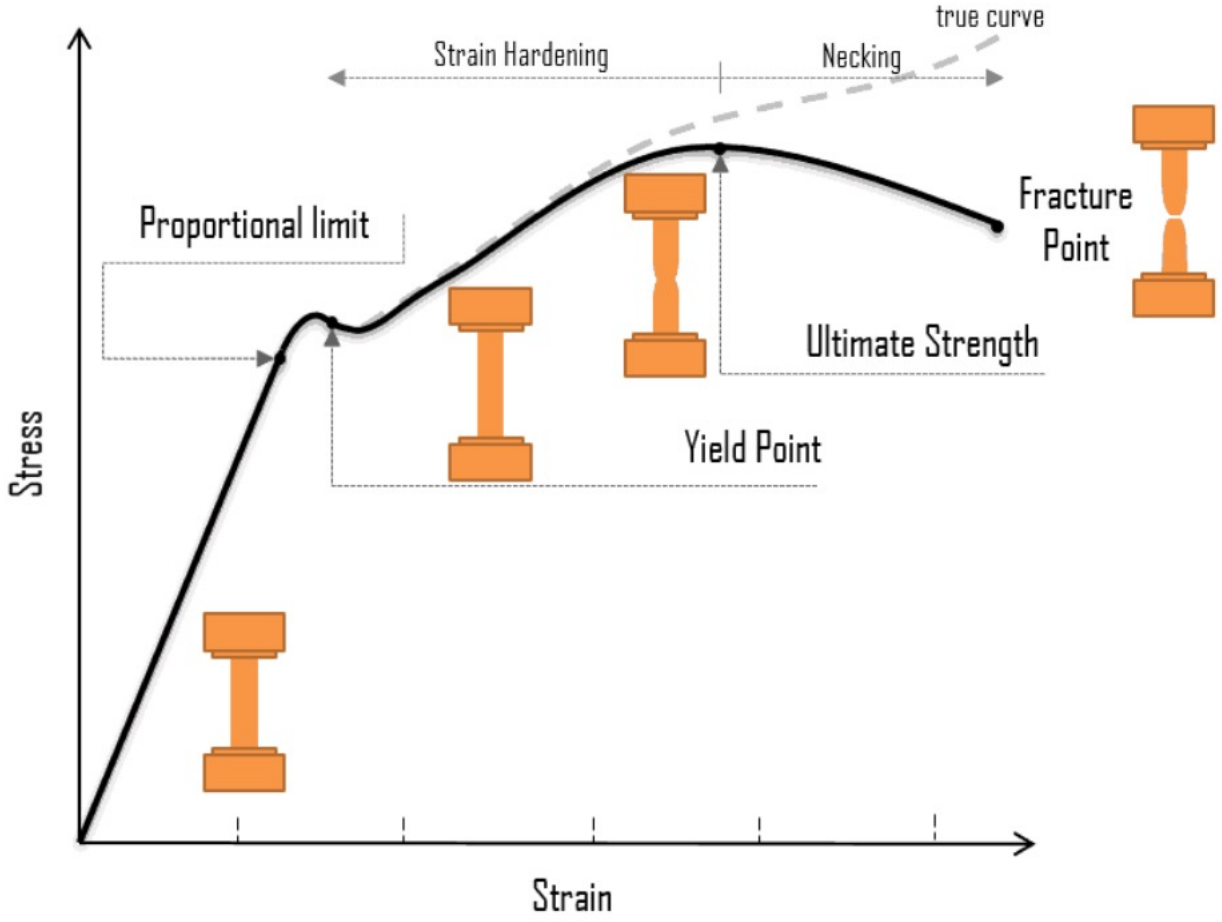
- ✓ Scaffolds are not intended as permanent implants. The scaffold must therefore be biodegradable so as to allow cells to produce their own ECM.
- ✓ The by-products of this degradation should be also non-toxic and able to exit the body without interference with other organs.

Scaffold

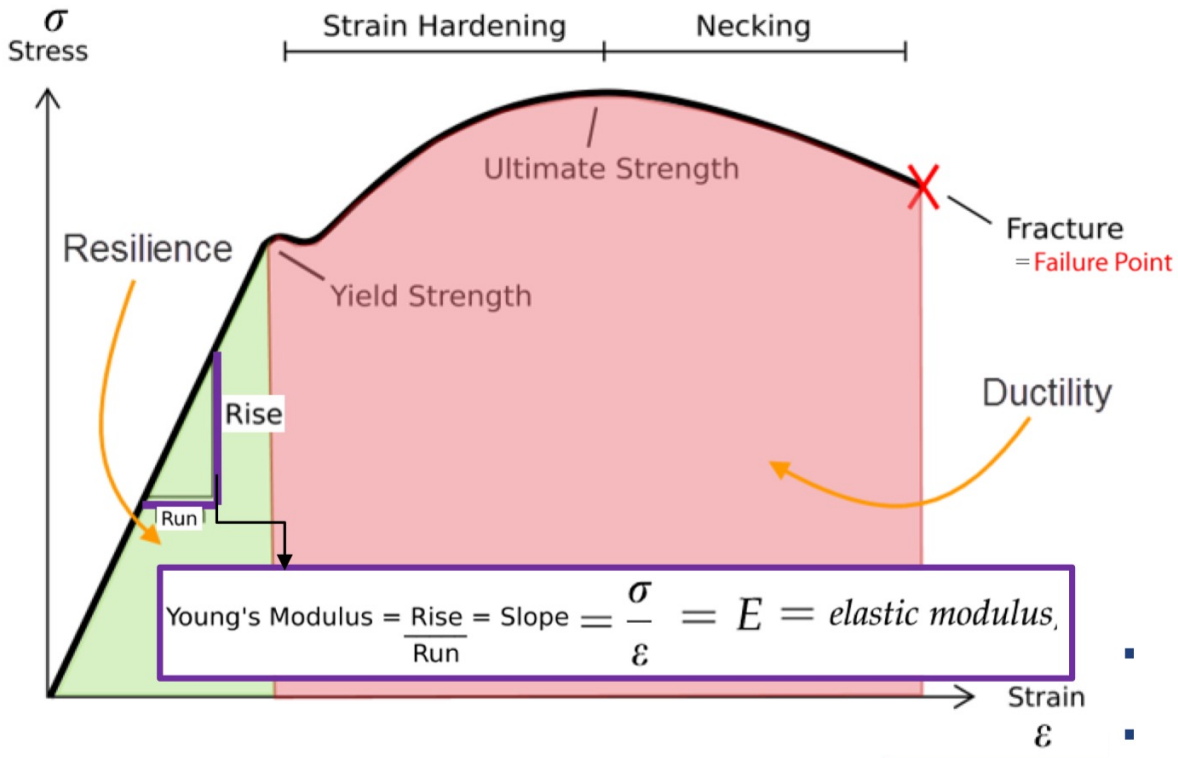
Characteristics of scaffolds:

3) Mechanical properties

- ✓ Able to maintain the structure and function immediately after implantation and during remodelling of the implants.



- **Strength:** Measures how much stress the material can handle before permanent deformation or fracture
- **Yield Strength:** The Stress at which material begins to deform plastically (=non-linear)
- **Ultimate strength:** Maximum stress before failure occurs.
- **Stiffness(E):** Resistance of the material to elastic deformation.

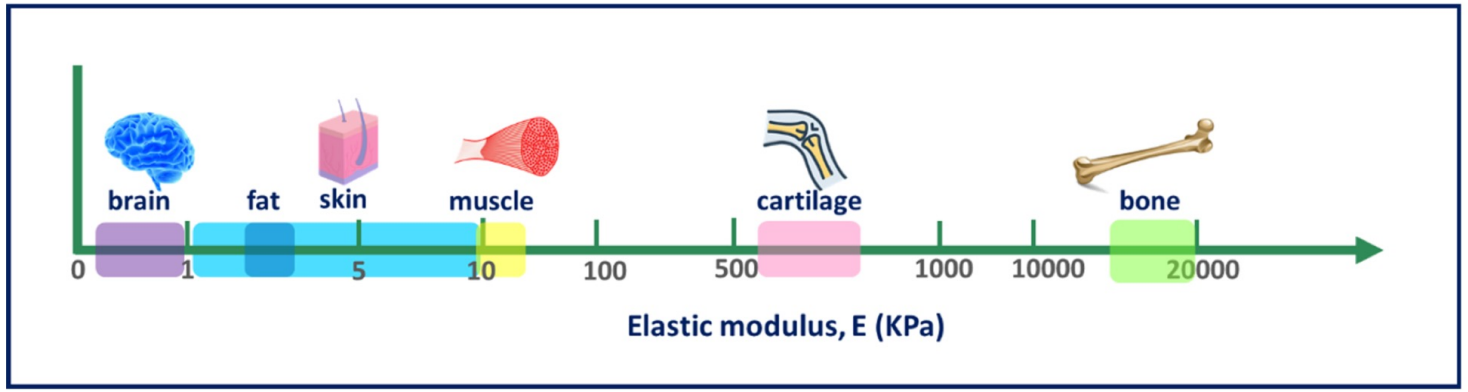


- **Toughness:** How well the material can resist fracturing when force is applied. Requires strength and ductility.

$$\text{Stress } \sigma = \frac{F}{A} \text{ [Pa]}$$

$$\text{Strain } \varepsilon = \frac{\Delta \ell}{\ell}$$

- **Resilience:** Ability of the material to spring back into shape; elasticity
- **Ductility:** Ability of a material to undergo permanent deformation through elongation



Scaffold

Characteristics of scaffolds:

4) Scaffolds architecture

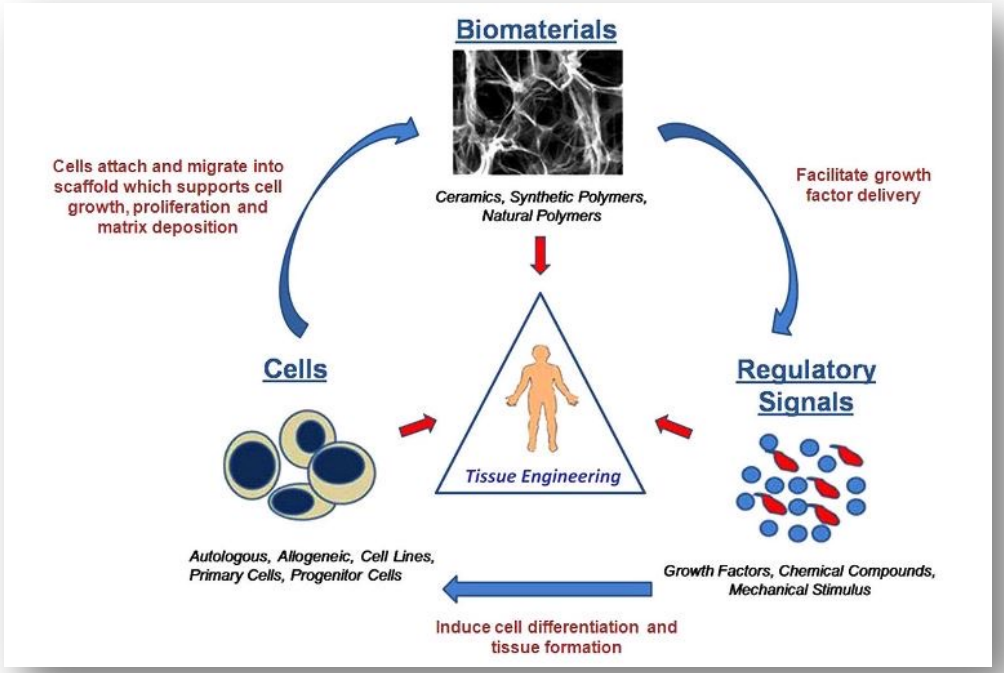
- ✓ Have an interconnected pore structure and high porosity to ensure cellular penetration and adequate diffusion of nutrients to cells within the constructs and to the ECM formed by these cells.
- ✓ The scaffold should mimic the ECM of the tissue to be regenerated or replaced. Df
- ✓ Biomimetics is defined as the application of methods and systems, found in nature, to technology and engineering.
- ✓ Mimicking the naturally occurring ECM, and how this is a promising approach to effectively tailor cell response and to successfully engineer replacement tissues.

