UNIVERSITÁ DEGLI STUDI DI TERAMO

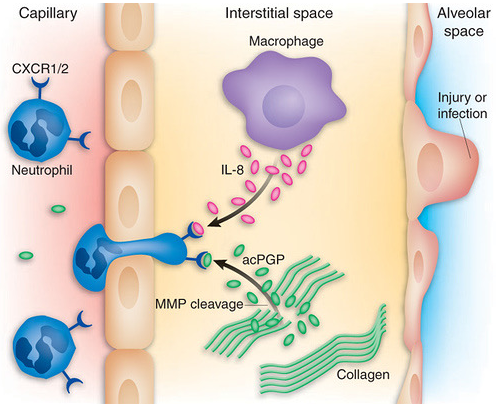
Corso di Laurea Magistrale in Biotecnologie Avanzate
AA 2021-2022

Scaffolds Fabrication Techniques in Tissue Engineering

Why?

In-vitro

In-vivo



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.

SCAFFOLDS

Image sourse: www.millipore.com/images
farm1.static.flickr.com

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.

Scaffold

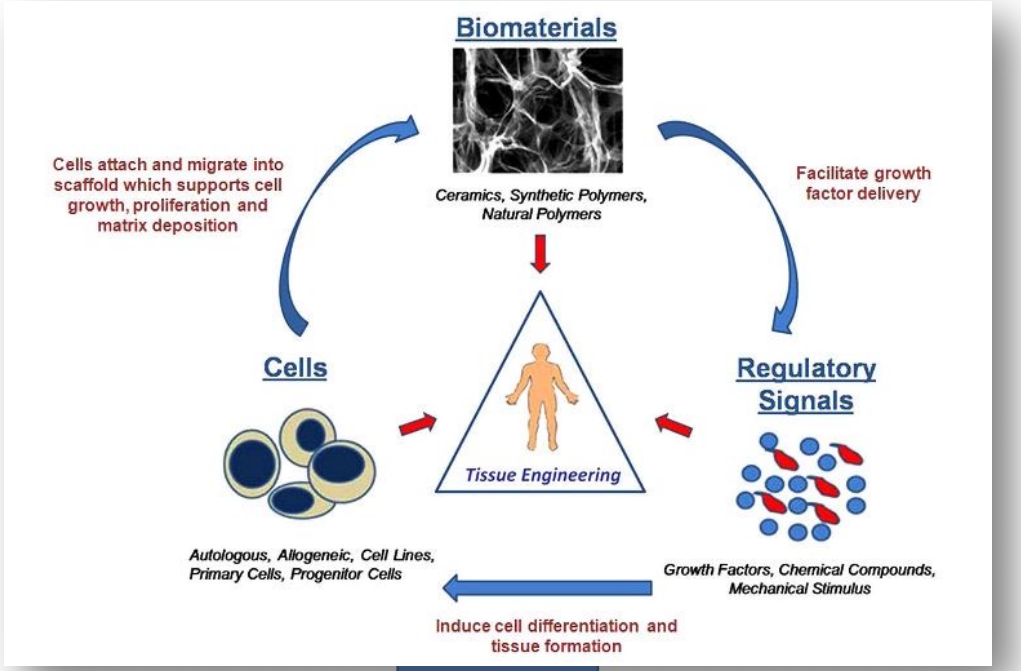
Characteristics of scaffolds:

4) Scaffolds architecture

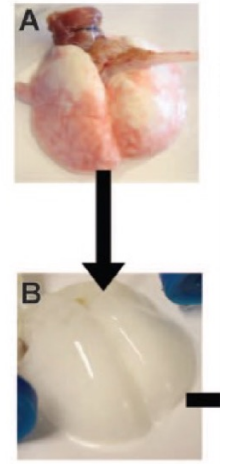
- ✓ Have an interconnected pore structure and high porosity to ensure cellular penetration and adequate diffusion of nutrients to cells within the constructs and to the ECM formed by these cells.
- ✓ The scaffold should mimic the ECM of the tissue to be regenerated or replaced. Df
- ✓ Biomimetics is 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**



Tissue engineering



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

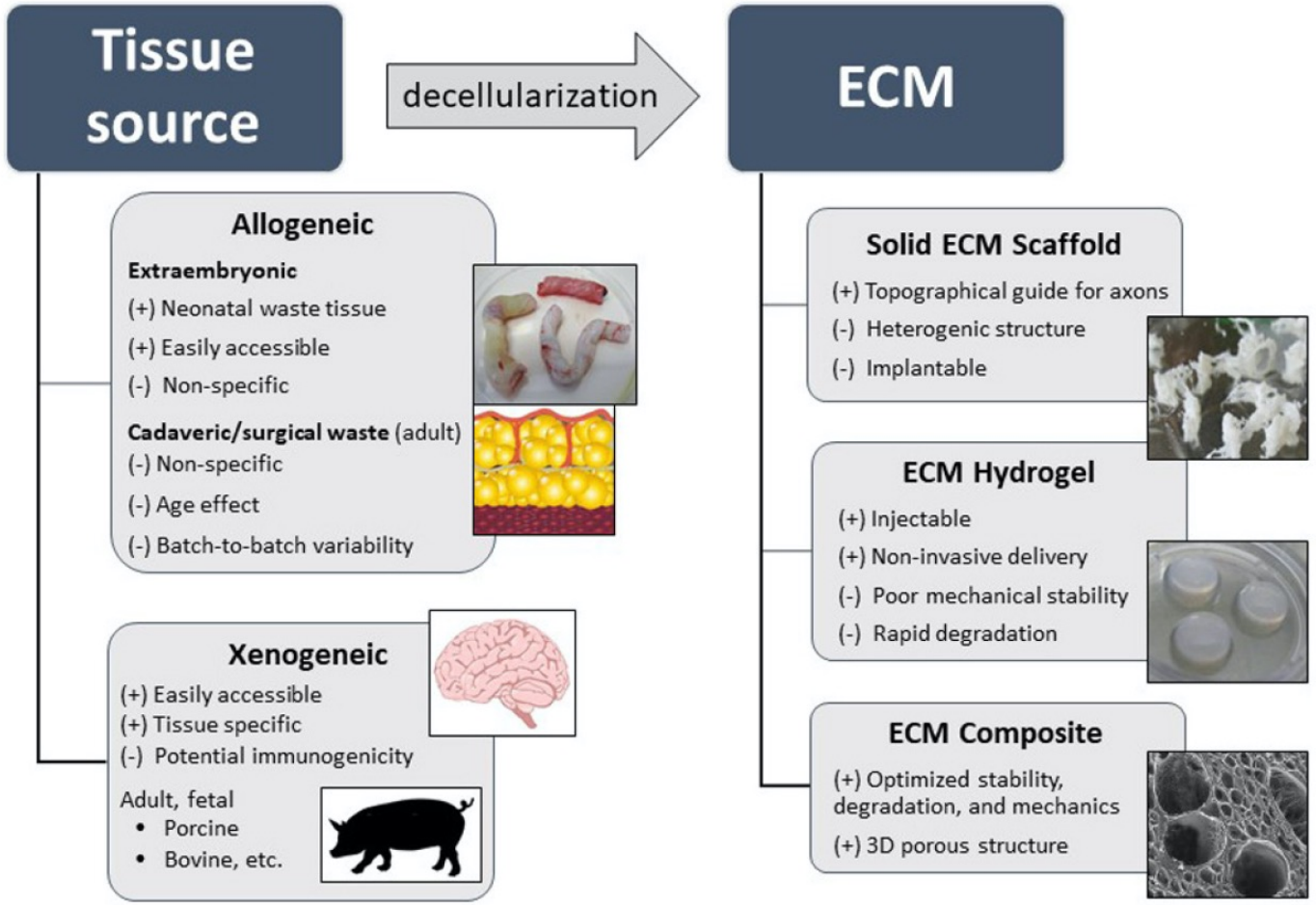
Natural scaffolds

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.



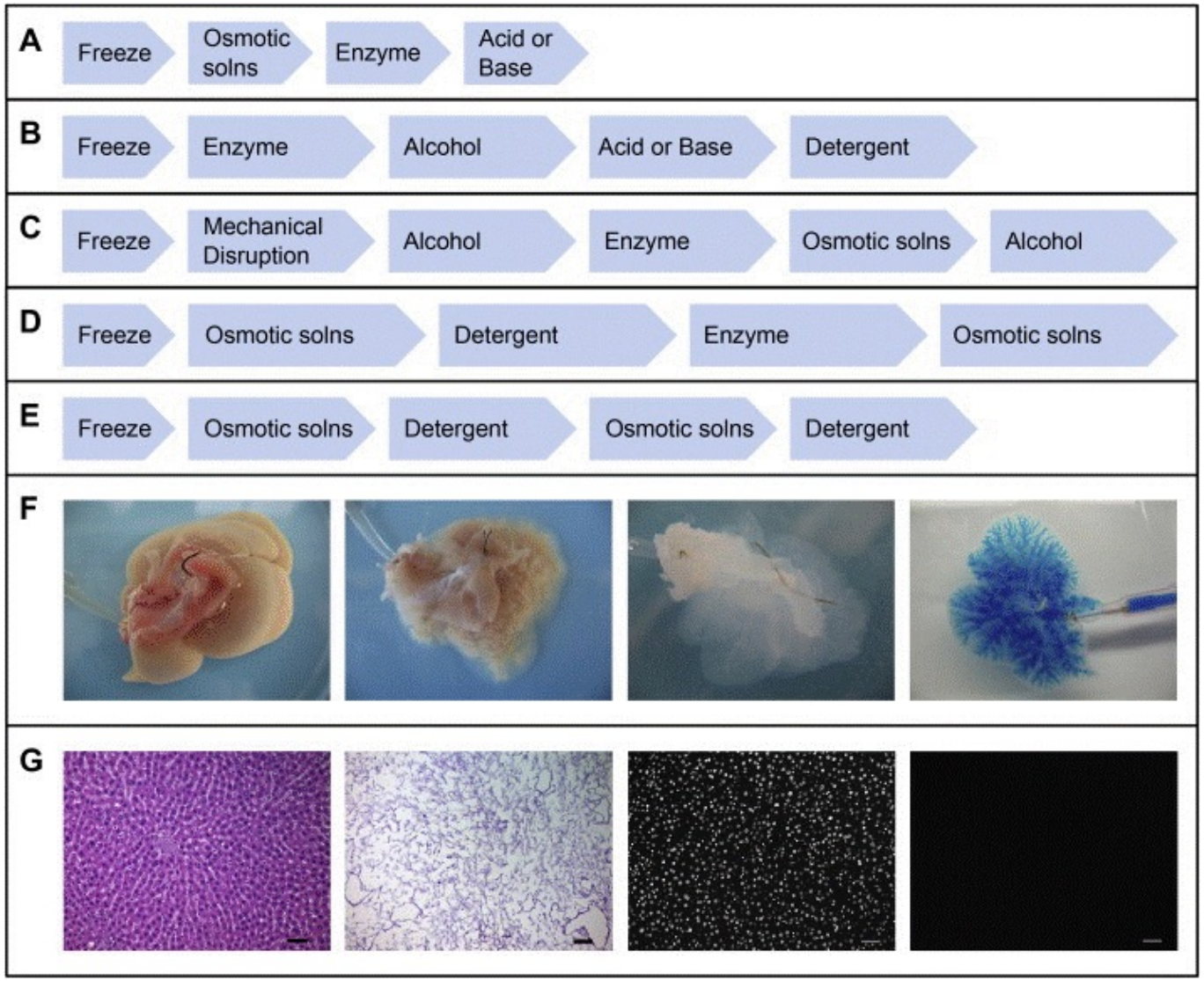
Strategies for decellularization and their problems

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).

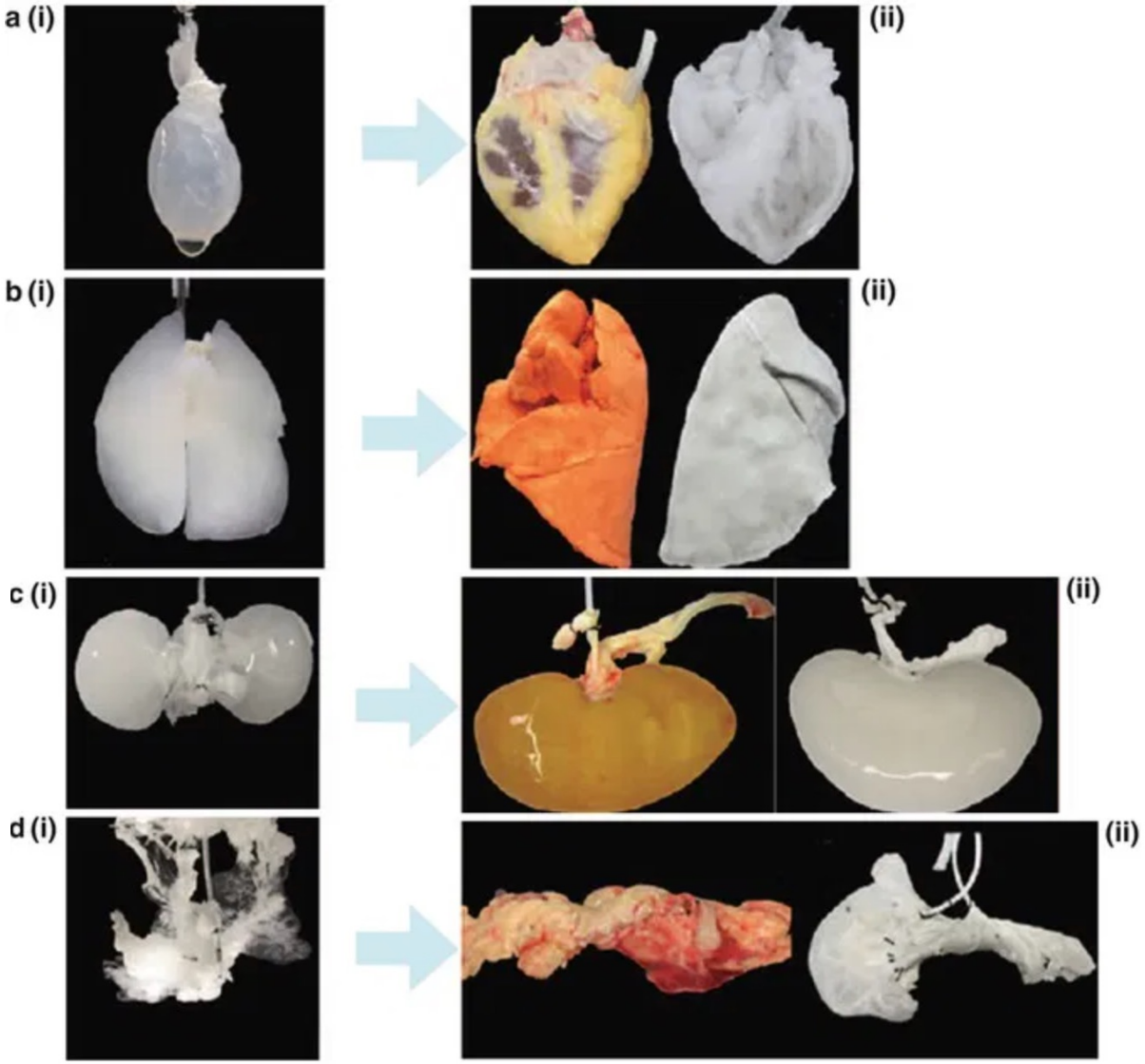
B) Enzymatic Decellularization
Enzymes used in decellularization of organs are the ones that cleave specific components of the cells. The list includes nucleases, tripsin, collagenase, lipase, dispase, thermolysin, and α-galactosidase

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.

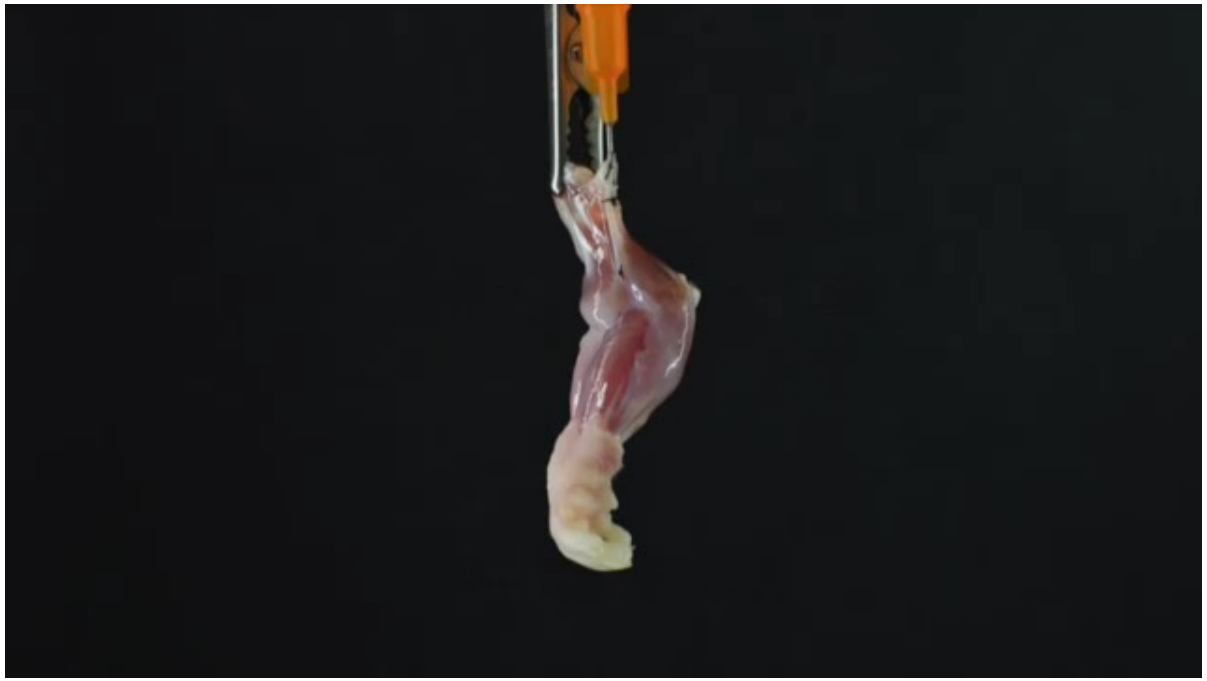


Natural Decellularized Scaffolds



https://abdominalkey.com/decellularization/

Forlimb of a rat

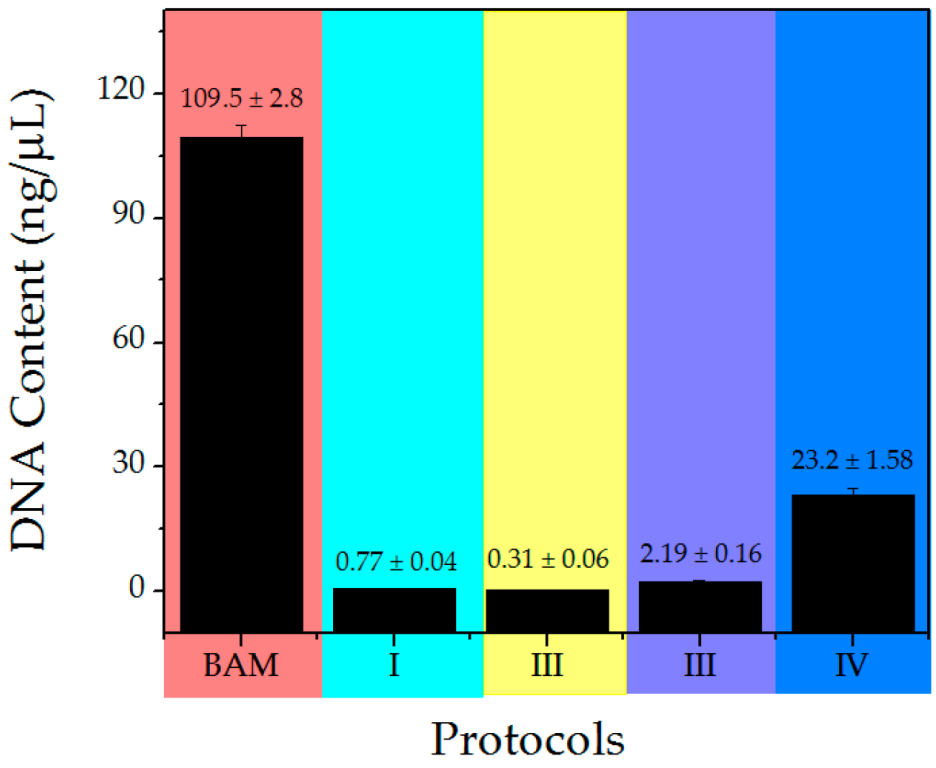


https://www.youtube.com/watch?v=p143bISuEJk

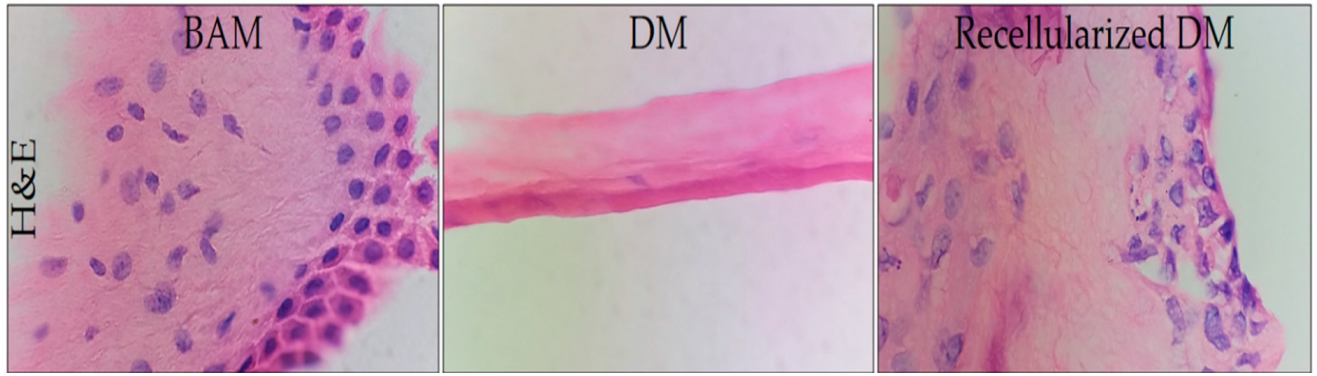
Table 1. Decellularization protocols for the bovine amniotic membranes (BAM).

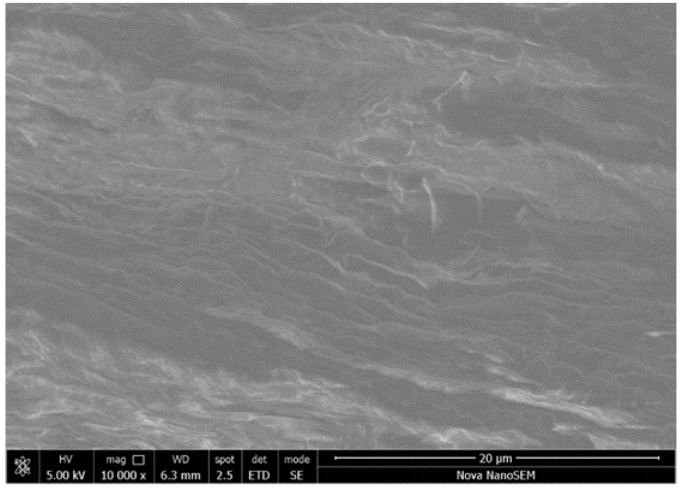
No.	Protocols
I	SDS 0.1% for 4 h NaOH 0.1 M for 1 h PAA + ascorbic acid 0.1 for 12 h Ethanol 70% for 1 h PBS for 2 h
II	SDS 0.1% for 4 h NaOH 0.1 M for 1 h PAA 0.15% + EtOH for 12 h NaOH 0.1 M for 1 h PAA for 1 h Ethanol 70% for 1 h PBS for 2 h
III	Tween 80 for 4 h NaOH 0.1 M for 1 h, PAA + ascorbic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

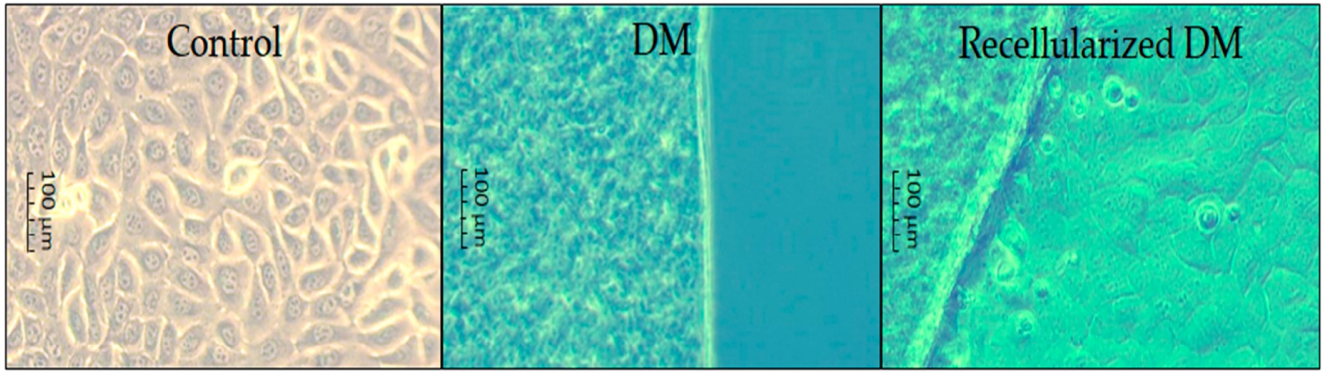
<50 ng/mg is considered decellularized.

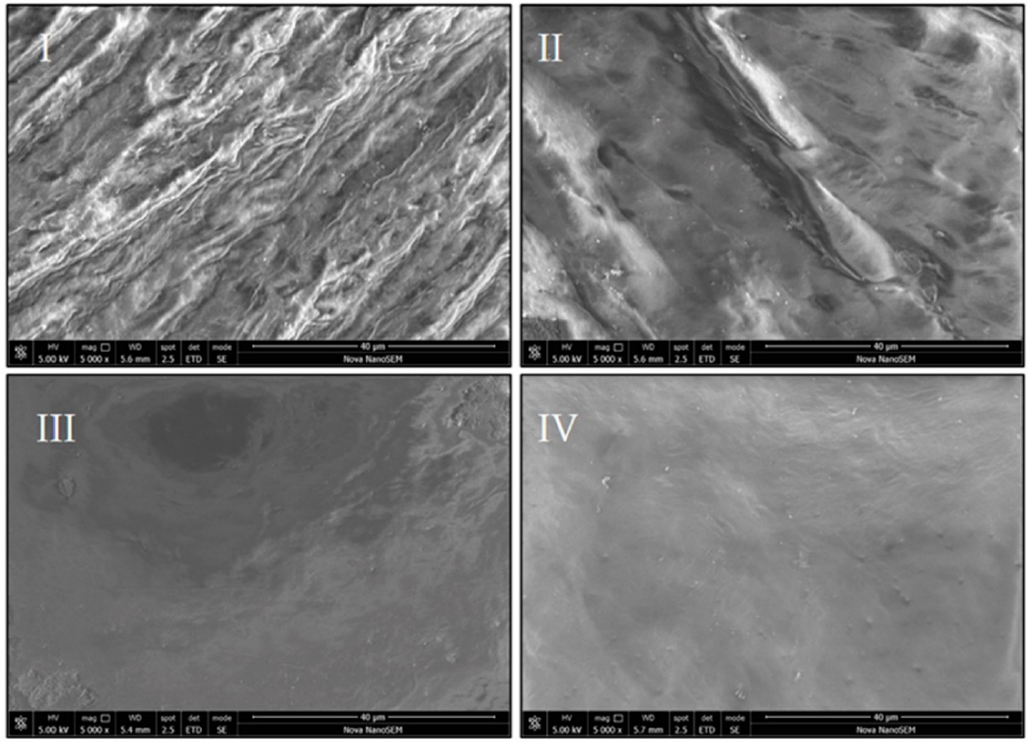


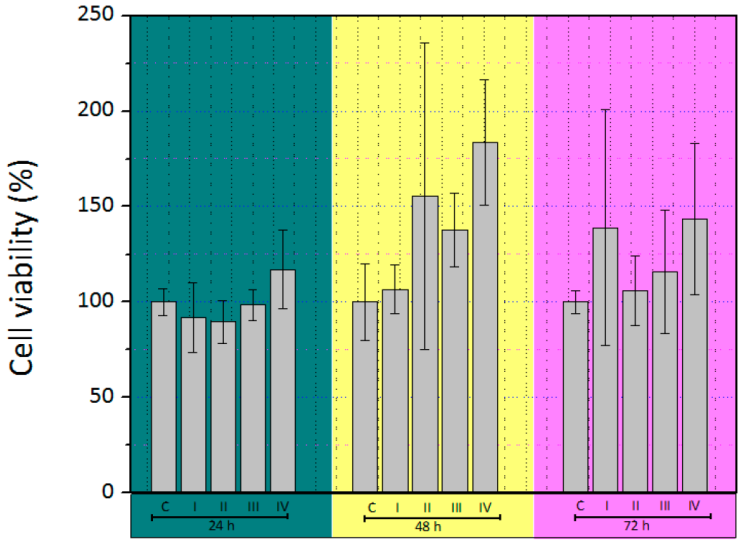
Villamil Ballesteros et al., Polymers 2020, 12, 590; doi:10.3390/polym12030590