BIO - MIMETIC

LIFE-LIKE **COPY**

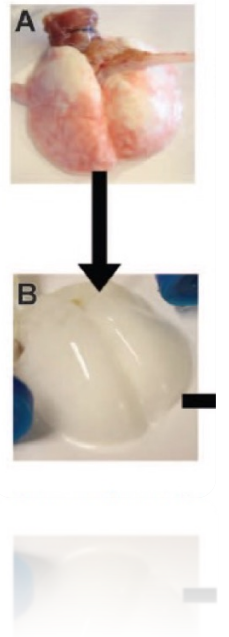
Restoration of lost body parts using scaffolds. Scaffolds are used to:

- Guide regeneration
- Growth and differentiation of cells in process of forming functional tissue
- Provide both physical and chemical signals



TE

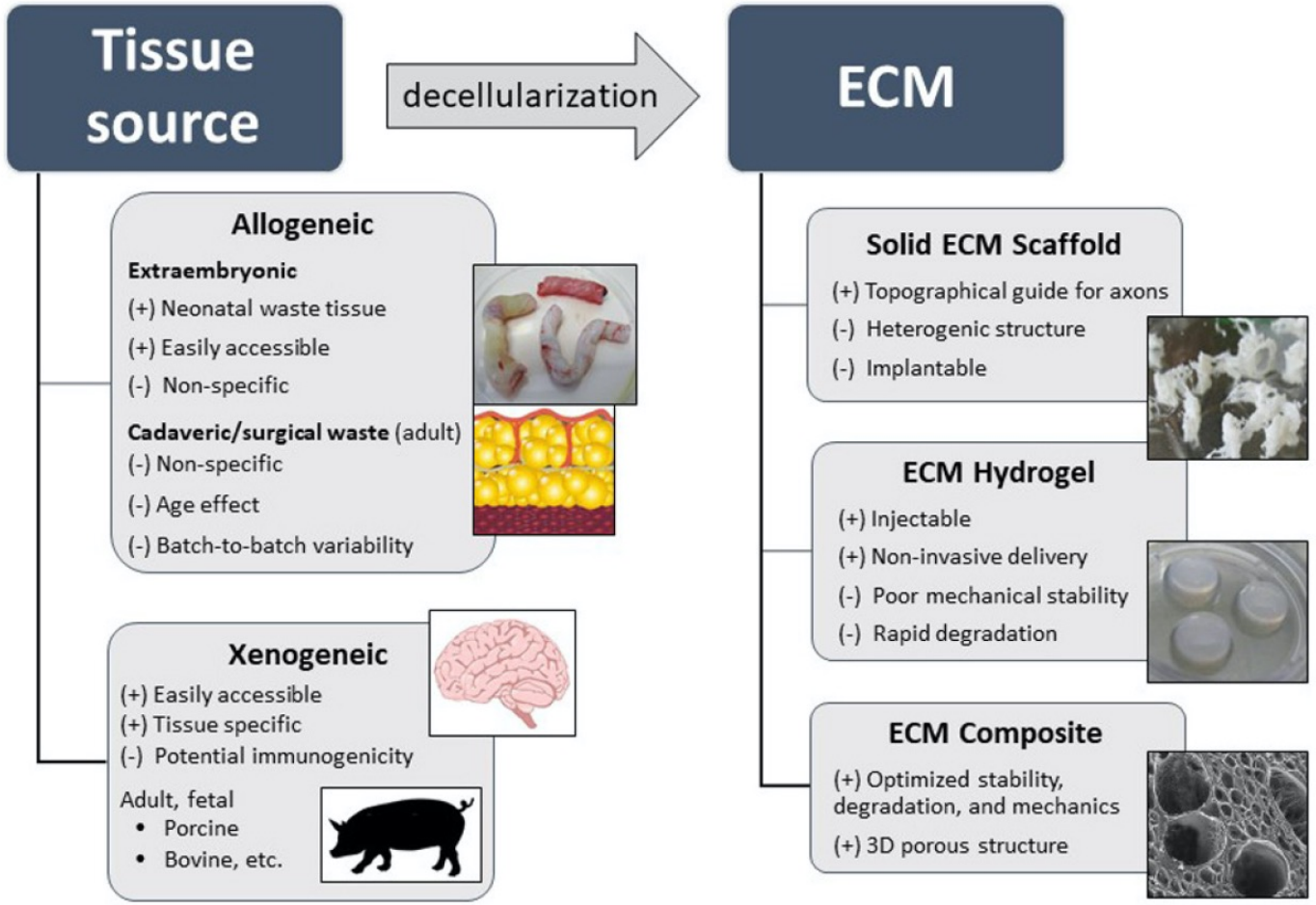
Natural scaffolds
made by extracellular matrixes (ECMs)



Artificial biomimetic scaffolds

Natural Scaffolds Composed of ECM

- The materials of scaffolds composed of ECM are commonly used for the repair and functional reconstruction of damaged and lost tissues.
- These bio-scaffolds are obtained after cell removal from the tissue sources conserving the structural and functional molecular units of the remaining ECM.



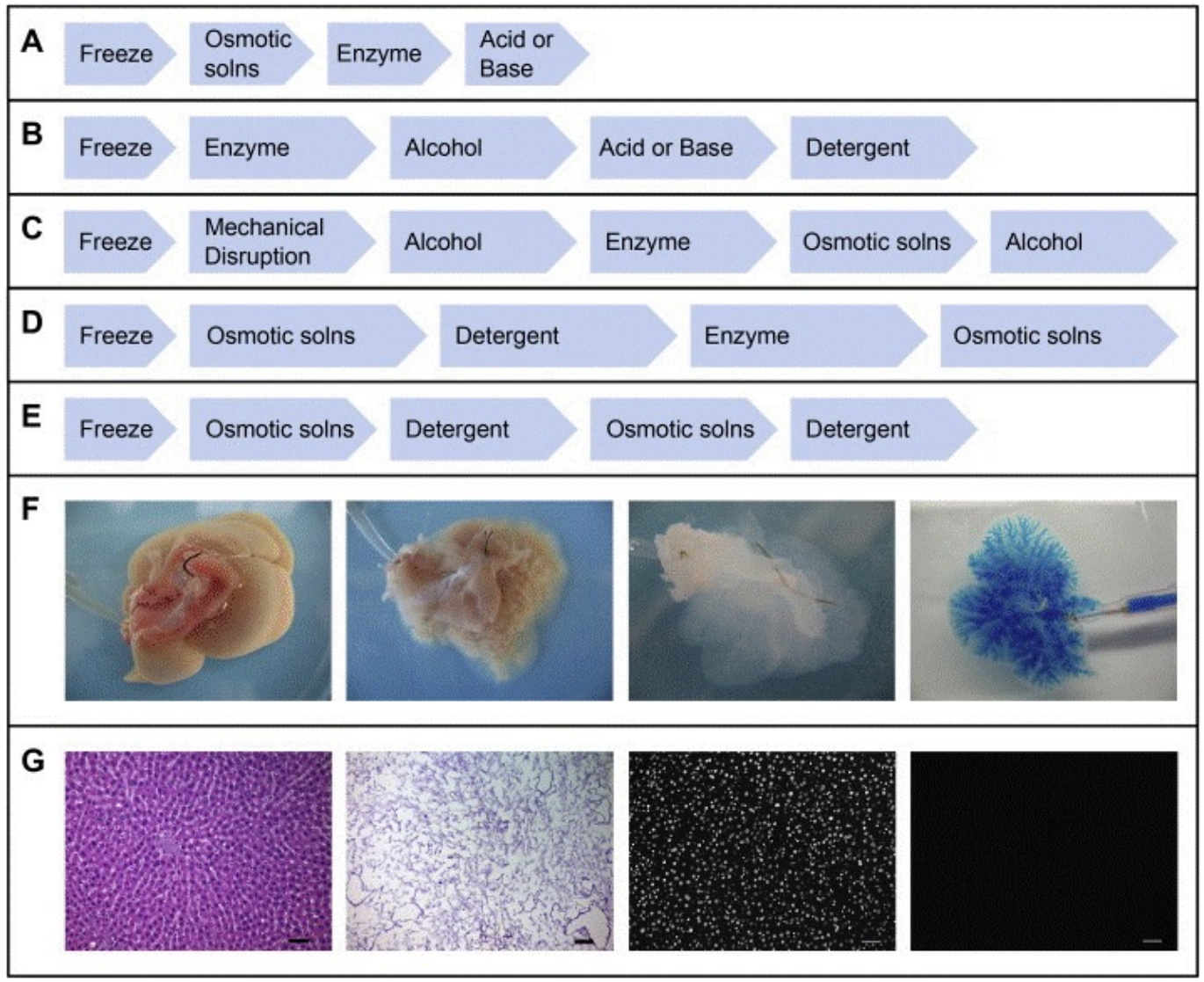
Strategies for decellularization and their problems

A) Chemical Decellularization
Break down the cells and the DNA component of the cell. The most widely used chemicals are detergents such as Triton X-100 and sodium dodecyl sulfate (SDS), acid, base, alcohol, and osmotic solutions.

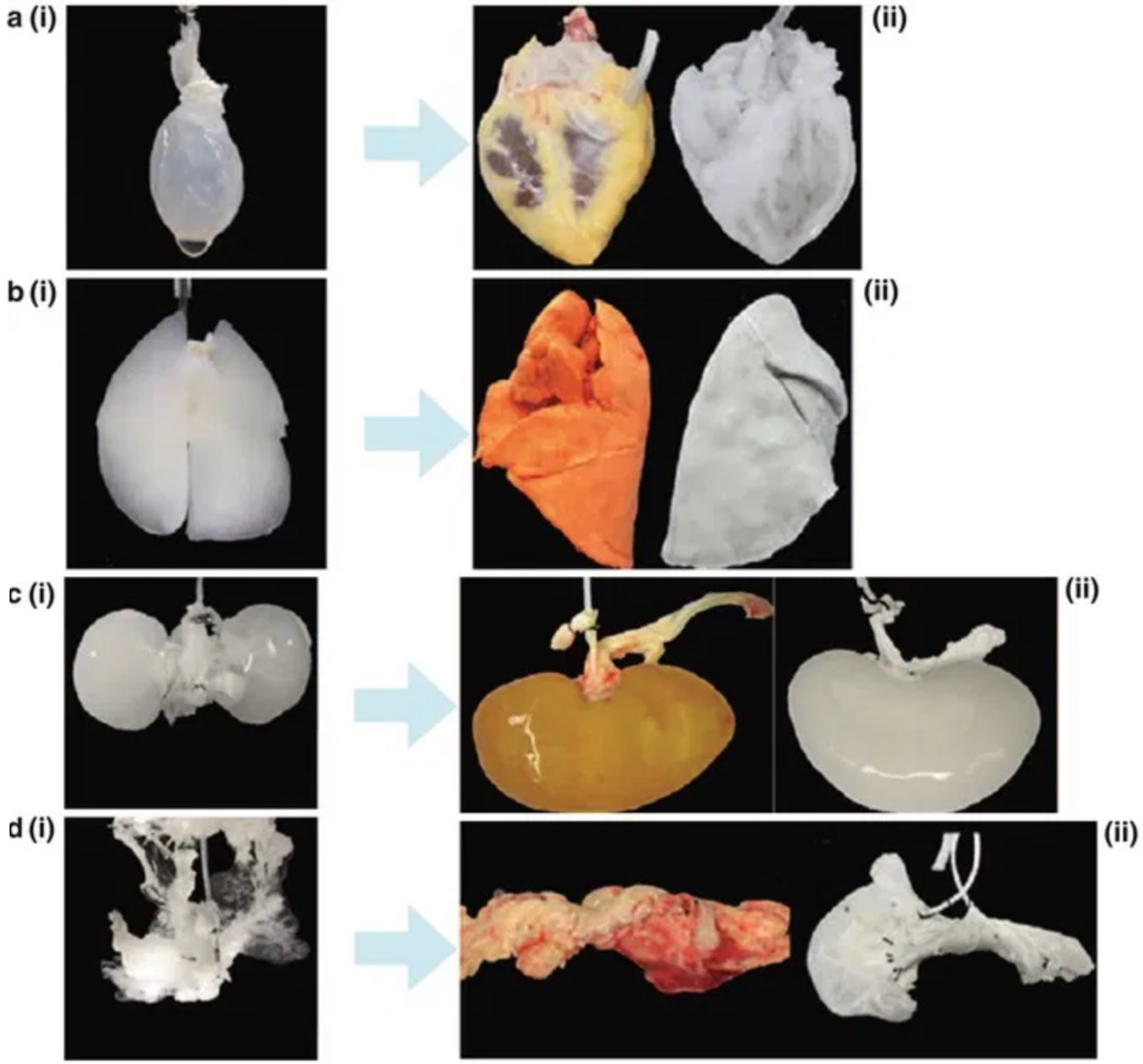
B) Enzymatic Decellularization
Enzymes used in decellularization of organs are the ones that cleave specific components of the cells. The list includes nucleases, trypsin, collagenase, lipase, dispase, thermolysin, and α-galactosidase

C) Physical Decellularization
Physical agents typically used in decellularization are temperature, pressure, sonication.

D) Combinations
Chemical, physical, and enzymatic agents can be used in combination to achieve complete decellularization of particular tissue and organ.