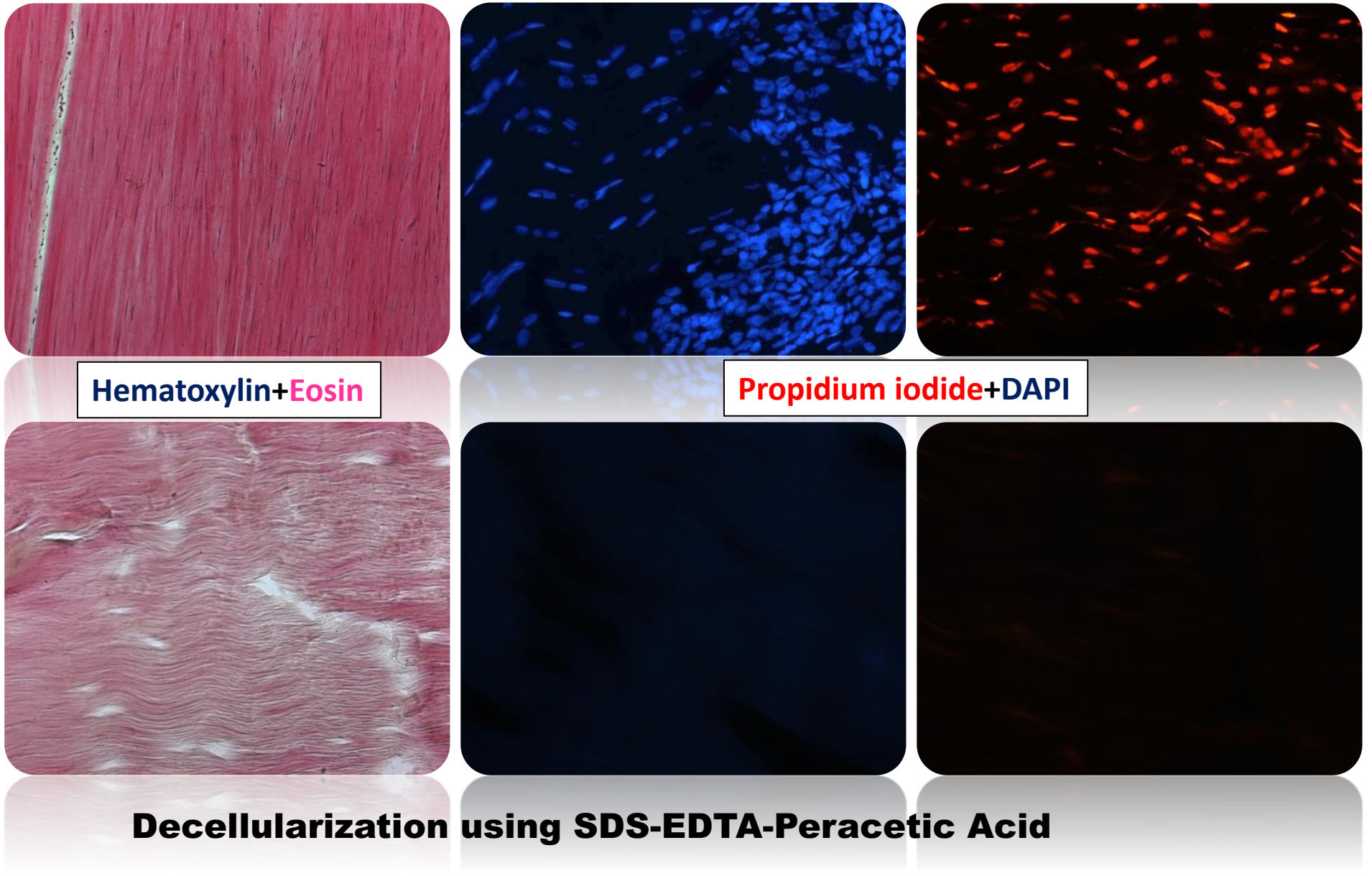


Villamil Ballesteros et al., Polymers 2020, 12, 590; doi:10.3390/polym12030590

Bio-scaffolds from a decellularized tendon

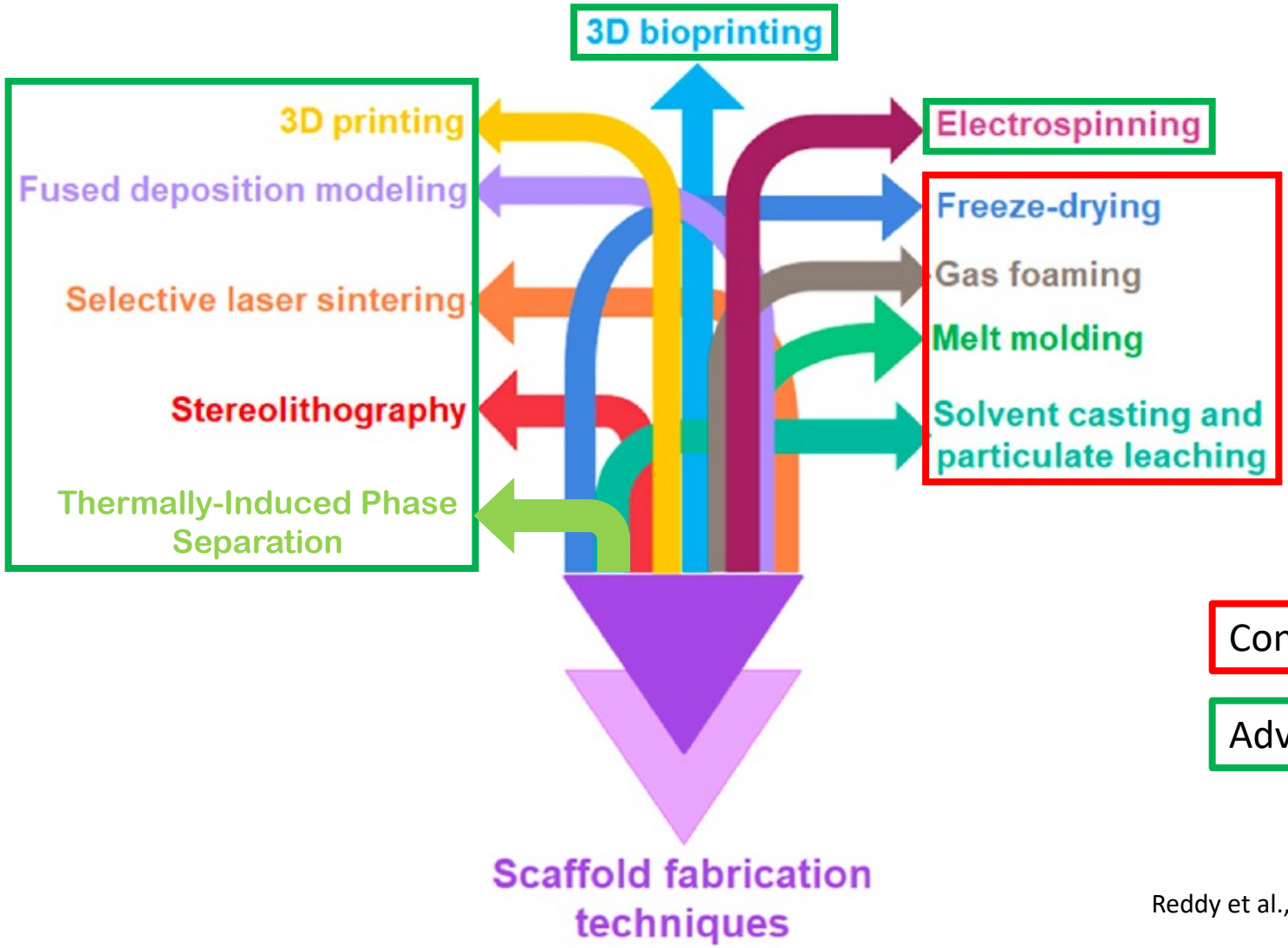
Adult ovine tendons

200x



Decellularization using SDS-EDTA-Peracetic Acid

Fabrication Techniques of Scaffolds



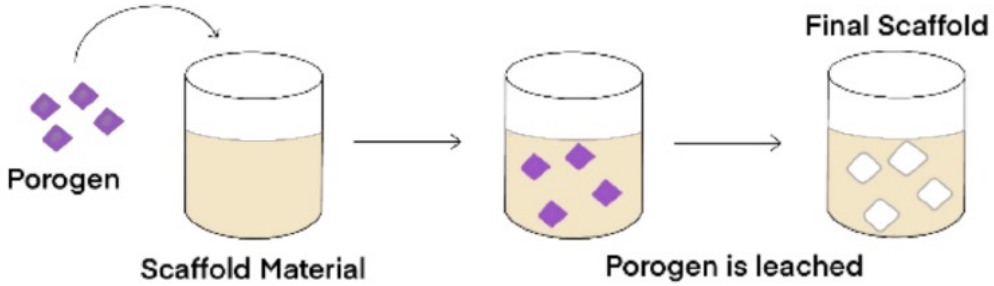
Conventional Techniques

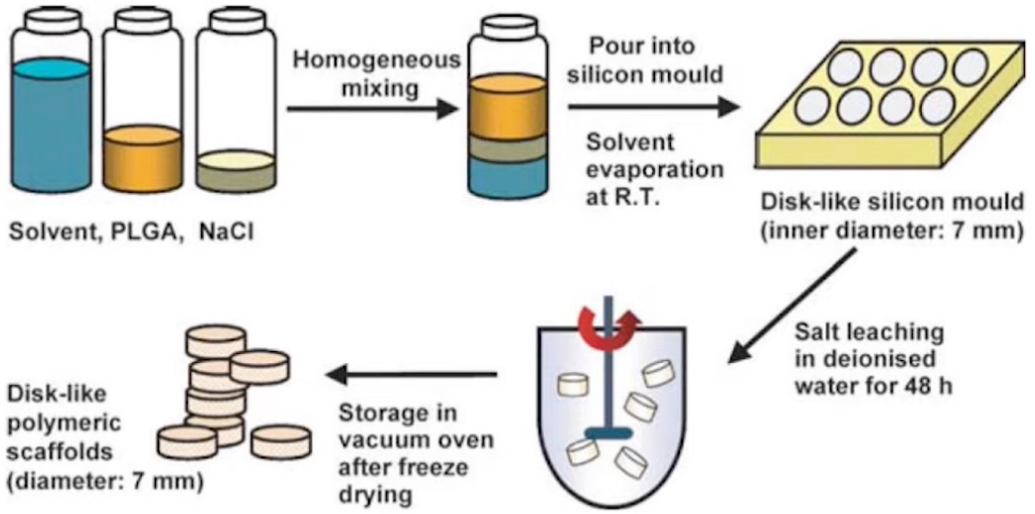
Advanced Techniques

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

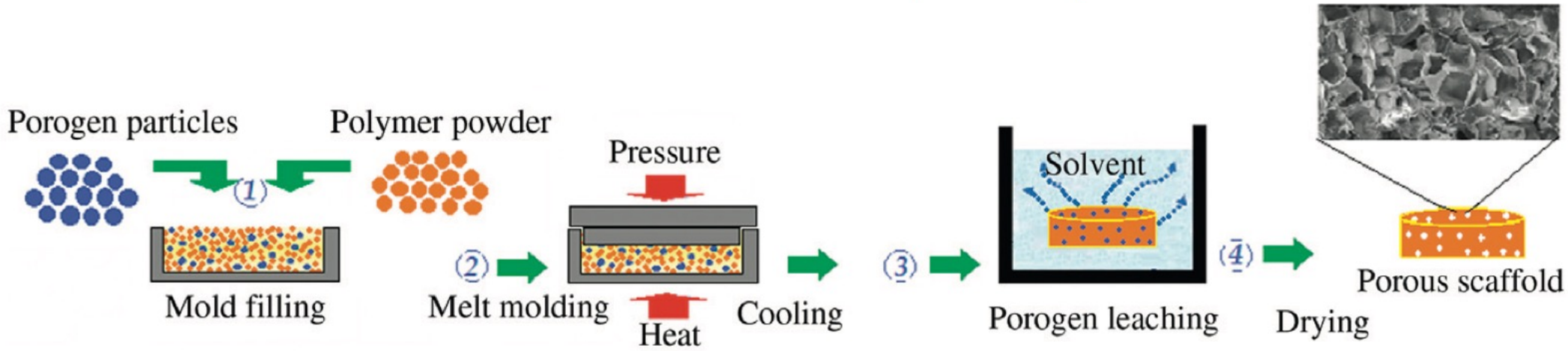




Reddy et al., 2021, polymers, 13, 1105

Melt Molding

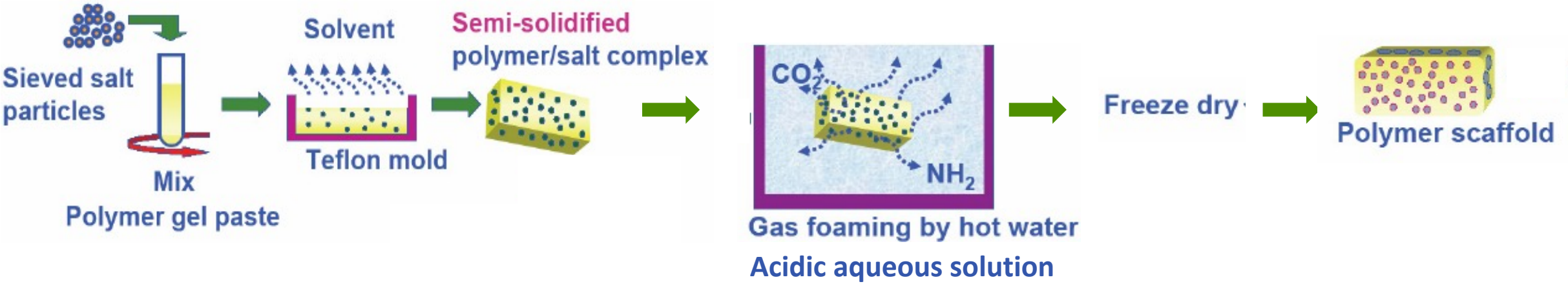
Fabrication Method	Advantages	Disadvantages	Materials
Melt molding: Both polymers and a suitable porogen are melted together, then by cooling the polymer mixture the scaffold is obtained. In this process, the porosity is attained by dissolving the porogen in water	• Independent control over porosity, 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

Gas Foaming

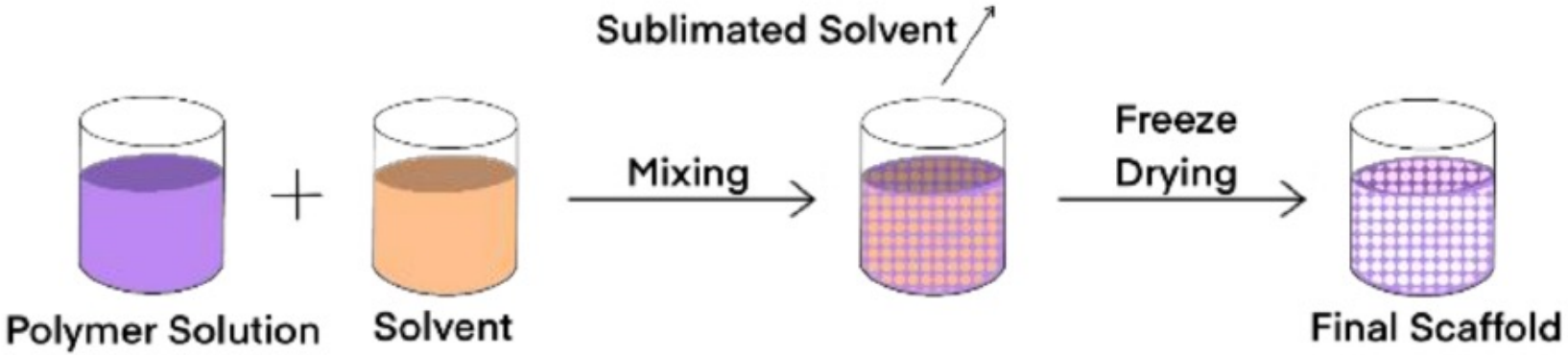
Fabrication Method	Advantages	Disadvantages	Materials
Gas foaming: Polymer gel paste along with sieved effervescent salt particles poured into a mold and immersed into hot water. Formation of the porous matrix after the evolution of ammonia and carbon dioxide gas from salt particles of the solidifying 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

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

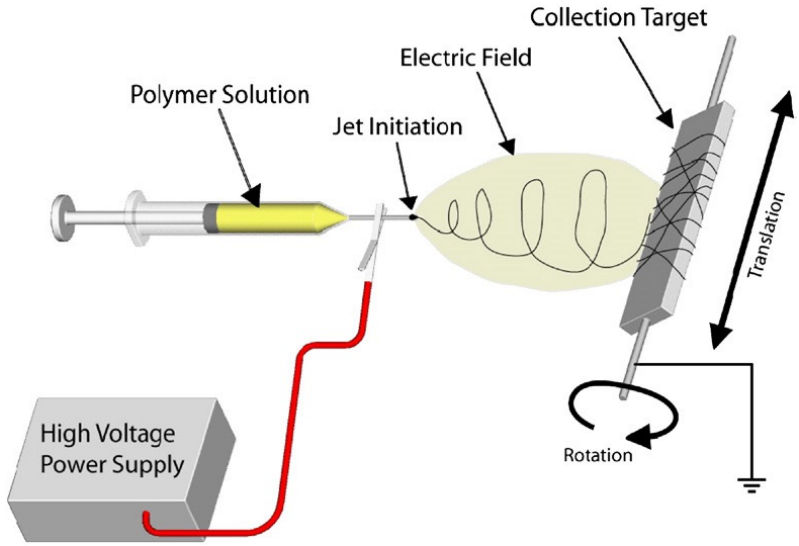
Electrospinning

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

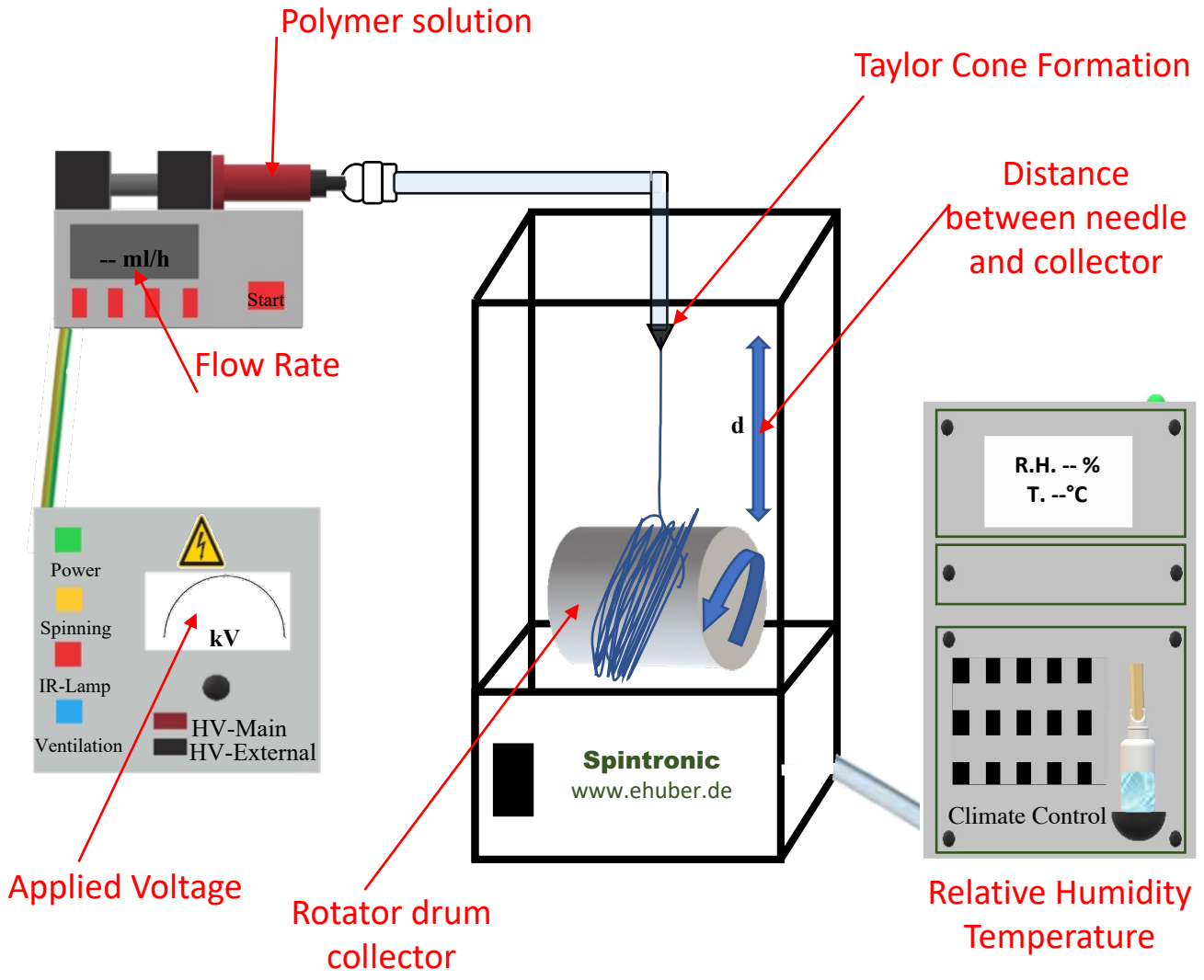
control over fiber diameter and scaffold architecture.



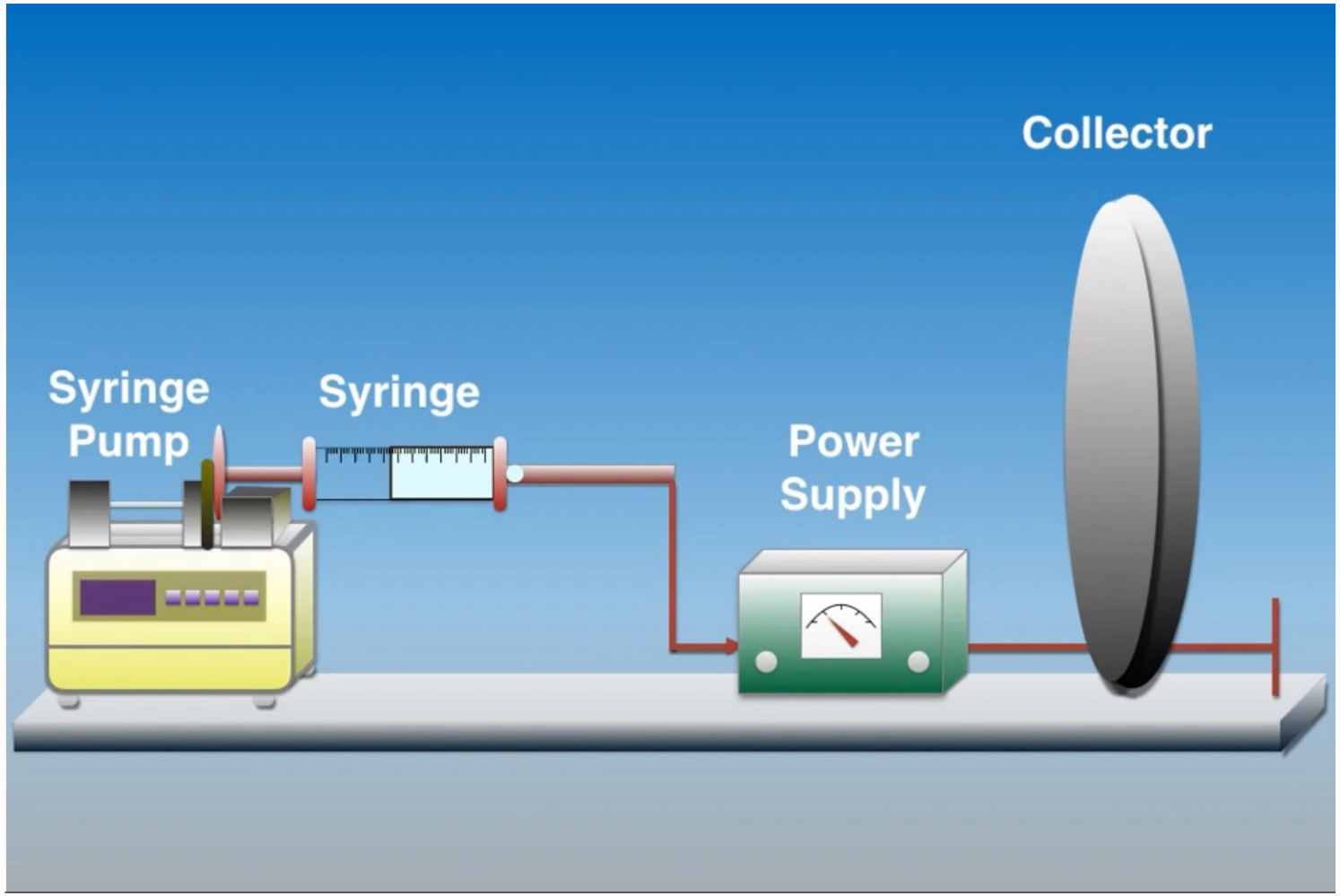
A schematic of the electrospinning process to illustrate the basic phenomena and process components

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

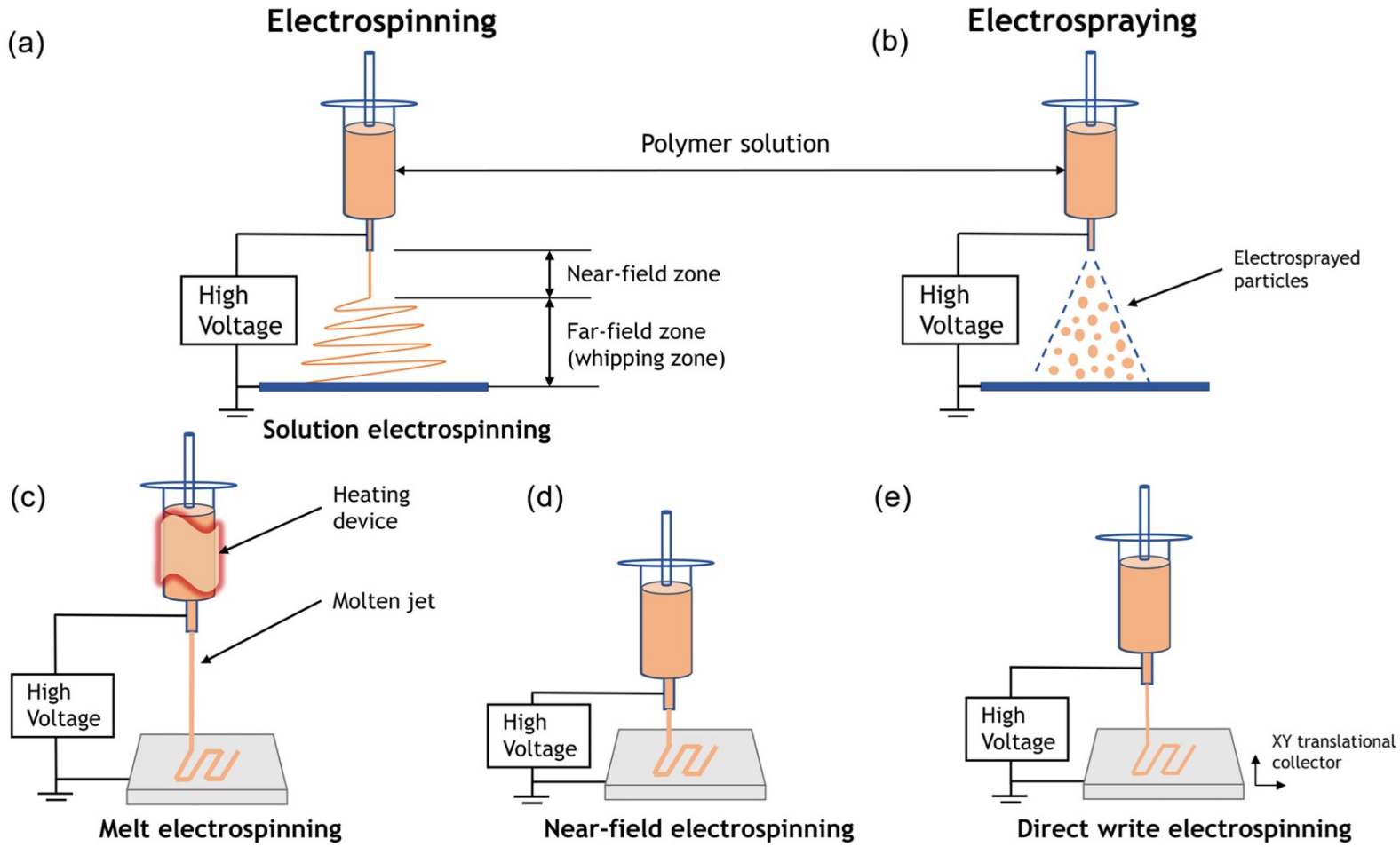
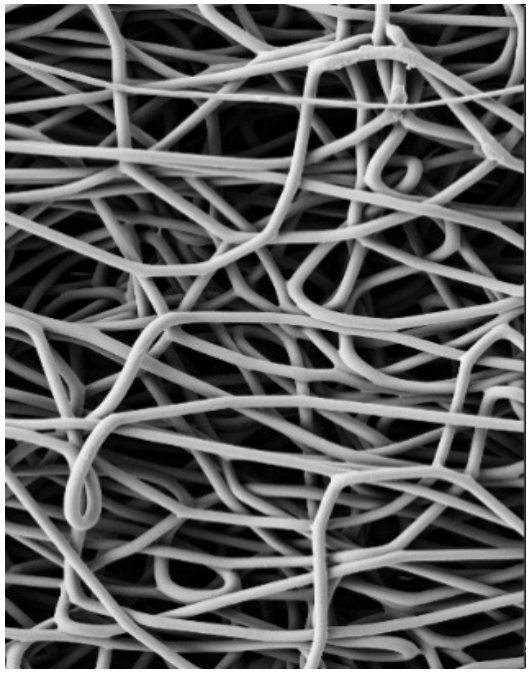


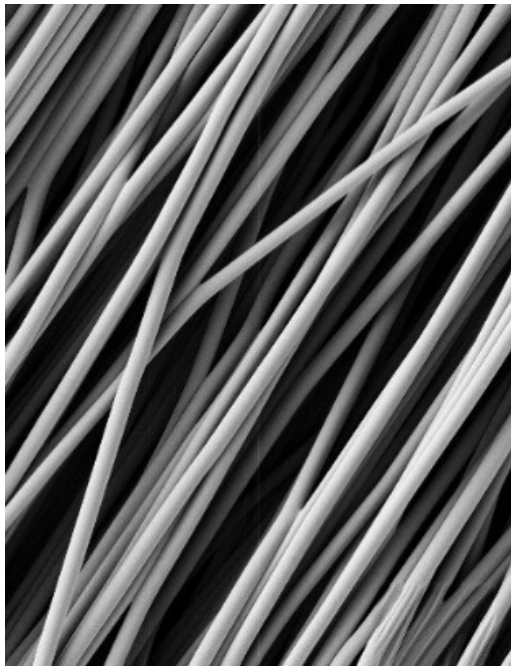
FIG. 1. Schematic illustrations of (a) electrospinning and (b) electrospraying. The charged jet can be kept in a continuous form to produce fibers in electrospinning, whereas it breaks into droplets to form particles in electrospraying. During electrospinning, the ejected jet initially follows a straight line in the near-field zone and undergoes stretching and thinning upon whipping motions in the far-field zone. (c) Schematic illustration of melt electrospinning. Unlike conventional solution electrospinning, a heating device is attached to maintain a molten jet in melt electrospinning. Normally, the jet travels in a straight line and generates micrometer scale fibers. (d) Schematic illustration of near-field electrospinning. The jet deposited on the collector within the straight segment, which shows higher spatial control of fiber placement but larger fiber diameter. (e) Schematic illustration of direct write electrospinning that integrates AM concept to electrospinning. A translational collector is used for predefined pattern construction.

Liu et al., APL Bioeng. 4, 030901 (2020); doi: 10.1063/5.0012309

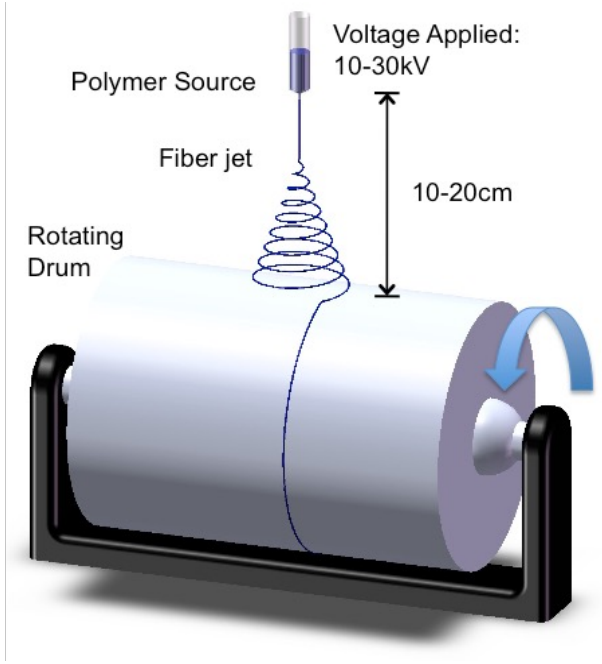
Modified Electrospinning Setups – Aligned fibers