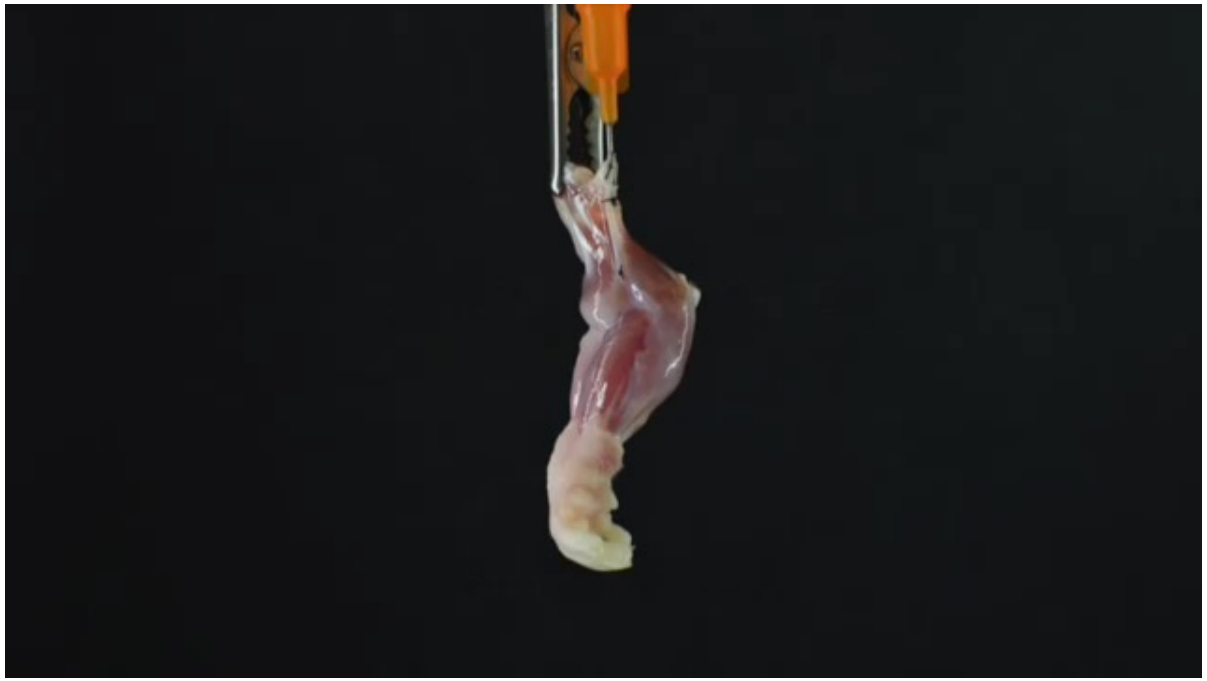


Natural Decellularized Scaffolds



https://abdominalkey.com/decellularization/

Forlimb of a rat

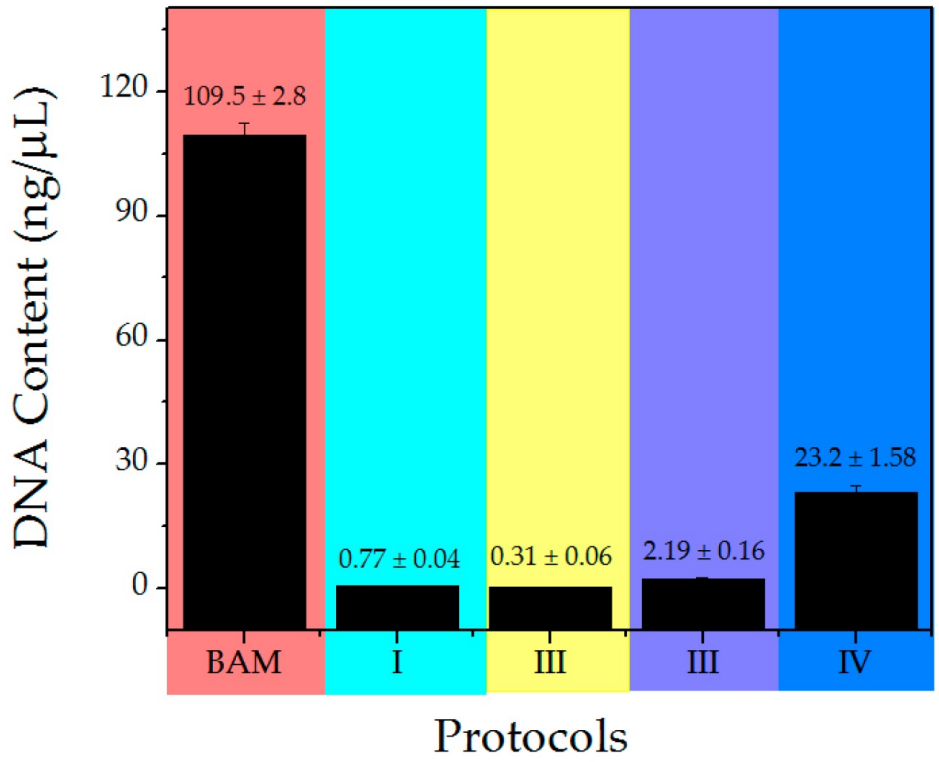


https://www.youtube.com/watch?v=p143bISuEJk

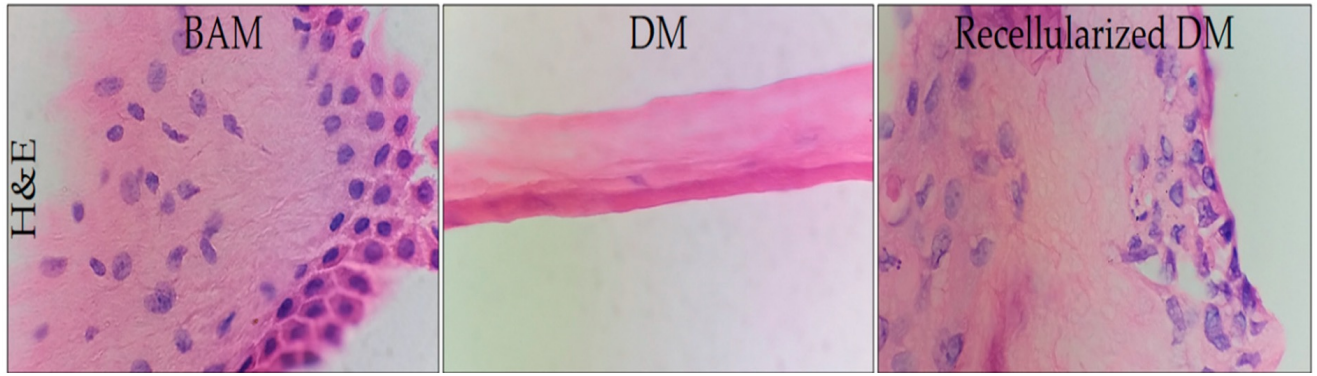
Decellularization of the Amniotic Membrane

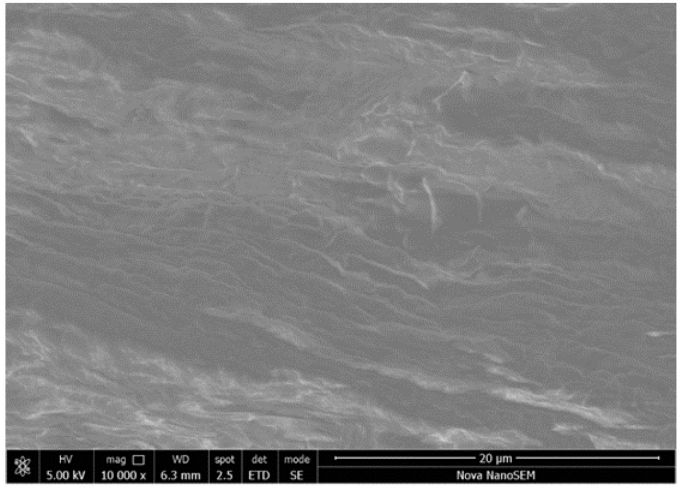
Table 1. Decellularization protocols for the bovine amniotic membranes (BAM).

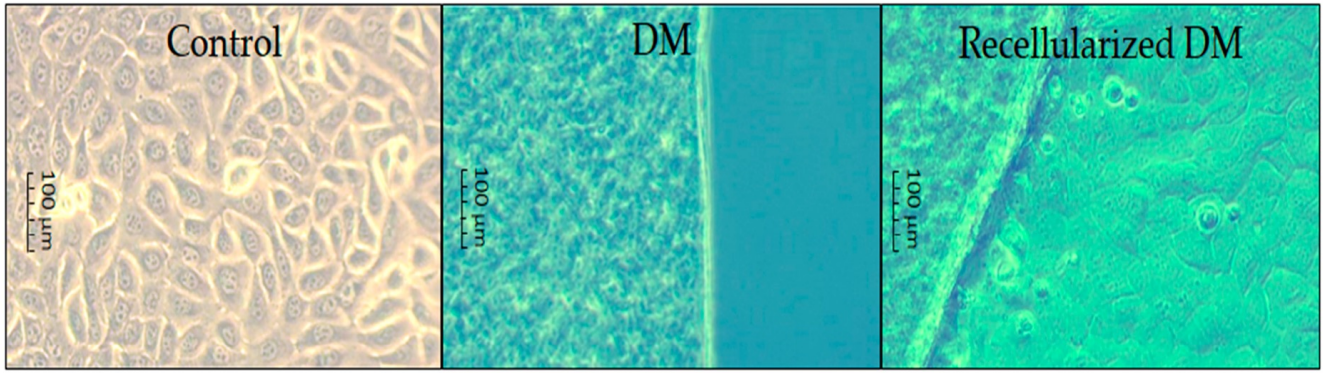
No.	Protocols
I	SDS 0.1% for 4 h NaOH 0.1 M for 1 h PAA + ascorbic acid 0.1 for 12 h Ethanol 70% for 1 h PBS for 2 h
II	SDS 0.1% for 4 h NaOH 0.1 M for 1 h PAA 0.15% + EtOH for 12 h NaOH 0.1 M for 1 h PAA for 1 h Ethanol 70% for 1 h PBS for 2 h
III	Tween 80 for 4 h NaOH 0.1 M for 1 h, PAA + ascorbic acid 0.1 for 12 h Ethanol 70% for 1 h PBS for 2 h
IV	Tween 80 for 4 h NaOH 0.1 M for 1 h PAA 0.15% + EtOH for 12 h NaOH 0.1 M for 1 h PAA for 1 h Ethanol 70% for 1 h PBS for 2 h

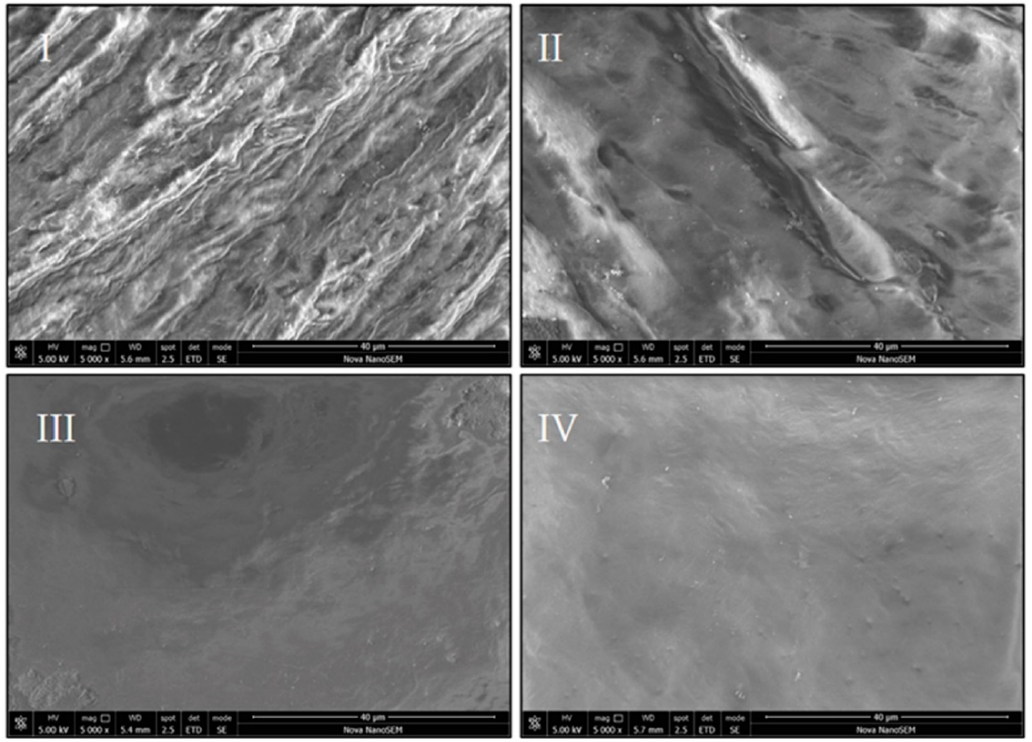


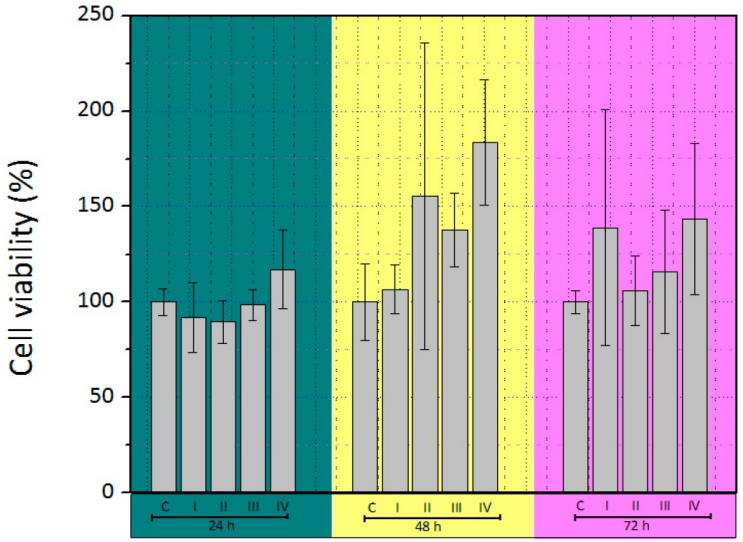
Villamil Ballesteros et al., Polymers 2020, 12, 590; doi:10.3390/polym12030590