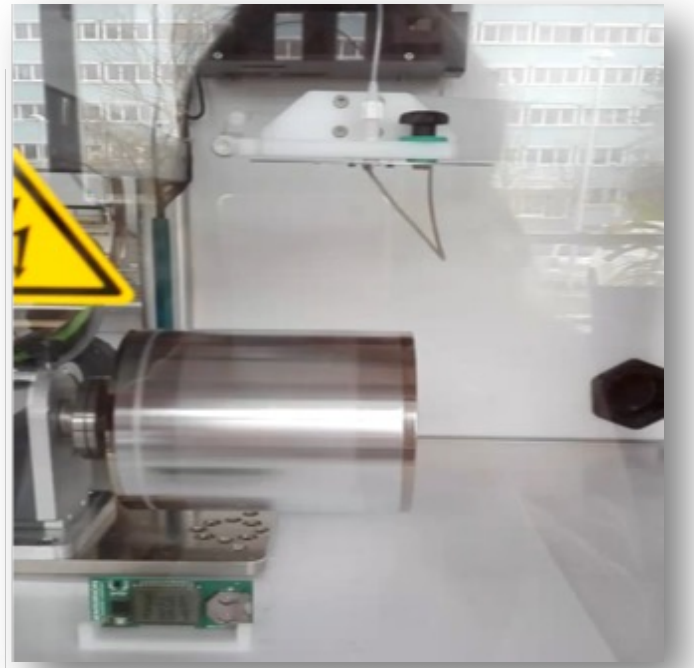


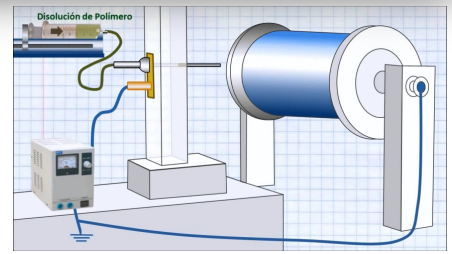
Standard Collector



Rotating Drum







Russo et al. 2020, molecules

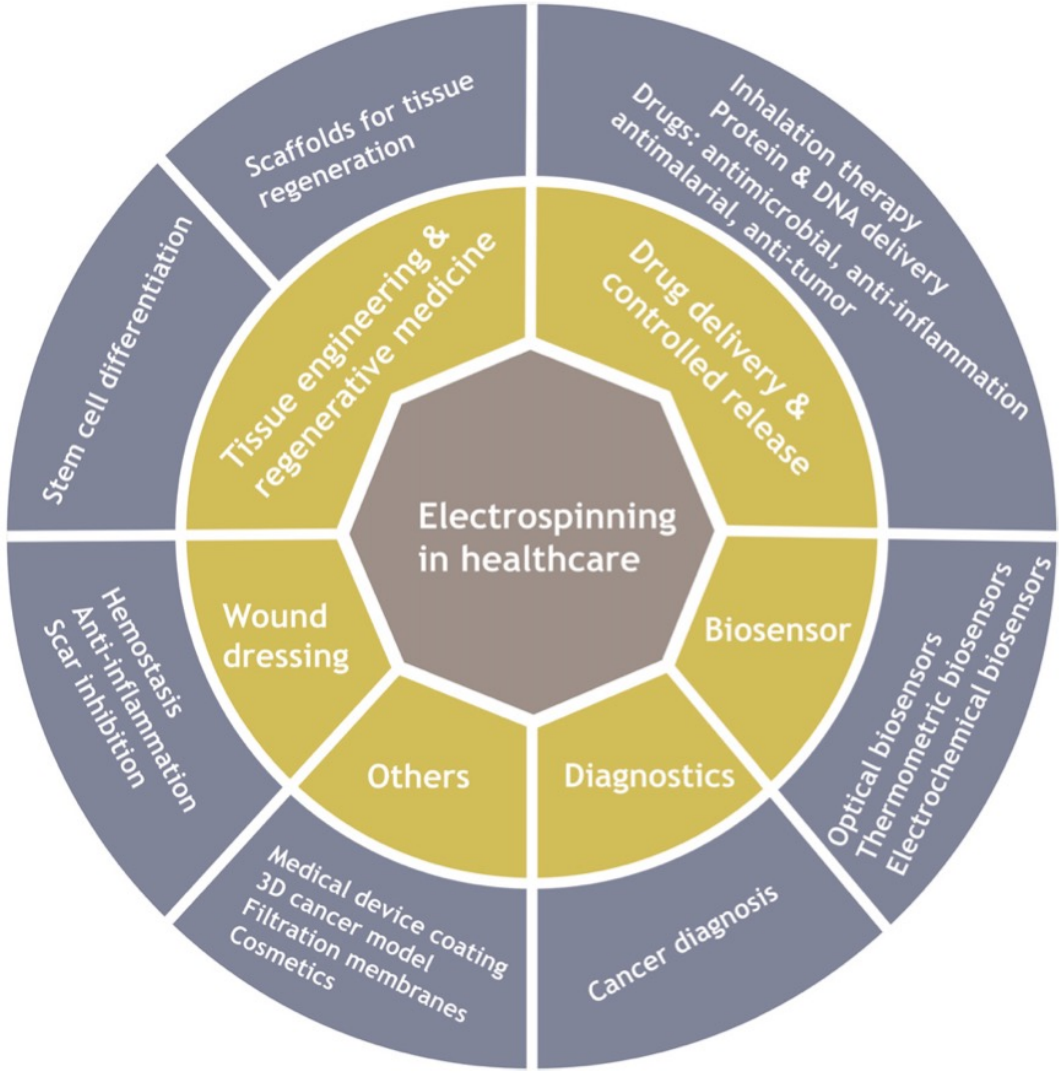


FIG. 2. Applications of electrospinning in healthcare.

Liu et al., APL Bioeng. 4, 030901 (2020); doi: 10.1063/5.0012309

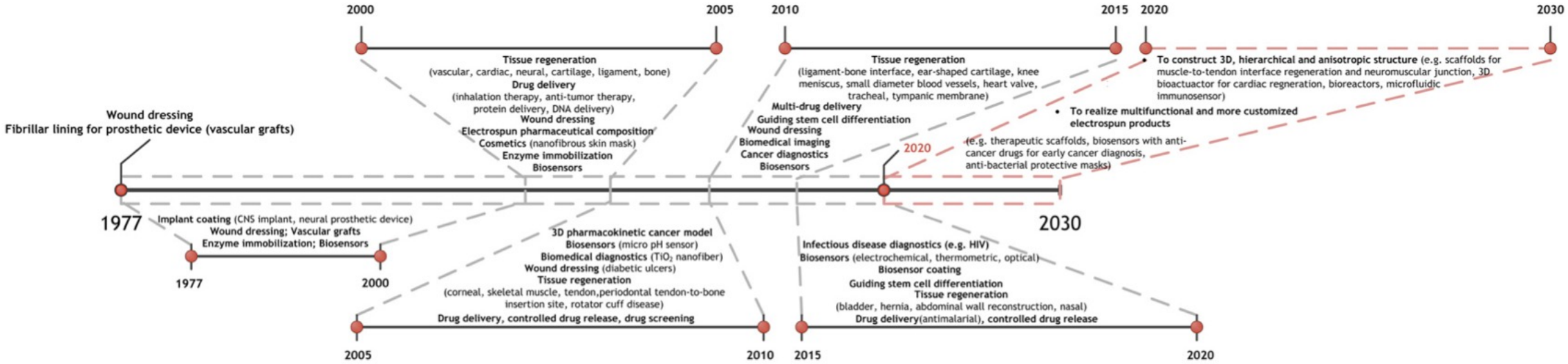


FIG. 3. Development of electrospinning in biomedical applications. Electrospun nanofibers have been utilized in biomedical applications mainly as wound dressing and implant coating since 1977. There were broad applications of electrospinning in healthcare in the past two decades. From 2000 to 2020, the key applications of electrospinning in healthcare are summarized and presented at 5-year intervals. From 2020 to 2030, two future trends of applying electrospinning in healthcare are suggested with examples. CNS implant: central nervous system implant.

Liu et al., APL Bioeng. 4, 030901 (2020); doi: 10.1063/5.0012309

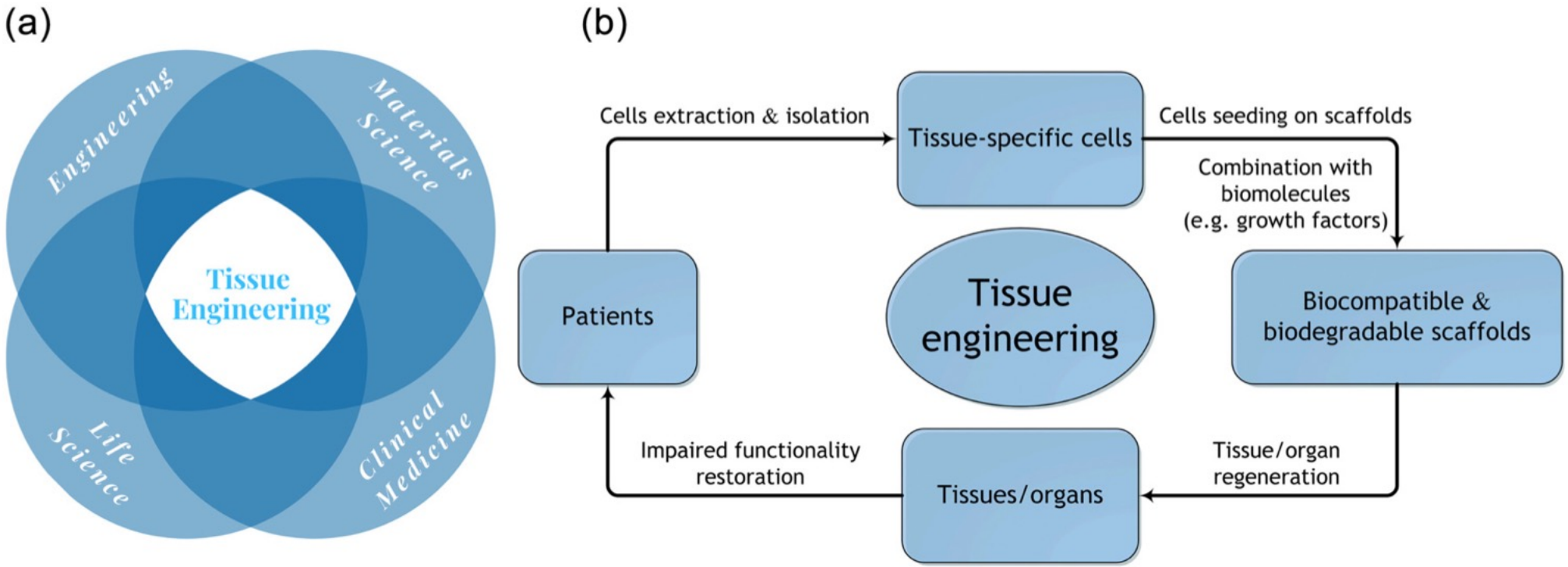


FIG. 4. Basic concept of tissue engineering approach for tissue regeneration. (a) Tissue engineering is a highly multidisciplinary field that recruits experts from engineering, materials science, life science, and clinical medicine. (b) In tissue engineering, biocompatible scaffolds act as a temporary template for tissue-specific cell growth and proliferation, and are occasionally incorporated with biomolecules for enhanced cell regulation and tissue regeneration. Upon implantation of the engineered tissue, scaffolds will gradually degrade leaving regenerated tissues or organs with restored functionality.

Liu et al., APL Bioeng. 4, 030901 (2020); doi: 10.1063/5.0012309

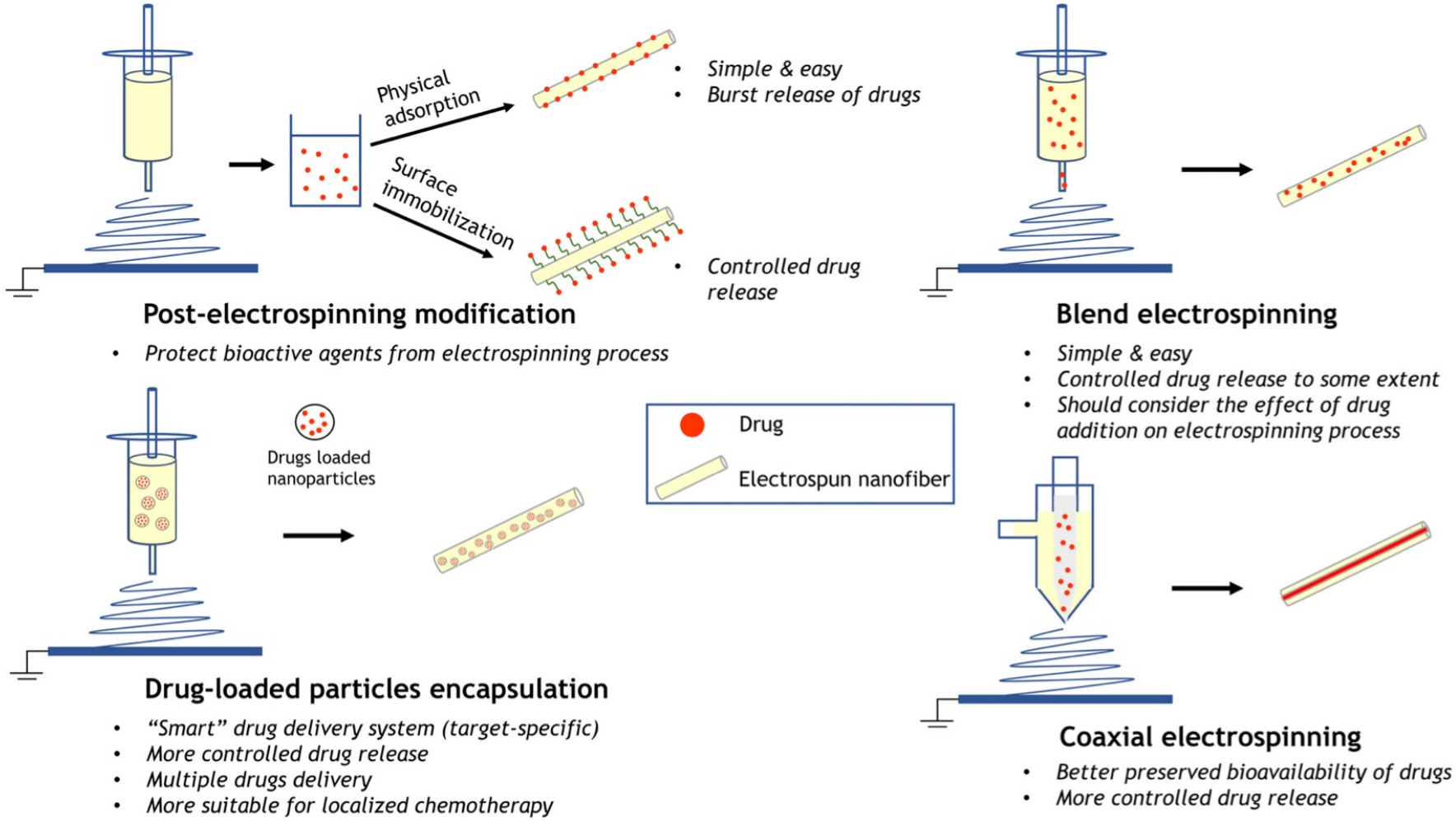
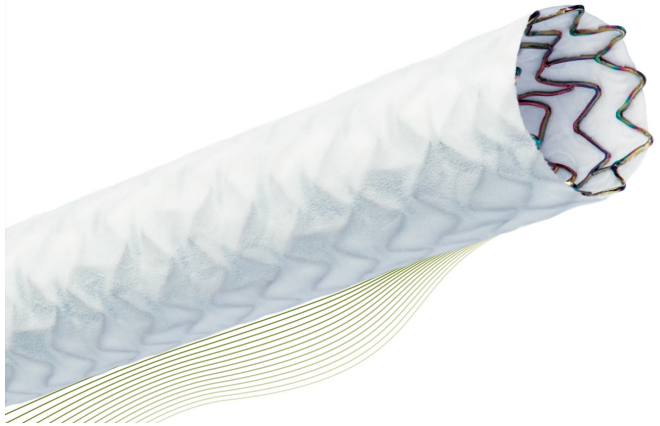


FIG. 5. Common approaches of loading drugs into electrospun nanofibers. Post-electrospinning modifications including simple physical adsorption and surface immobilization for more controlled drug release. Blend electrospinning and coaxial electrospinning allow drug encapsulation to as-spun nanofibers. Loading drugs into particles followed by particle incorporation to nanofibers permits a more versatile drug delivery system by tailoring the characteristics of both nanofibers and particles. Characteristics of each approach are presented.