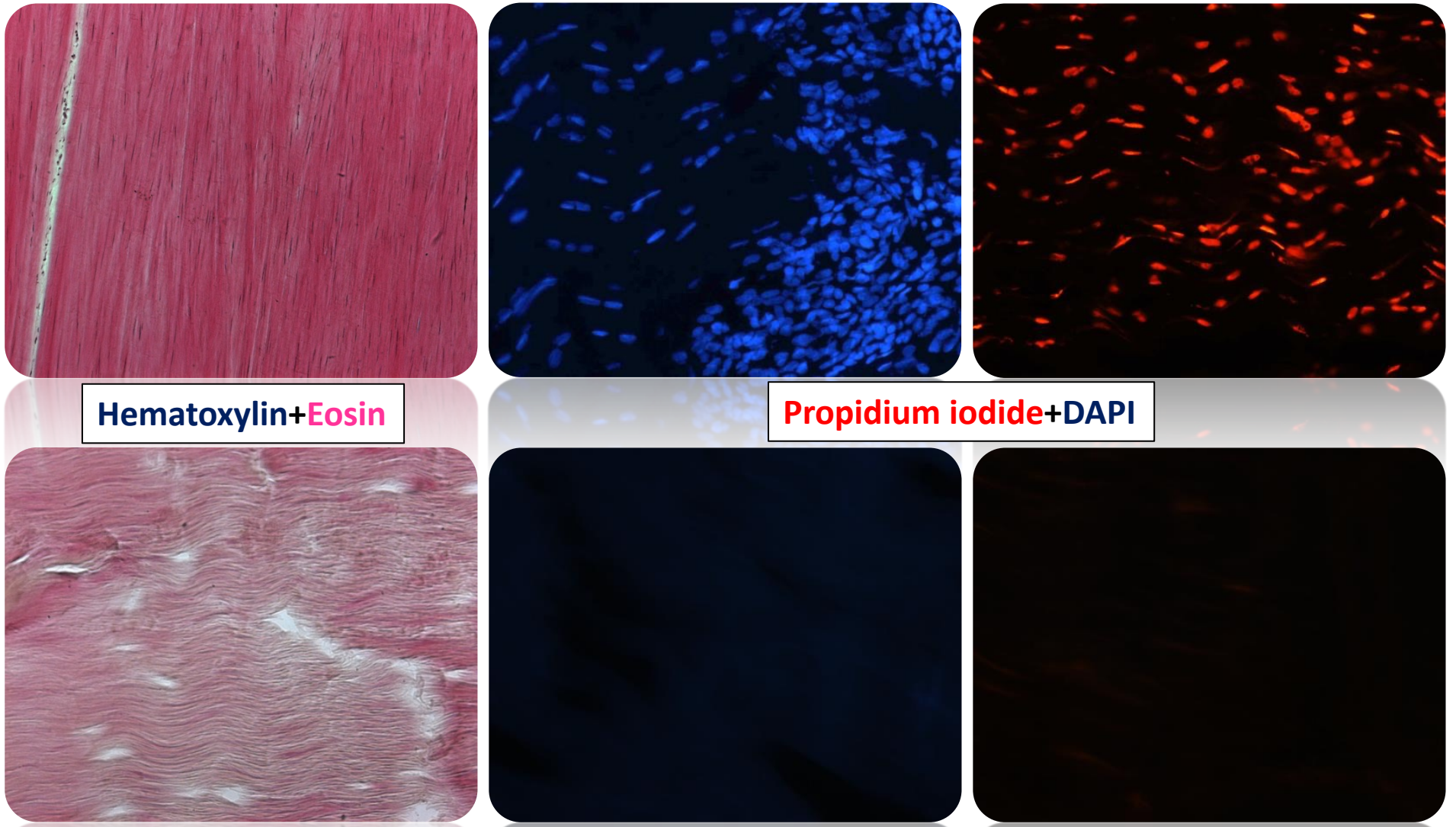


Villamil Ballesteros et al., Polymers 2020, 12, 590; doi:10.3390/polym12030590

Bio-scaffolds from a decellularized tendon

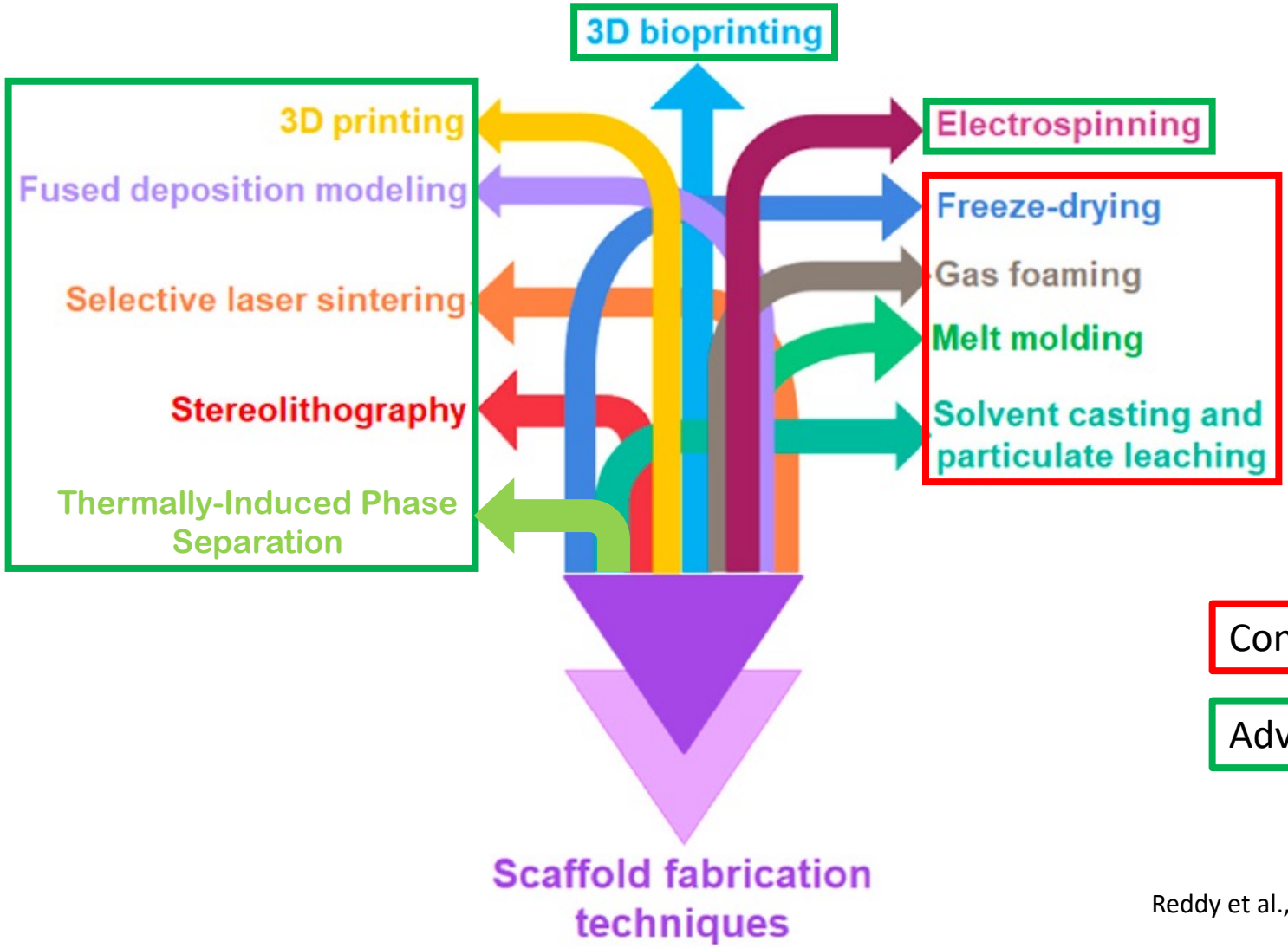
Adult ovine tendons

200x



Decellularization using SDS-EDTA-Peracetic Acid

Fabrication Techniques of Scaffolds



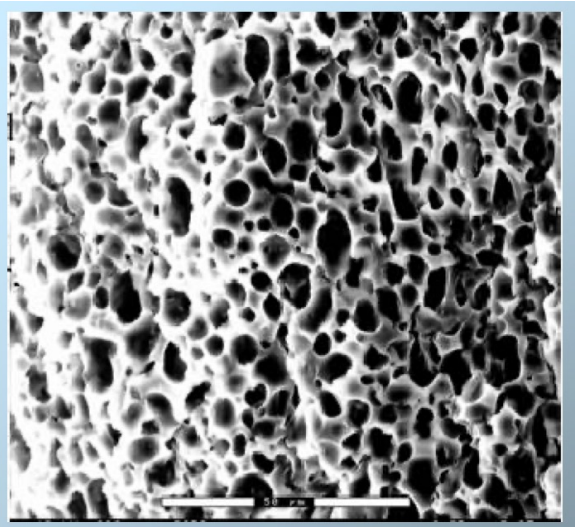
Conventional Techniques

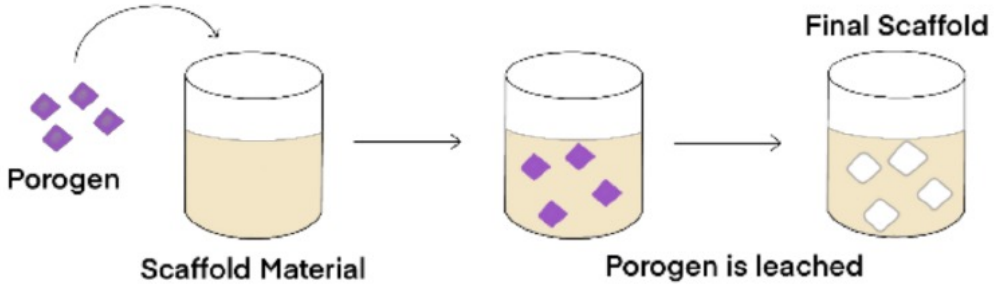
Advanced Techniques

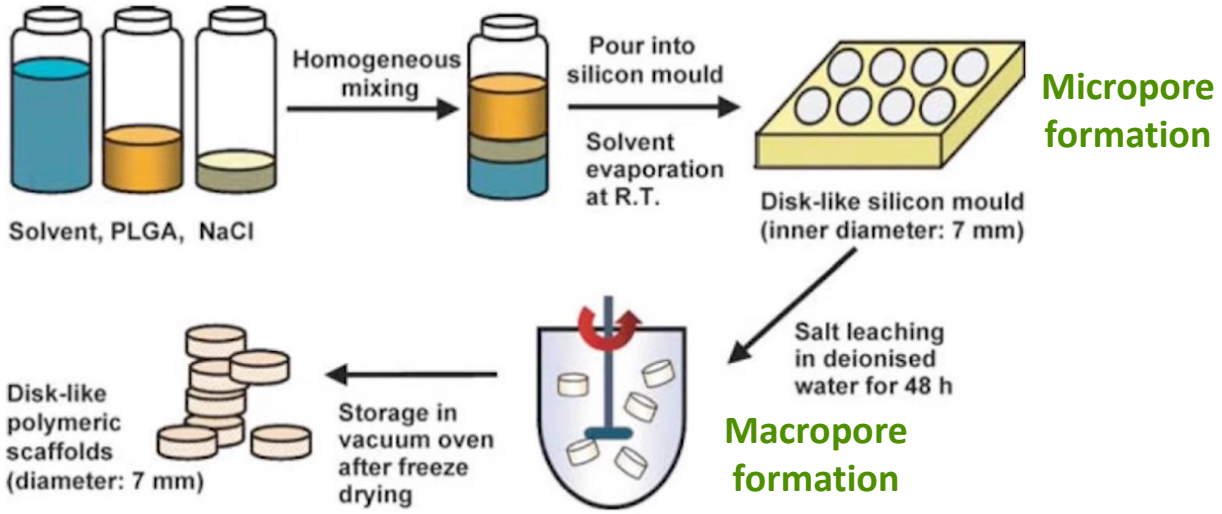
Reddy et al., 2021, polymers, 13, 1105

Solvent Casting and Particulate Leaching

Fabrication Method	Advantages	Disadvantages	Materials
Solvent casting and particulate leaching: Polymer solution poured into the mold along with an appropriate porogen. A porous scaffold is obtained at high pressure and after evaporation of organic solvents	• Control over porosity, pore size, and crystallinity. • Highly porous materials with interconnected pores. • Simple and reproducible technique.	• Limited mechanical properties, residual solvents, and porogen material. • Longer processing time. • This technique is mainly applied to produce thin membranes.	Different classes of synthetic polymers (e.g., PLLA, PLGA, or PEG) and natural polymers

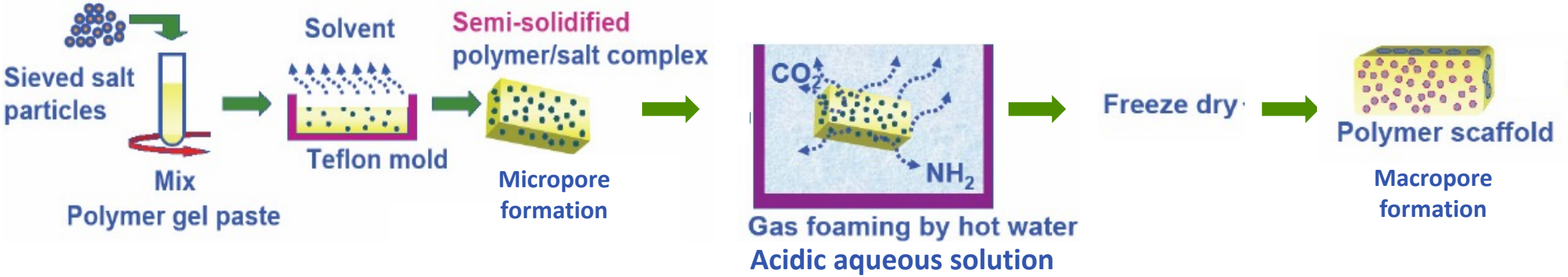




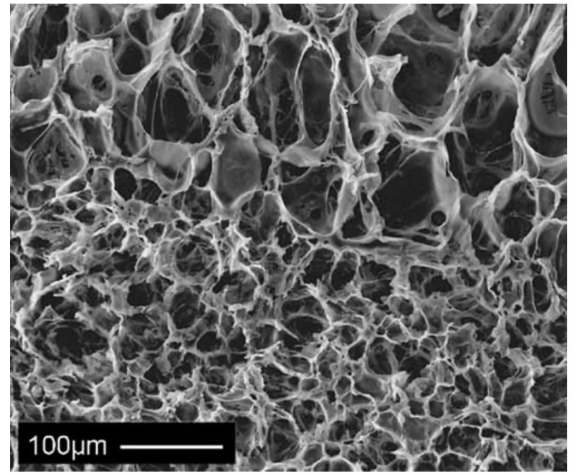


Reddy et al., 2021, polymers, 13, 1105

Gas Foaming



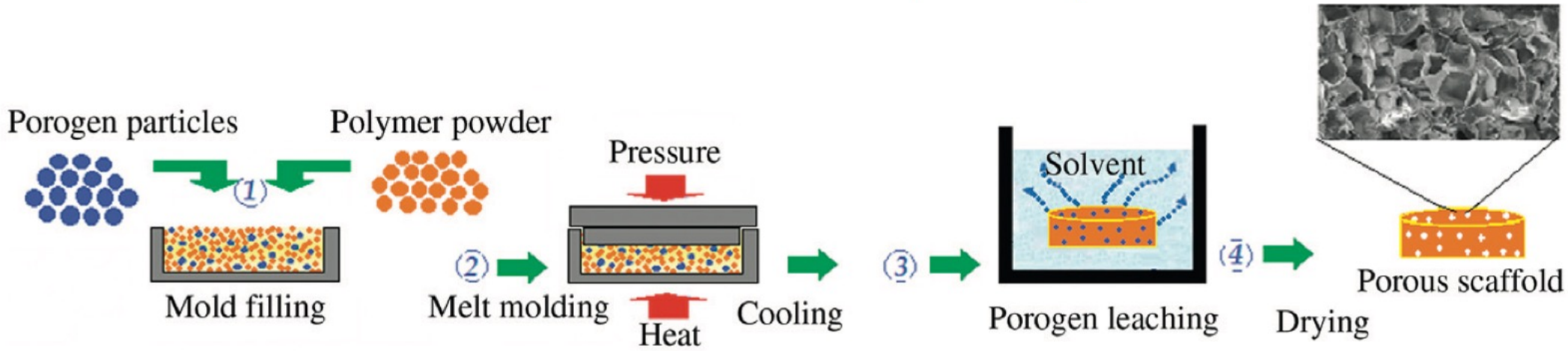
Fabrication Method	Advantages	Disadvantages	Materials
Gas foaming: Polymer gel paste along with sieved effervescent salt particles poured into a mold and immersed into hot water. Formation of the porous matrix after the evolution of ammonia and carbon dioxide gas from salt particles of the solidifying polymer matrix	• Free of harsh organic solvents. • Control over porosity and pore size. • Minimum loss of bioactive molecules. • No need for the leaching process. • High porosity > 90%.	• Limited mechanical properties, inadequate pore interconnectivity. • Longer processing time.	PLA, PLLA, or PLGA



Reddy et al., 2021, polymers, 13, 1105

Melt Molding

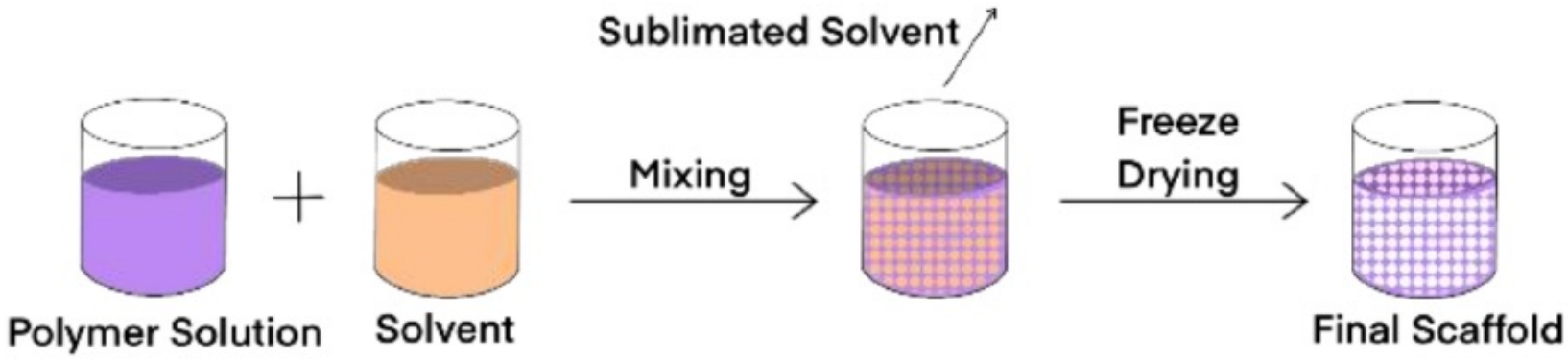
Fabrication Method	Advantages	Disadvantages	Materials
Melt molding: Both polymers and a suitable porogen are melted together, then by cooling the polymer mixture the scaffold is obtained. In this process, the porosity is attained by dissolving the porogen in water	• Independent control over porosity, pore size, pore interconnectivity, and geometry.	• The requirement of high temperature for the non-amorphous polymer. • Requires a residual porogen. • Longer processing time. • Limited mechanical properties. • Expensive technique.	PLA, PGA, PLGA–gelatin, PA

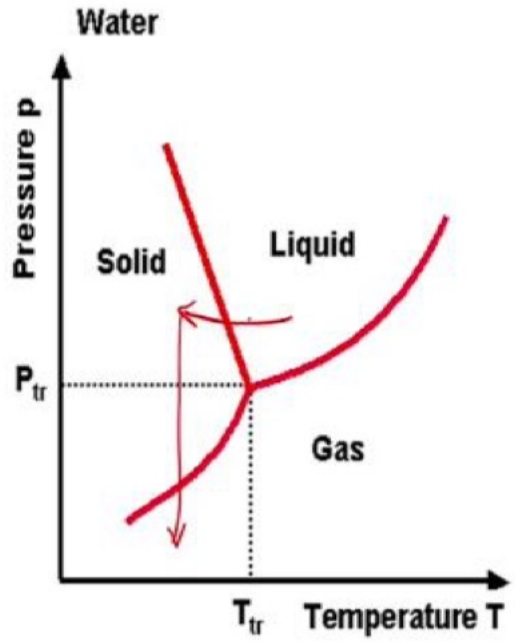


Reddy et al., 2021, polymers, 13, 1105

Freeze-Drying

Fabrication Method	Advantages	Disadvantages	Materials
Freeze-drying: A polymer solution is poured into a suitable mold and solvents are removed using a lyophiliser. This technique is mainly based on the sublimation process	• High temperature and a separate leaching step not required. • Highly porous materials, with random or oriented pores.	• Pore size is relatively small and porosity is often irregular. • Long processing time. • Expensive technique.	Natural polymers like alginate, agarose, gelatin, chitosan, etc., and PGA, PLLA, PLGA, PLGA/PPF blends

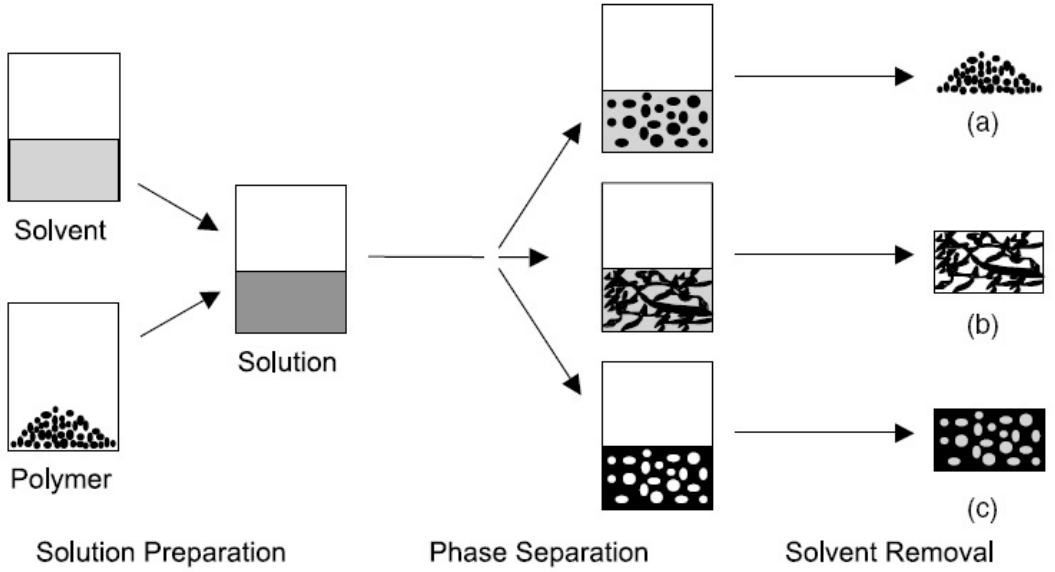




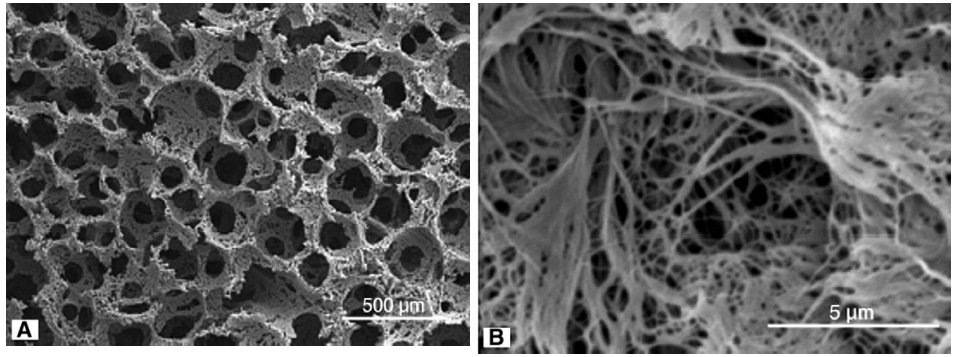
Reddy et al., 2021, polymers, 13, 1105

Thermally-Induced 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 and dimensions include pits, islands, fibers, and irregular pore structures.