Liu et al., APL Bioeng. 4, 030901 (2020); doi: 10.1063/5.0012309

Vascular Intervention // Coronary
Covered Coronary Stent System

PK Papyrus

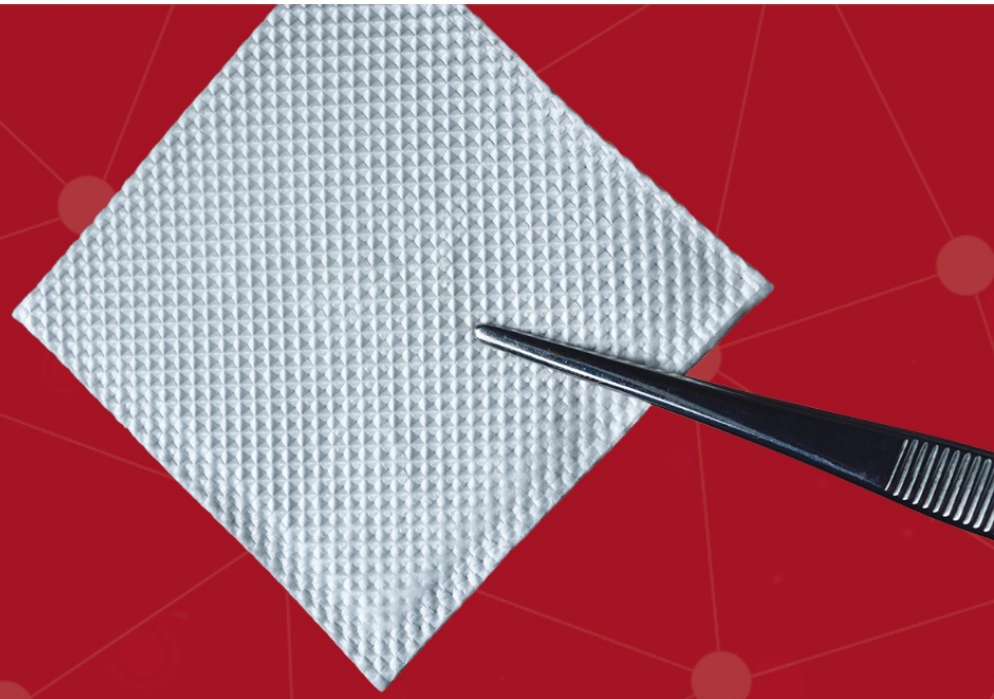


IMPLANT:
Electrospun polyurethane fibers on stent surface; thin and highly elastic membrane

BIOTRONIK
excellence for life

SurgiCLOT® - Fibrin Sealant Patch

SurgiCLOT is the first and only fibrin sealant designed specifically for bone bleeding, utilizing the dextran nanofibers to deliver a bolus of human fibrinogen and thrombin, augmenting the clotting cascade to promote a FAST, STRONG, and NATURAL fibrin clot in order to aid in the bone healing process.



Dissolvable

Resorbable

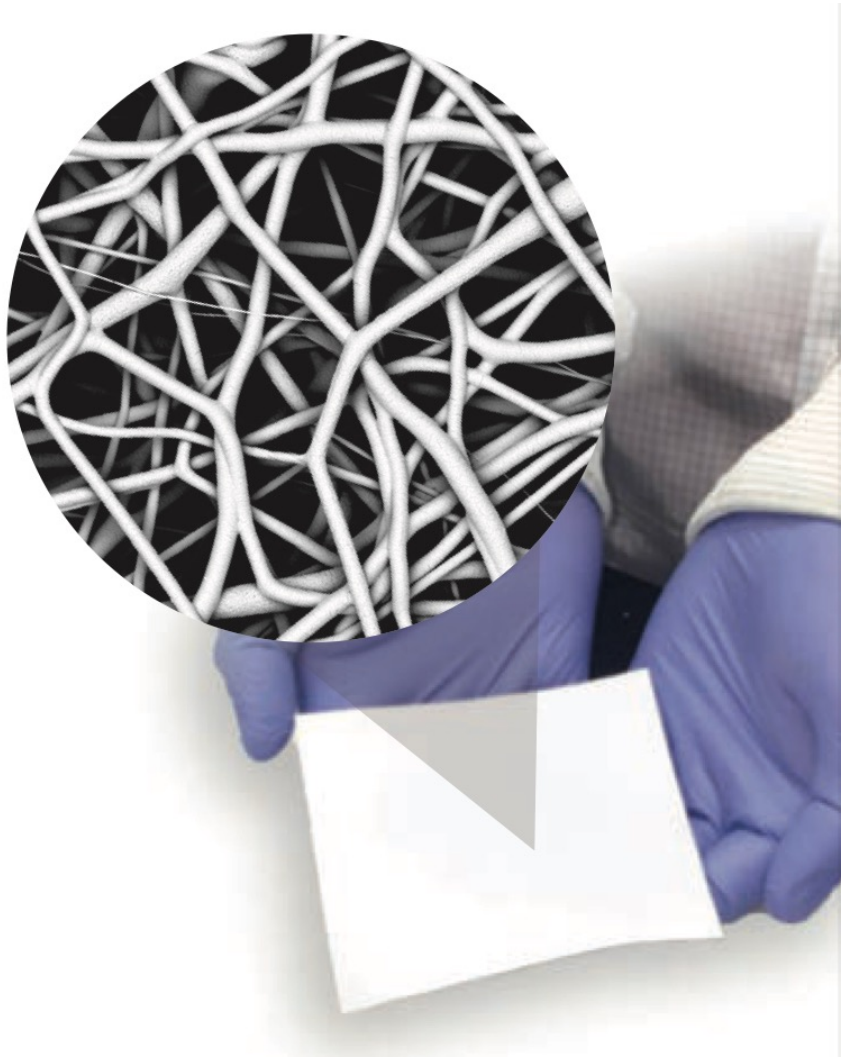
Biocompatible

Shrinks Upon Contact

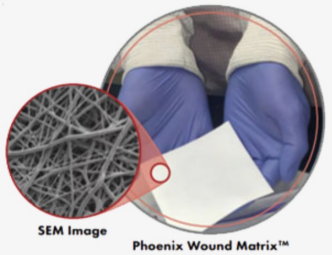
Shrinks Upon Contact

WOUND DRESSIN:

Dextran nanofibers; fibrin sealant designed specifically for bone bleeding;



PHOENIX™
WOUND MATRIX
Powered by Electrospun Synthetic Polymer Technology



A cutting-edge, multi-dimensional wound healing solution

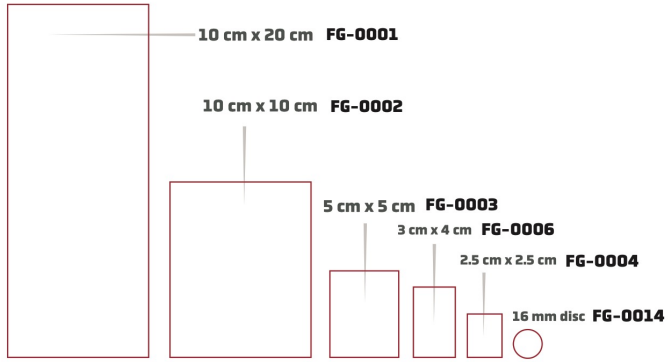
Phoenix Wound Matrix is a 3D electrospun synthetic polymer matrix designed to improve wound healing outcomes by addressing chronicity and persistent inflammation.

INDICATIONS

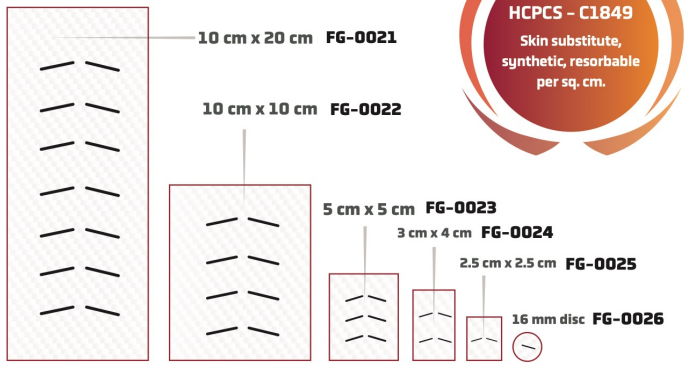
- PHOENIX Wound Matrix is indicated for the management of partial to full-thickness acute and chronic wounds, and burns.

SIZING AND REIMBURSEMENT

PHOENIX™ Wound Matrix

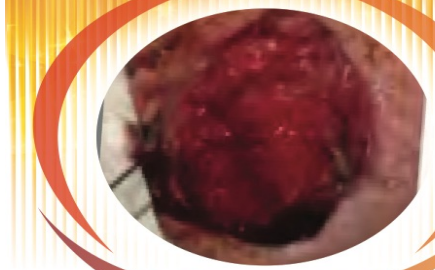


PHOENIX™ Wound Matrix Fenestrated



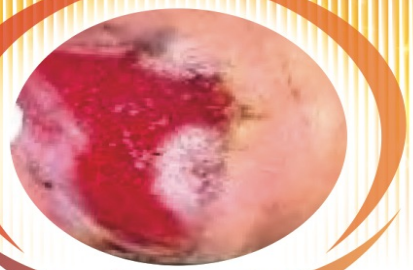
HCPCS – C1849
Skin substitute, synthetic, resorbable per sq. cm.

Pressure Ulcer



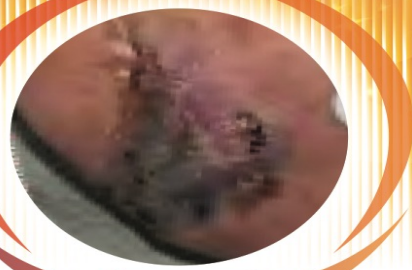
Day 1

Plan. area: 11.8 cm²
1st PHOENIX applied



Day 42

Plan. area: 3.6 cm²
70% decrease



Day 77

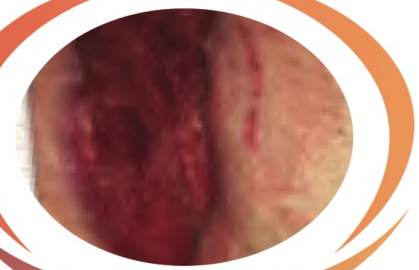
Wound Closure

4 months in duration prior to treatment with PHOENIX

Necrotizing Faciitis

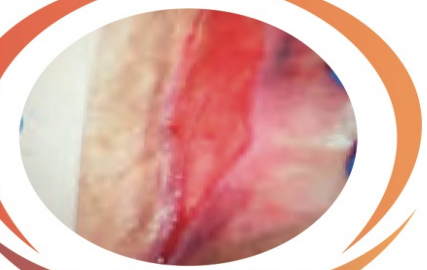
Day 0

Plan. area: 256.9 cm² (anterior)
1st PHOENIX applied



Day 32

Plan. area: 58.4 cm²
3rd PHOENIX applied
77% decrease



Day 67

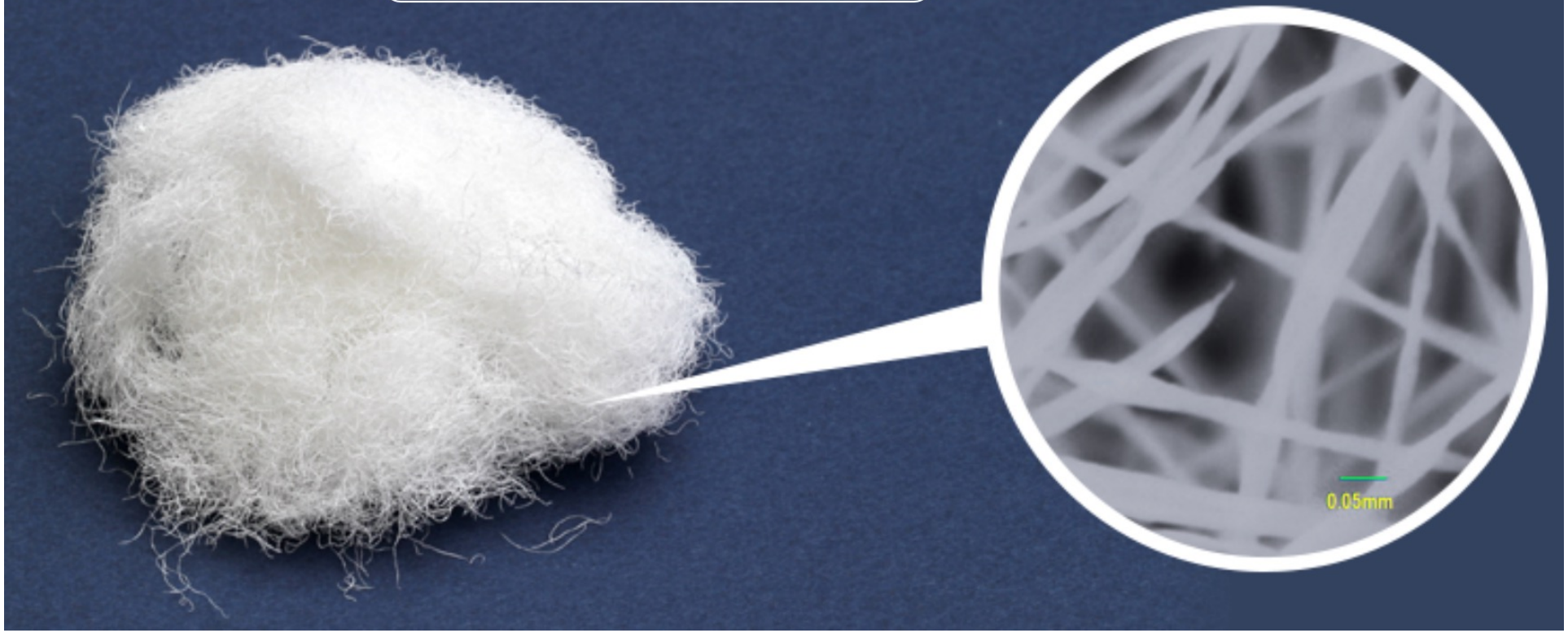
Plan. area: 11.4 cm²
96% decrease

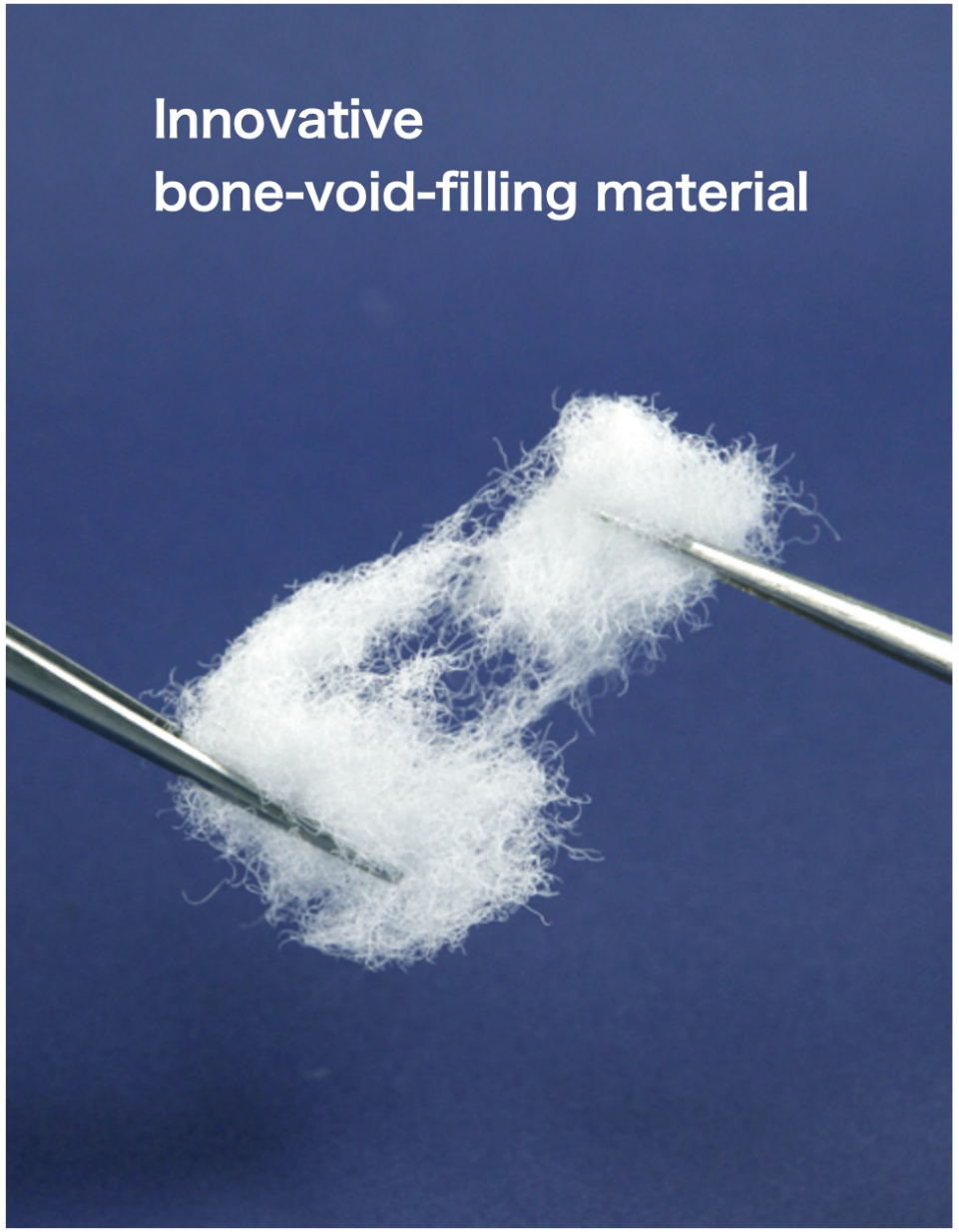
Bioweb™ Products

Biocompatible Composites

Advantage of ReBOSSIS

- Handling Ability
- Moldability
- Fluid Absorption and Retention
- Osteogenic Potential
- Bioresorbability