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



SEM of nanofibrous scaffold with interconnected spherical macropores

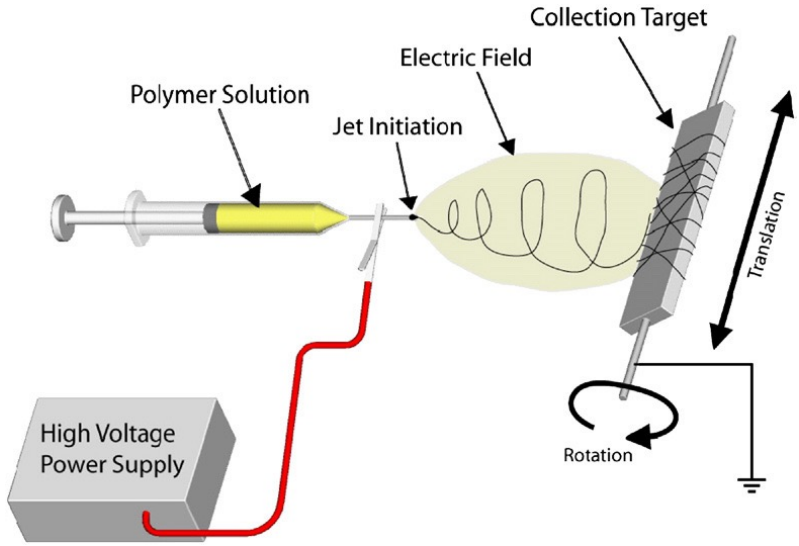
Electrospinning

Fabrication Method	Advantages	Disadvantages	Materials
Electrospinning: The electrospinning process draws a continuous narrow stream of material from a reservoir of polymer melt or solution to a collecting plate, where the material accumulates, producing the fibrous mat. This is accomplished by inducing charge buildup on the surface of the solution through the application of strong voltages	• Control over porosity, pore size, and fiber diameter. • High surface area. • Cheap and simple.	• Limited mechanical properties, pore size decreases with fiber thickness. • Not applicable for all polymers. • Not sufficient for cell seeding. • Not sufficient for cell infiltration.	Synthetic polymers (PEO, PLGA, PLLA, PCL, PVA) and natural polymers (collagen, silk fibroin, elastin, fibrinogen, chitosan) and their composites

This process involves the ejection of a charged polymer fluid onto an oppositely charged surface.

multiple polymers can be combined

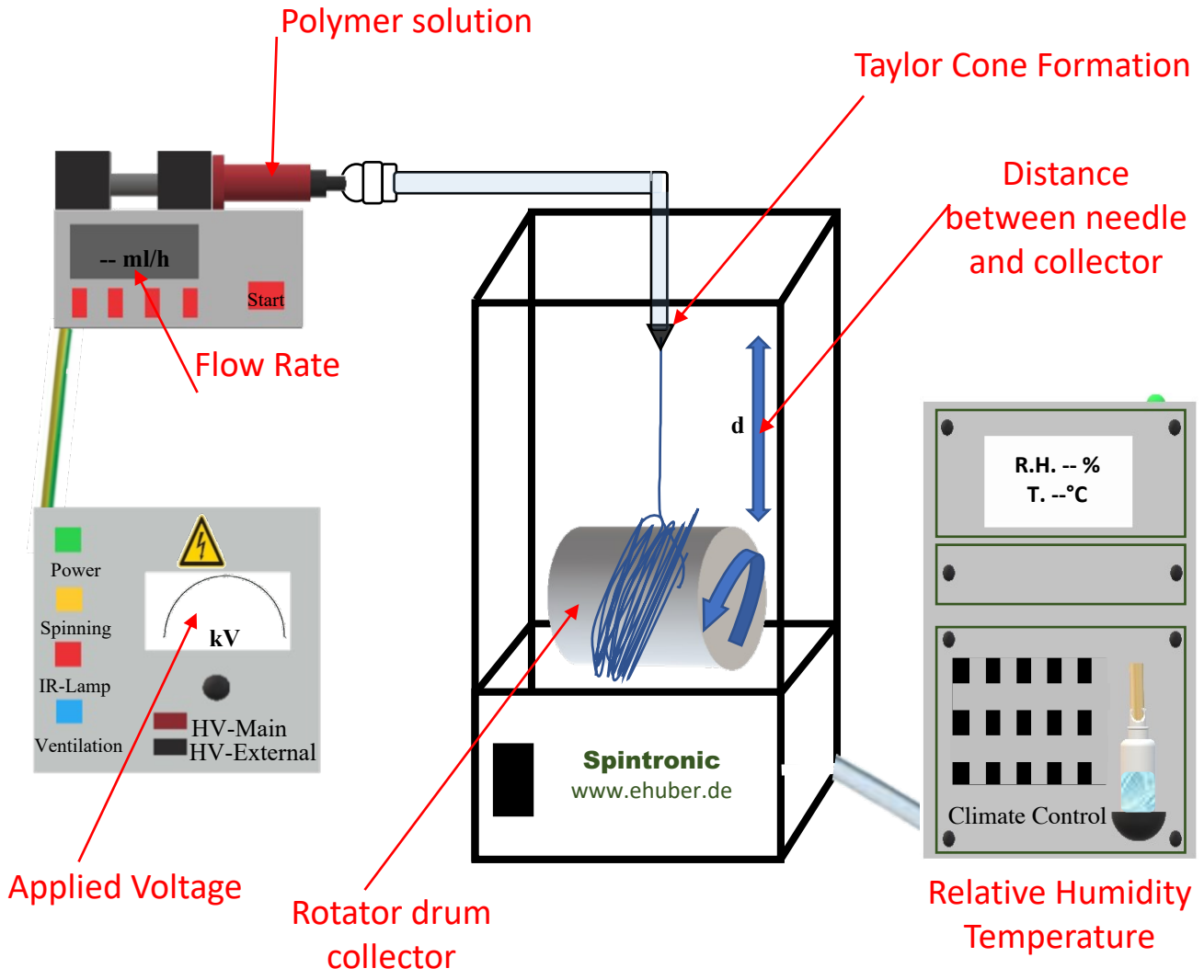
control over fiber diameter and scaffold architecture.



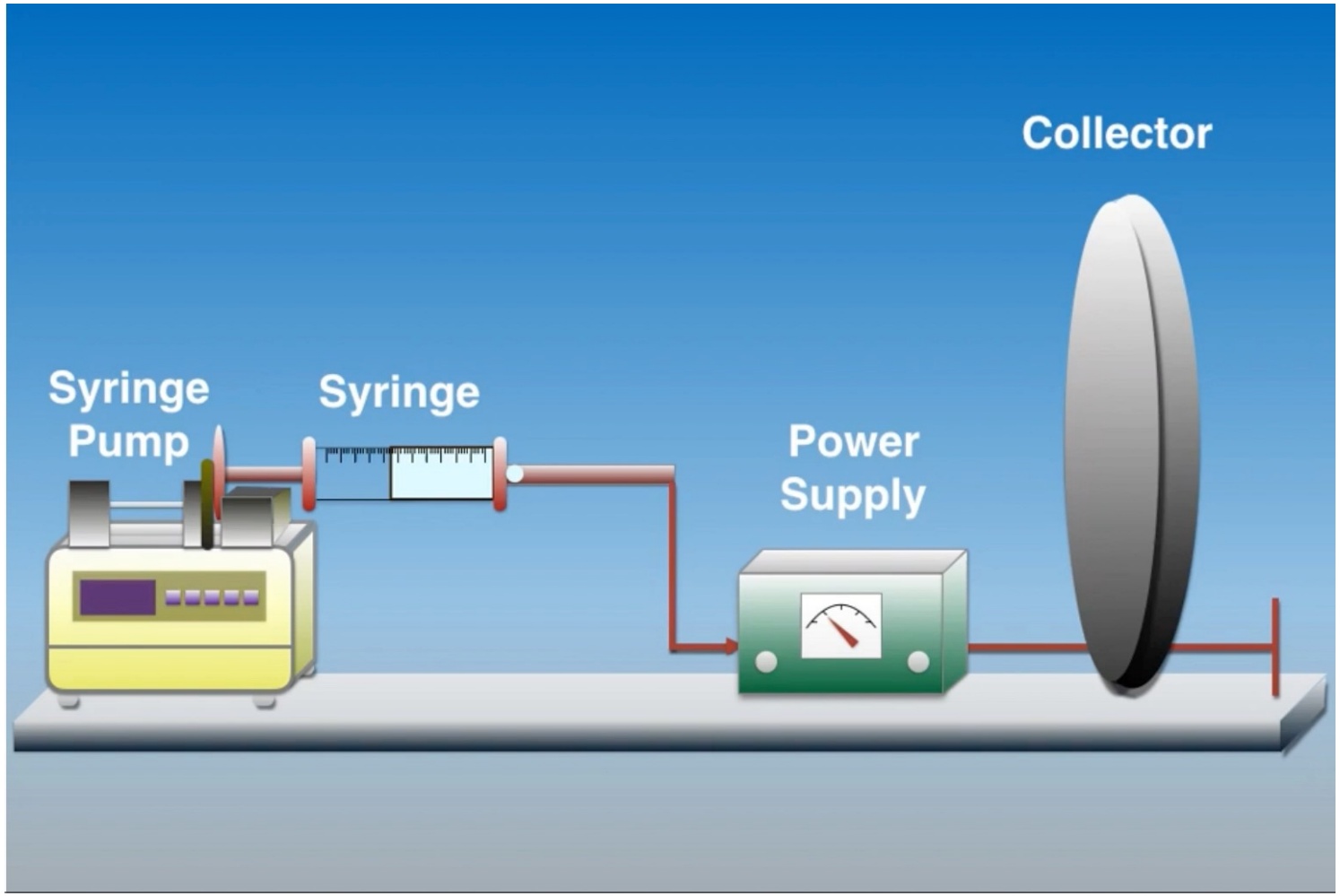
A schematic of the electrospinning process to illustrate the basic phenomena and process components

Electrospinning

1. A high voltage **power supply** (normally working in a range between 10 and 30kV);
2. A **polymer reservoir** that can maintain a constant flow rate of solution, commonly a syringe connected to either a mechanical or a pneumatic syringe pump;
3. A conductive dispensing **needle** as polymer source connected to the high voltage power supply;
4. 4. A conductive substrate, normally grounded, which serves as a **collector** for the electrospun fibers.

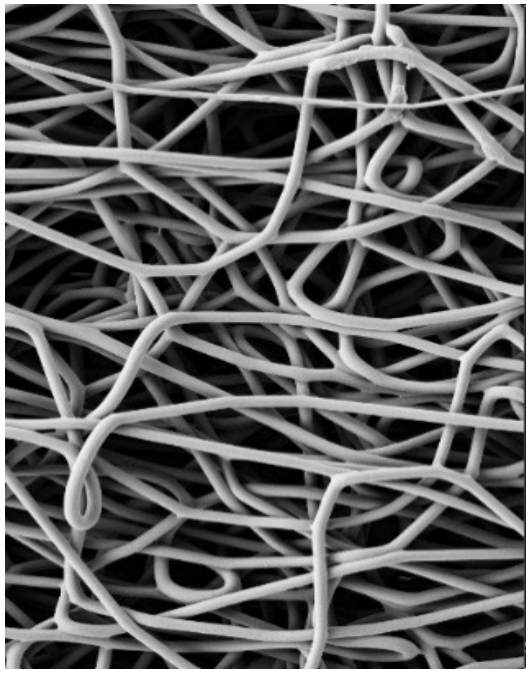


Electrospinning

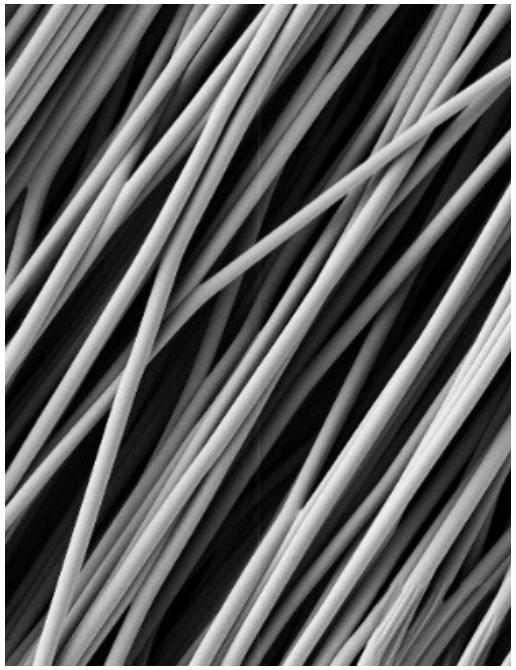


https://www.youtube.com/watch?v=ZZ9iExn5VtI

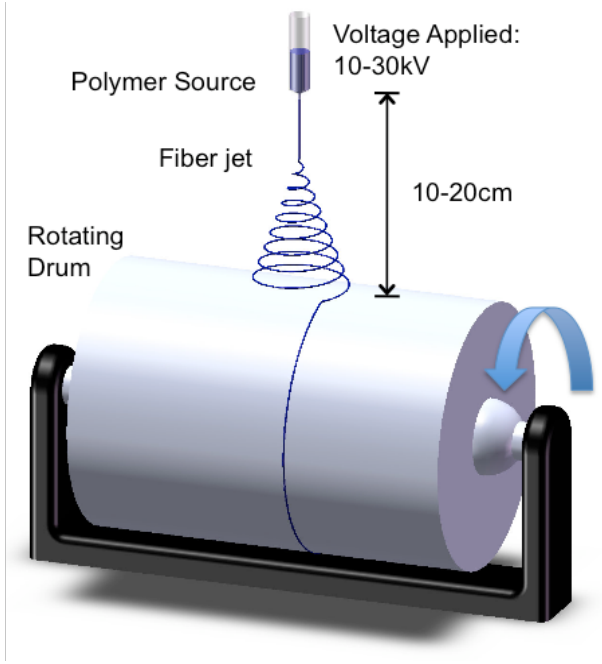
Modified Electrospinning Setups – Aligned fibers