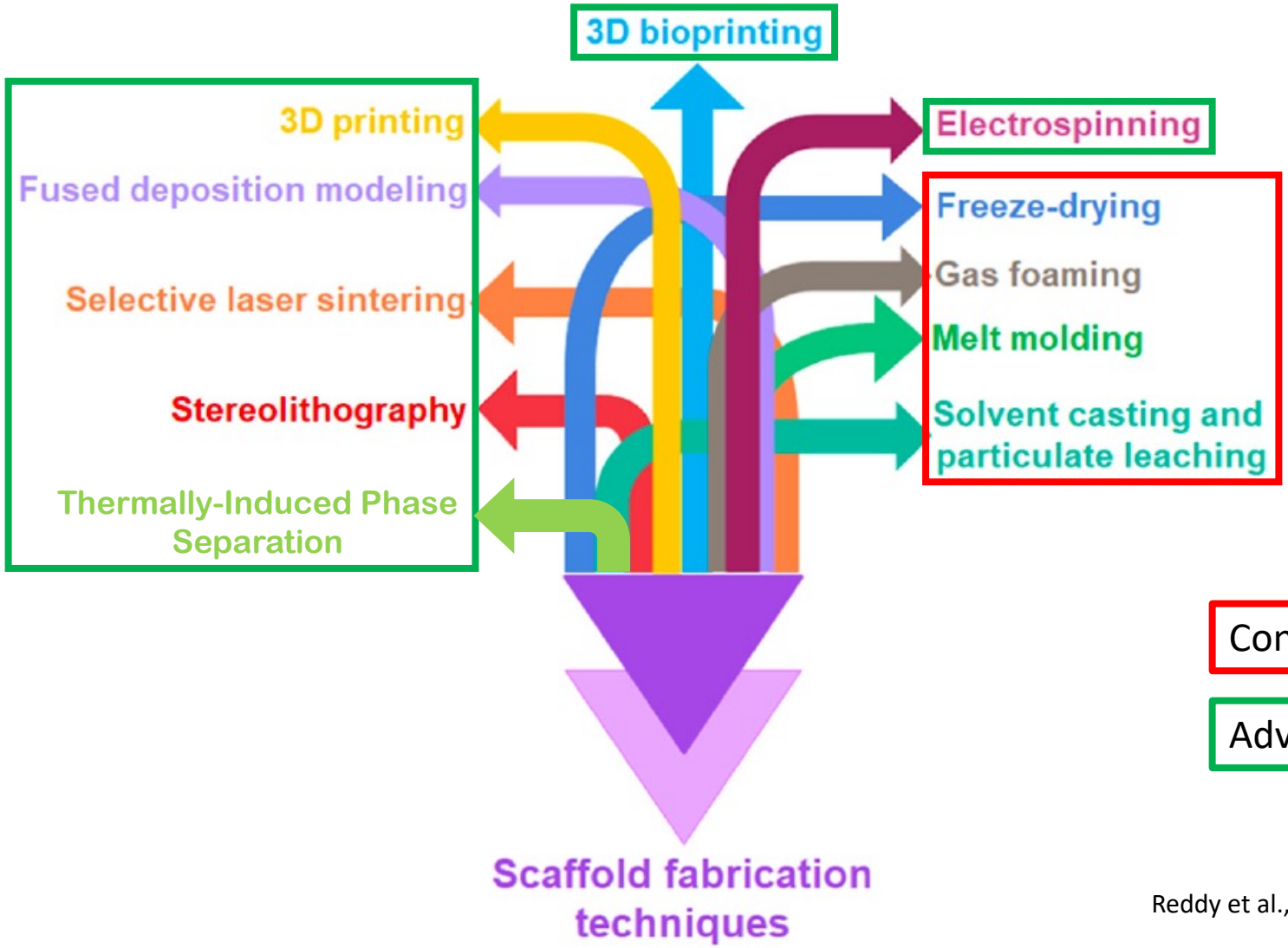


ReBOSSIS is an innovative-type synthetic bone-void/defect-filling material. ReBOSSIS feels like cotton. Its main ingredients are β-TCP (β-Tricalcium Phosphate), Bioabsorbable Polymer and SiV (Silicone-containing Calcium Carbonate that promotes the bone formation). Being cottony type and using these major ingredients are the greatest advantage of ReBOSSIS.

Being cottony-type, ReBOSSIS is much easier-to-handle at the time of operations comparing to existing types of artificial bones. For example, unlike block-type solid artificial bone, doctors don't need to process ReBOSSIS to make it fit into the shape or condition of different bone defects. Also, unlike granular-type artificial bone, ReBOSSIS does not fall from a bone-defect/void after filling. In addition to its good handling property, ReBOSSIS is featured with good elasticity and resilient capability, which is a great difference from the existing types of artificial bones.

Being elastic and resilient, ReBOSSIS is designed to perfectly fill a bone void of any part of a patient's body and any sizes in a shorter time. Plus, ReBOSSIS can stay in a void firmly without any risk of falling from the void. Then ReBOSSIS replaces itself with the patient's bone after healing.

Fabrication Techniques of Scaffolds



Conventional Techniques

Advanced Techniques

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

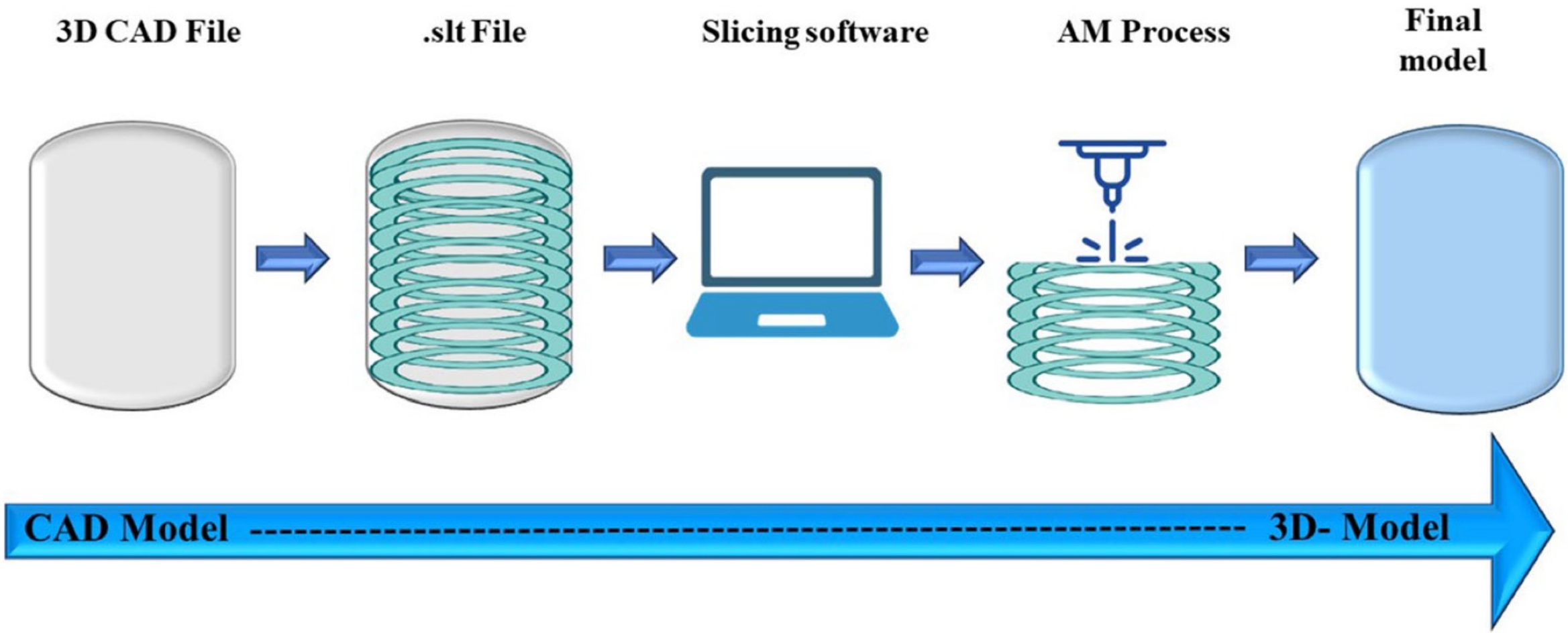
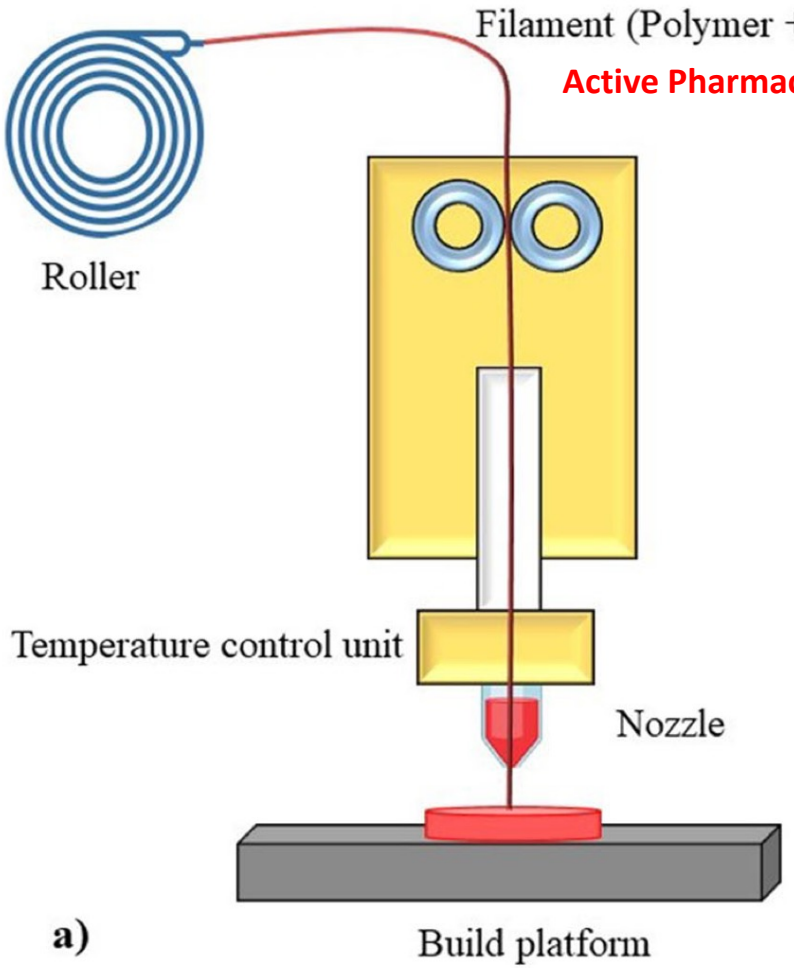
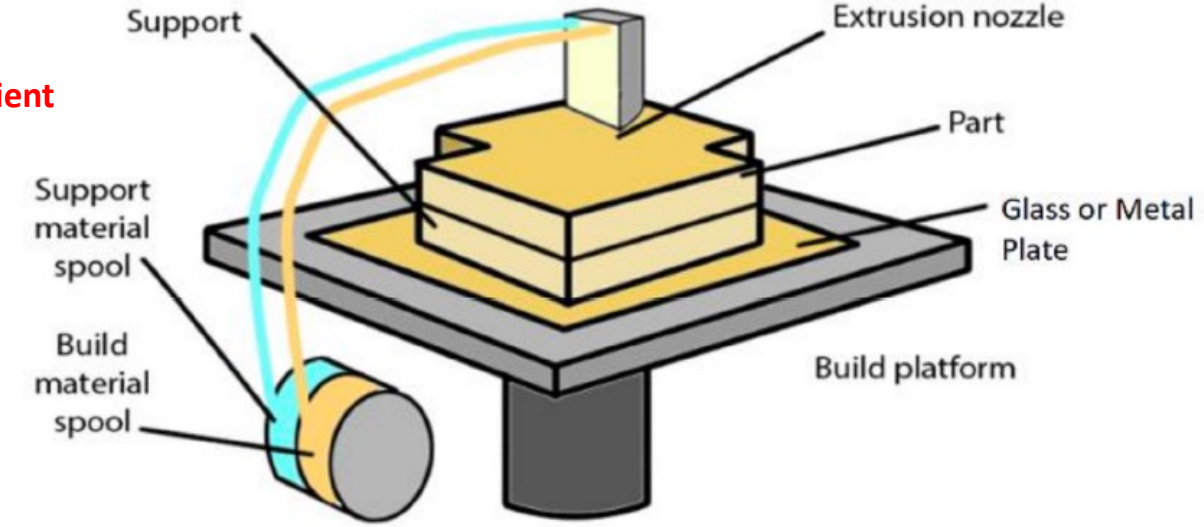


Fig. 1 Blueprint of Additive Manufacturing (AM)

Pavan Kalyan et al., AAPS PharmSciTech (2022) 23: 92. DOI: 10.1208/s12249-022-02242-8

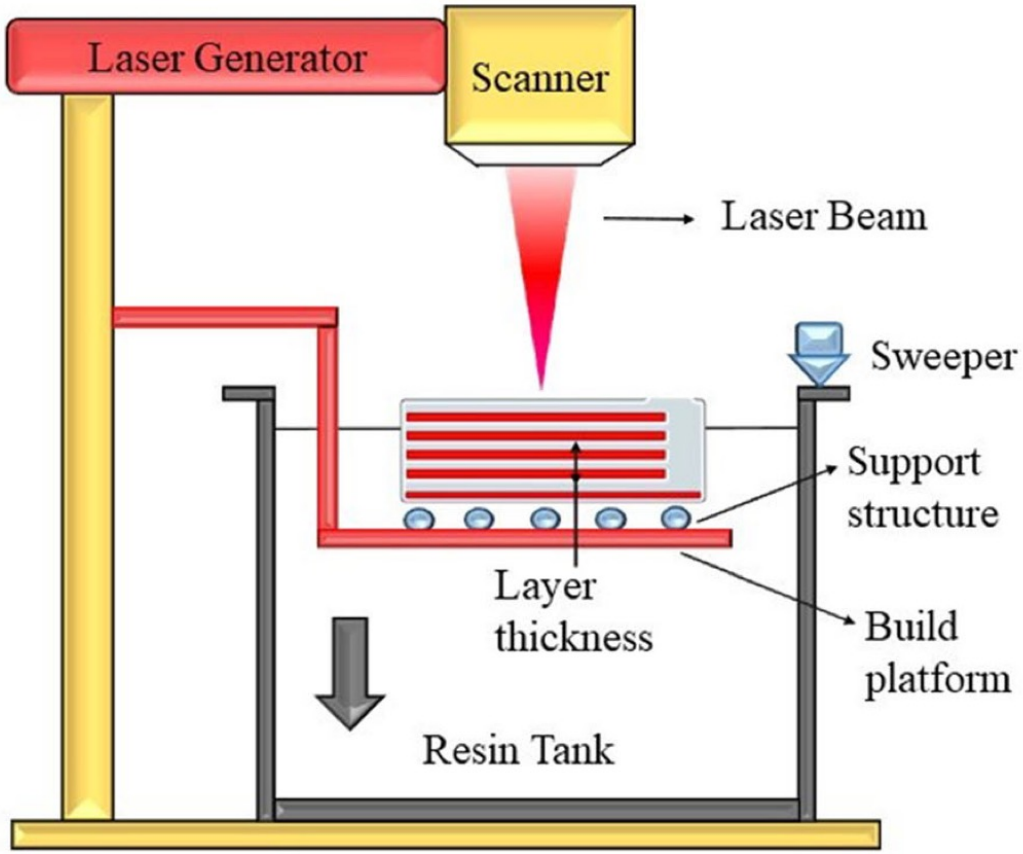
Fused deposition modelling (FDM)