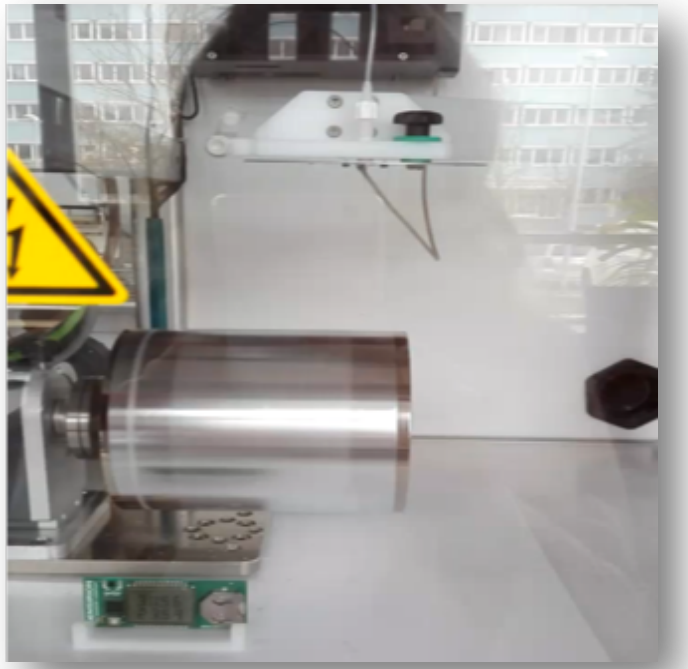


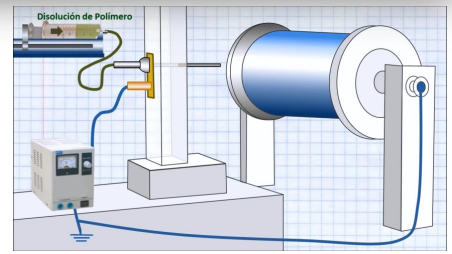
Standard Collector



Rotating Drum



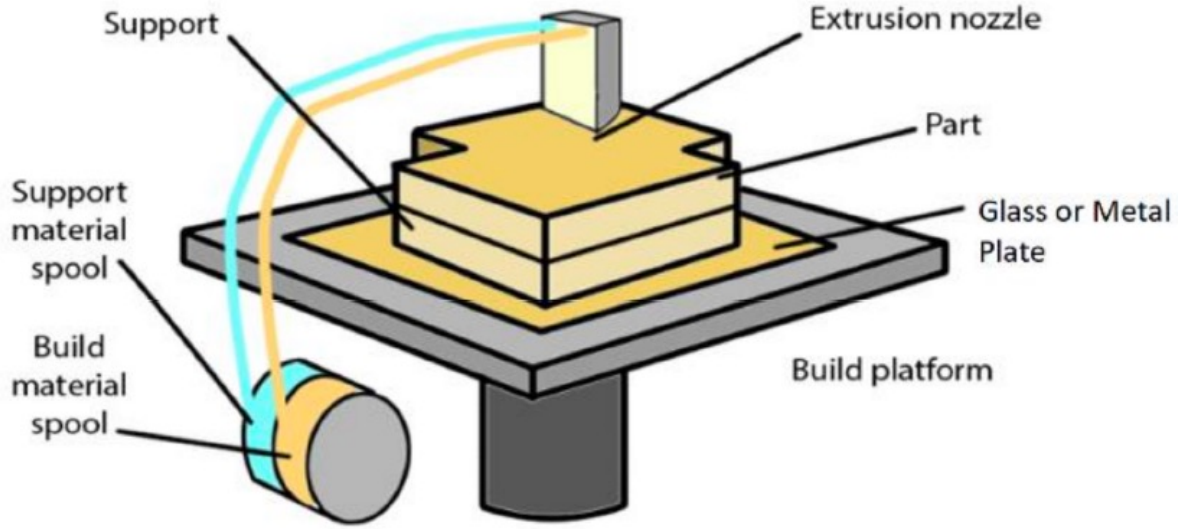




Russo et al. 2020, molecules

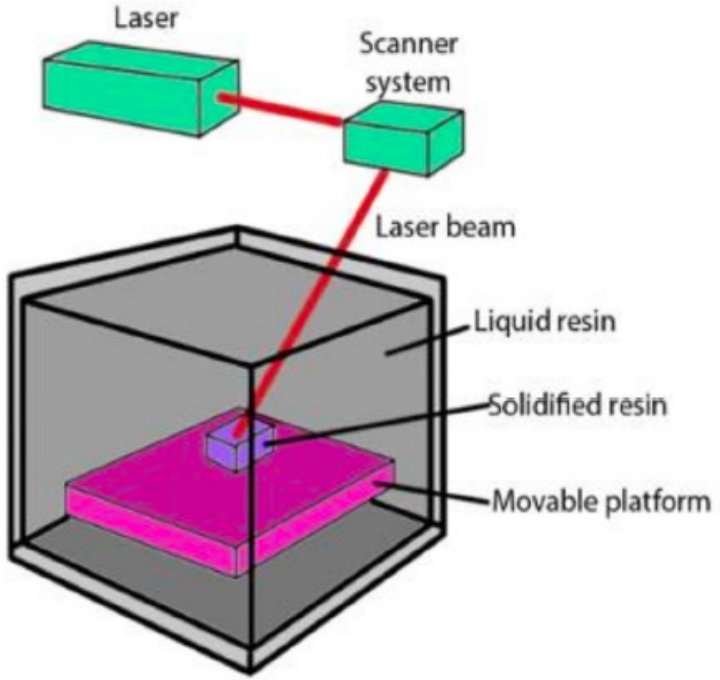
Fused deposition modelling (FDM)

Fabrication Method	Advantages	Disadvantages	Materials
Fused deposition modeling (FDM): FDM uses a layer-by-layer deposition technique, in which molten polymers or ceramics are extruded through a nozzle with a small orifice and merge with the material on the previous layer	• 3D models of custom-made implants cast for individual patients. • FDM processes can achieve pore sizes ranging from 160 to 700 microns, with porosities ranging from 48% to 77%.	• Pore anisotropy and the geometry of pore connectivity are substantially limited due to the continuous deposition process. • FDM is typically limited to synthetic thermoplastic polymers, thereby eliminating many natural biomaterials and thermoset synthetic polymers.	Biodegradable materials used for this method include PCL, PLGA, polycarbonate, polypropylene, and various polyesters



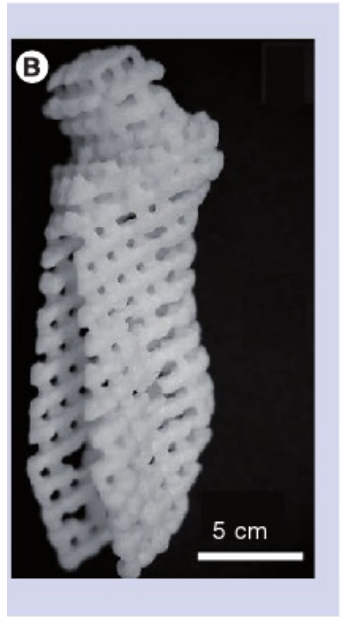
Stereolithography (SLA)

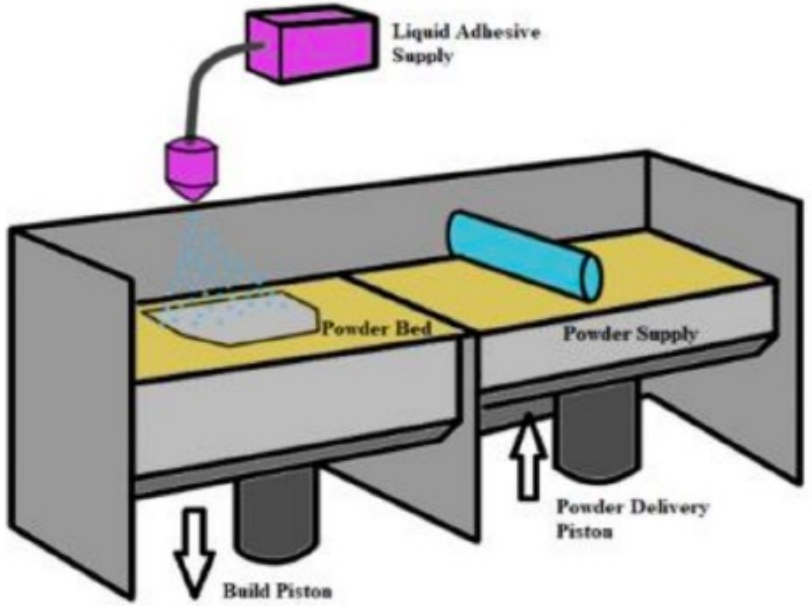
Fabrication Method	Advantages	Disadvantages	Materials
Stereolithography (SLA): In SLA, an object is created by selectively curing a polymer resin layer-by-layer using an ultraviolet (UV) laser beam	• Creates 3D scaffolds for tissue engineering with complex geometries. • Pores of multiple sizes, which can ensure a selective transport of cells versus smaller molecules.	• The time required for fabrication increases cubically as resolution increases.	PPF, PEO, PEG

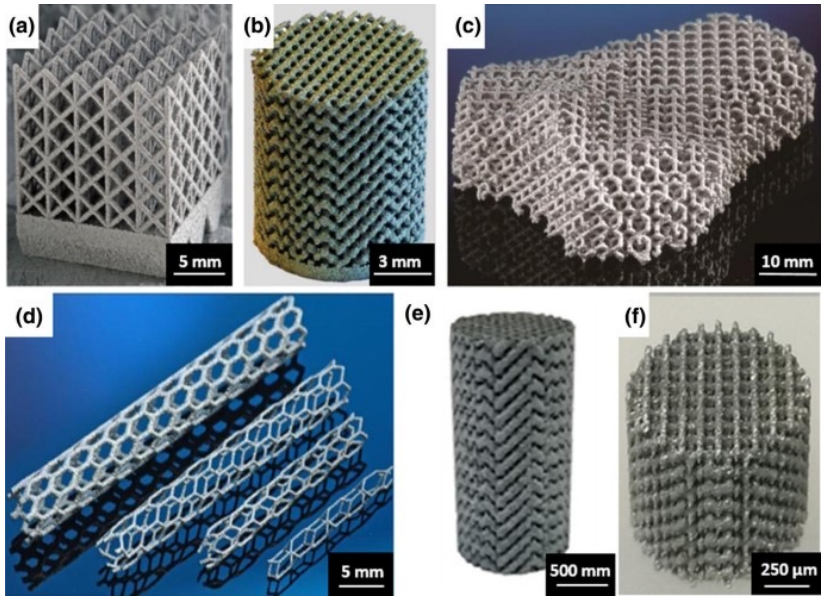


Selective laser sintering (SLS)

Fabrication Method	Advantages	Disadvantages	Materials
Selective laser sintering: This method selectively sinters thin layers of polymer-based mixtures in the powder form, creating solid 3D composite objects with macro-and microscale features	• Highly capable of producing objects with intricate structures and shapes containing channels, overhanging features, and gradient structures. • TE scaffolds with controlled porosity and customized architecture.	• Incapability to use polymers in the hydrogel form. • Impossibility to encapsulate cells in scaffolds. • Limitation in forming sharp corners and clear boundaries, making it impossible to create small details.	Nondegradable or degradable biopolymers (e.g., PE, PCL, PLLA, PLGA, etc.), and composites can be processed into scaffolds for TE

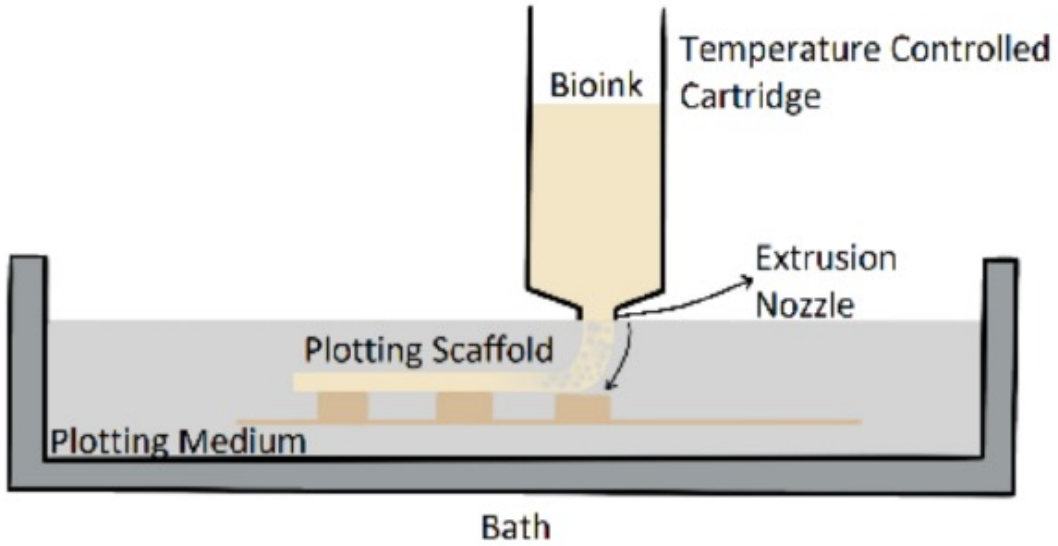






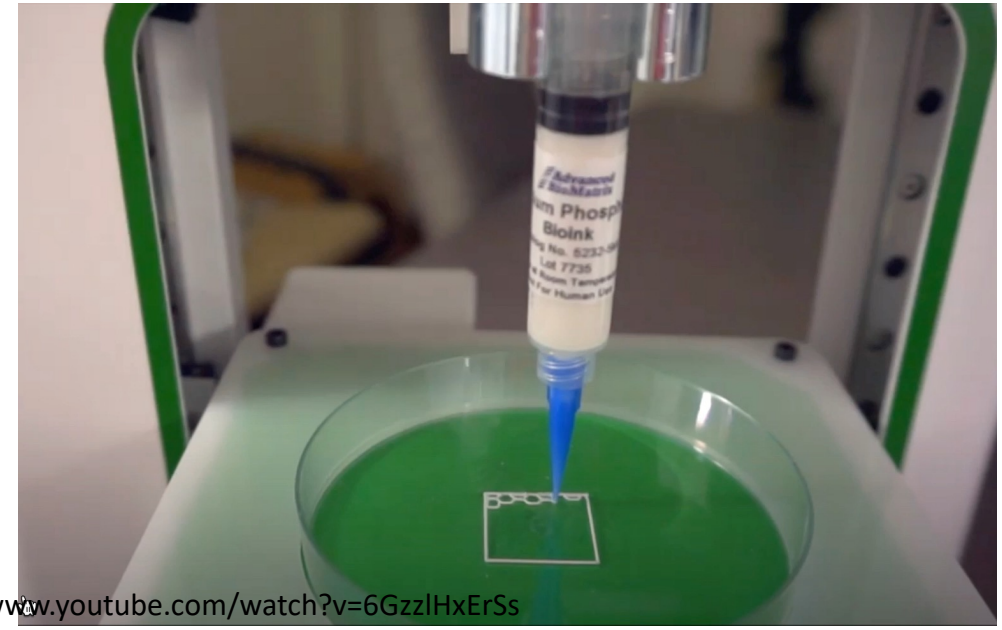
3D printing

Fabrication Method	Advantages	Disadvantages	Materials
3D printing: It is a process of reconstruction of a 3D physical model by the successive addition of material layers resulting in a 3D solid object based on CAD model design	• Able to create almost any shape or geometric feature, allows defined internal architectures for implants.	• The addition of a chemical binder. • Post-fabrication efforts to remove the residual solvent such as vacuum drying are not completely effective; therefore, the issue of cytotoxicity in 3D printing (3DP)-fabricated scaffolds remains.	PEO, PCL, and PLGA



3D bioprinting

Fabrication Method	Advantages	Disadvantages	Materials
3D bioprinting: It is the 3D printing process of generating layer-by-layer 3D tissue-like structures using viable cells, an encapsulation biomaterial, and growth and differentiation factors to create a bio-printed pre-tissue that is further transferred to an incubator where it matures into a tissue	• Biomimicry. • Autonomous self-assembly. • Small tissue building blocks.	• The development of biomaterials for 3D bioprinting is still in its early stages.	Common biomaterials include natural and/or synthetic polymers and decellularized ECM



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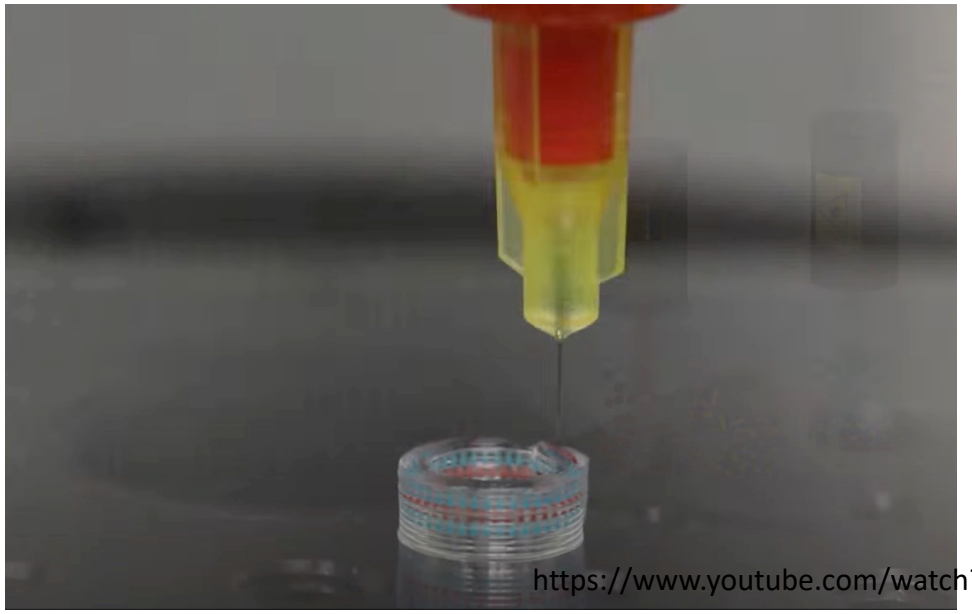

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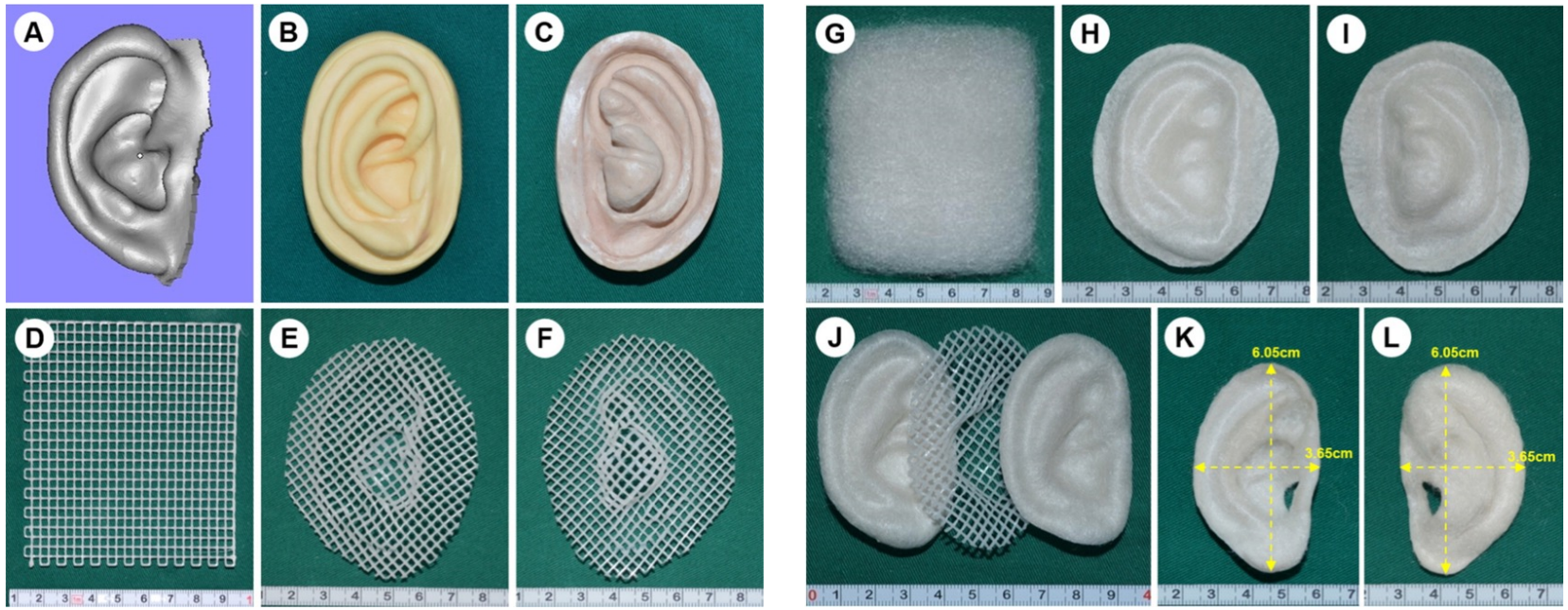

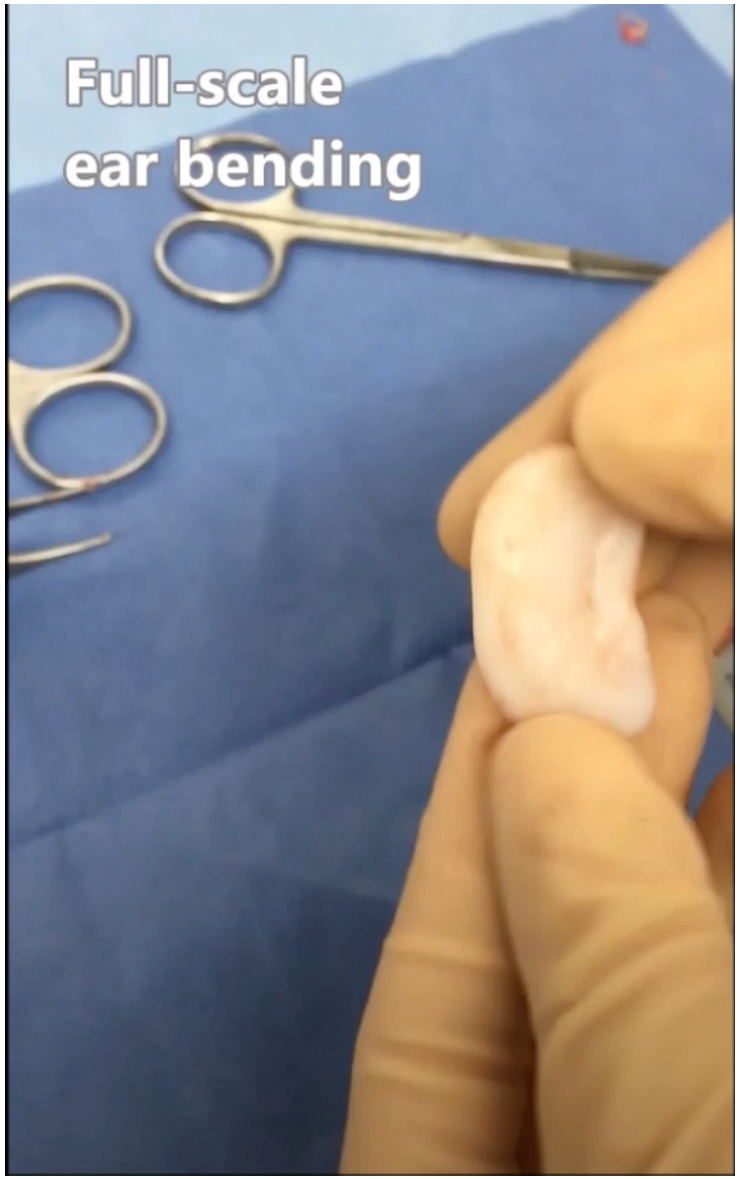

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