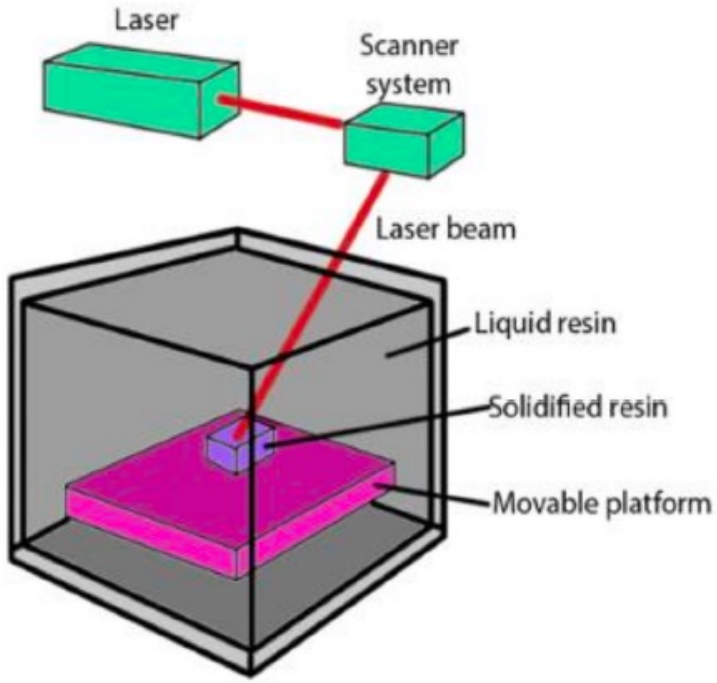


Fabrication Method	Advantages	Disadvantages	Materials
Fused deposition modeling (FDM): FDM uses a layer-by-layer deposition technique, in which molten polymers or ceramics are extruded through a nozzle with a small orifice and merge with the material on the previous layer	• 3D models of custom-made implants 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)

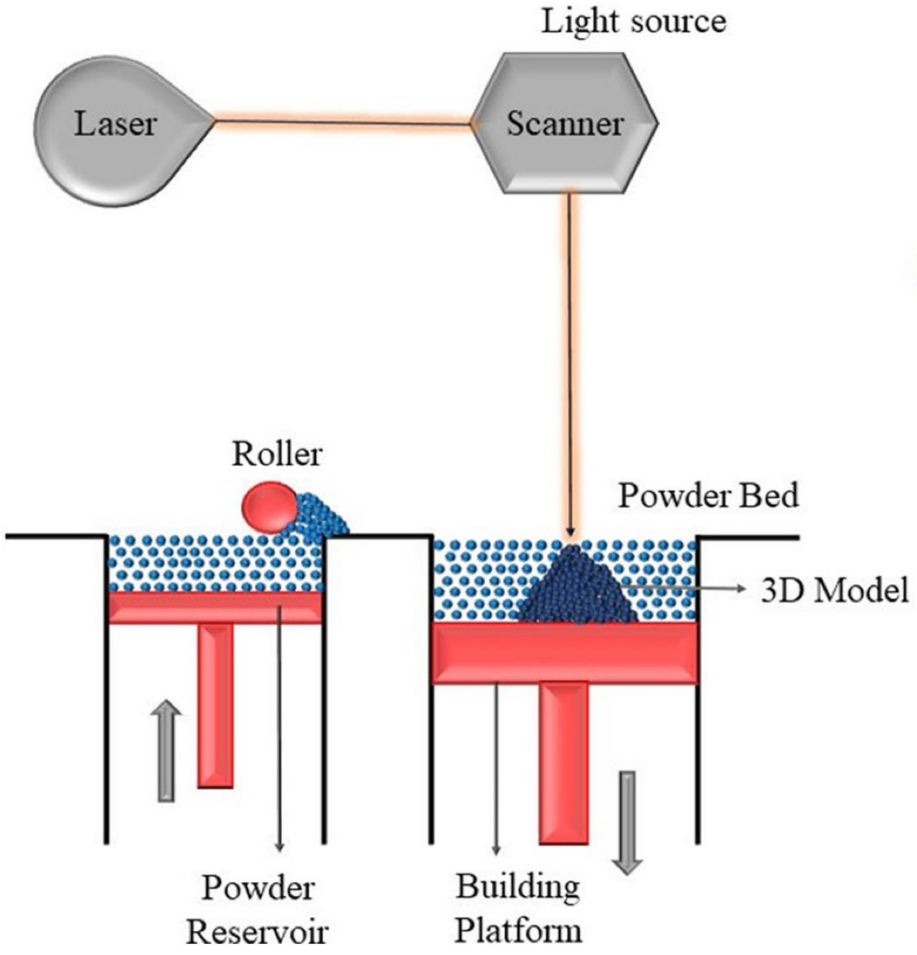


b)

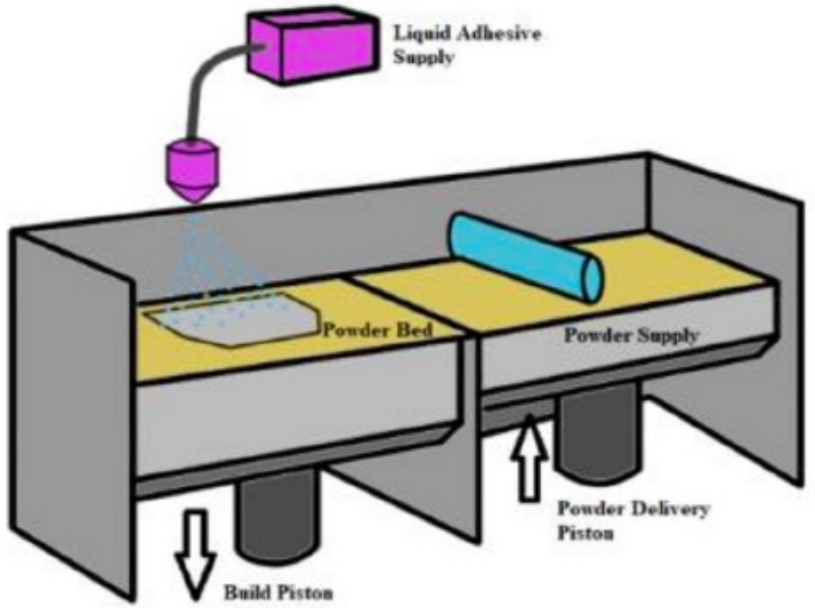


Fabrication Method	Advantages	Disadvantages	Materials
Stereolithography (SLA): In SLA, an object is created by selectively curing a polymer resin layer-by-layer using an ultraviolet (UV) laser beam	• Creates 3D scaffolds for tissue engineering with complex geometries. • Pores of multiple sizes, which 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)

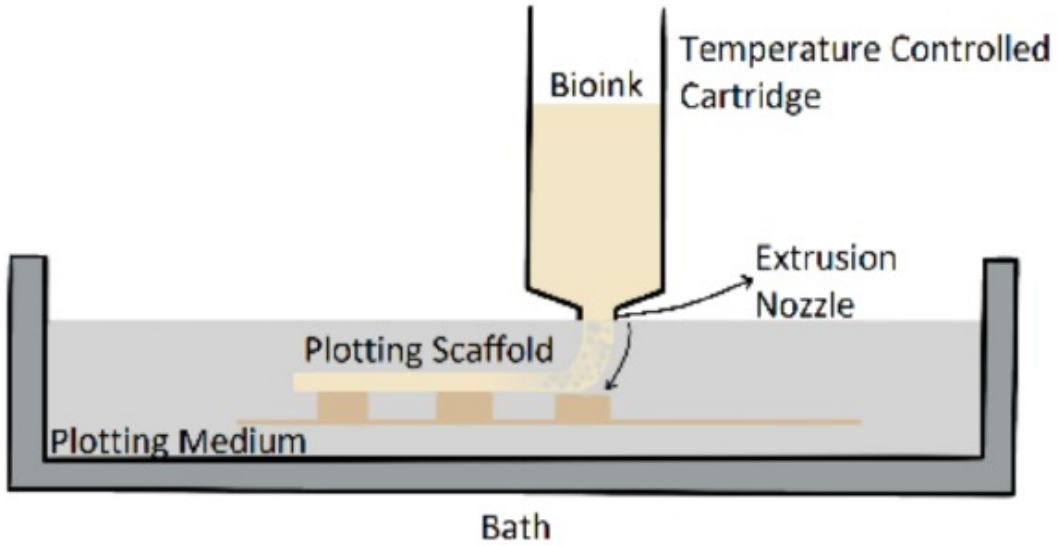


Fabrication Method	Advantages	Disadvantages	Materials
Selective laser sintering: This method selectively sinters thin layers of polymer-based mixtures in the powder form, creating solid 3D composite objects with macro-and microscale features	• Highly capable of producing objects with intricate structures 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



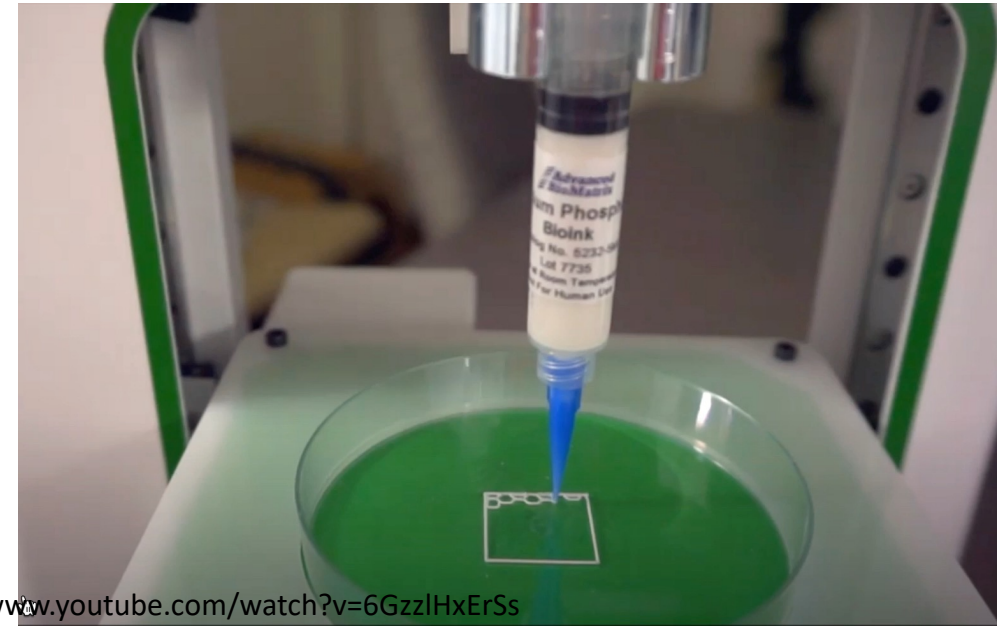
3D printing

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

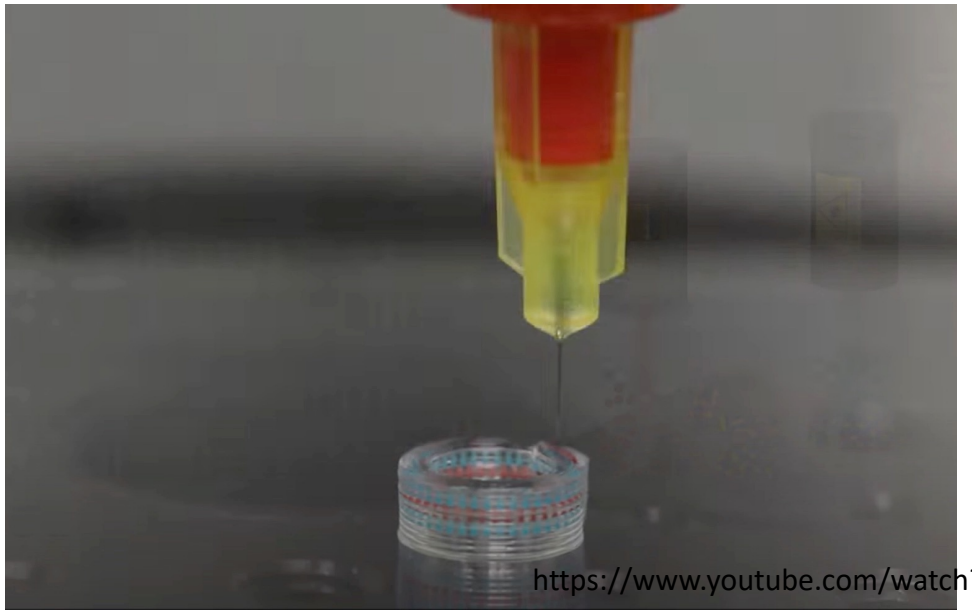


3D bioprinting

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



https://www.youtube.com/watch?v=6GzzlHxErSs



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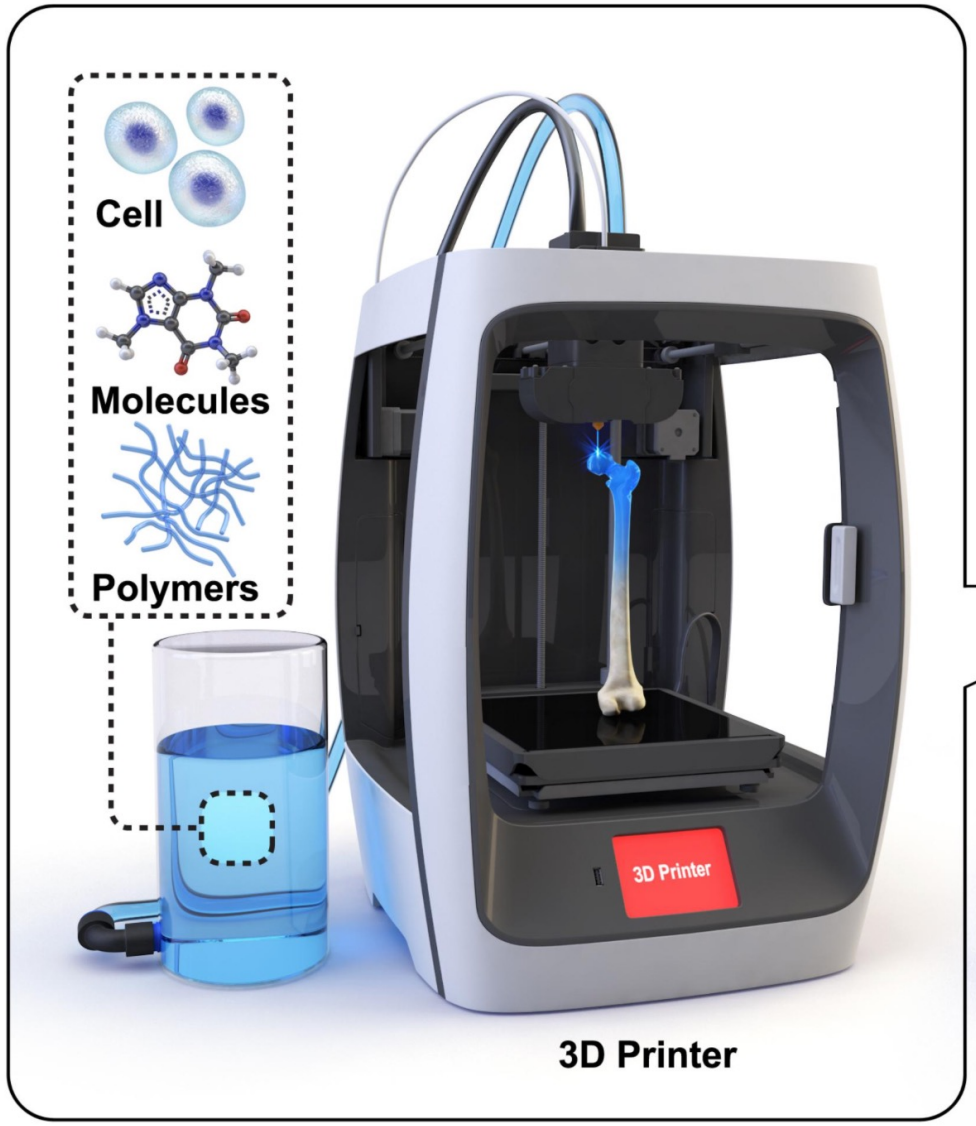
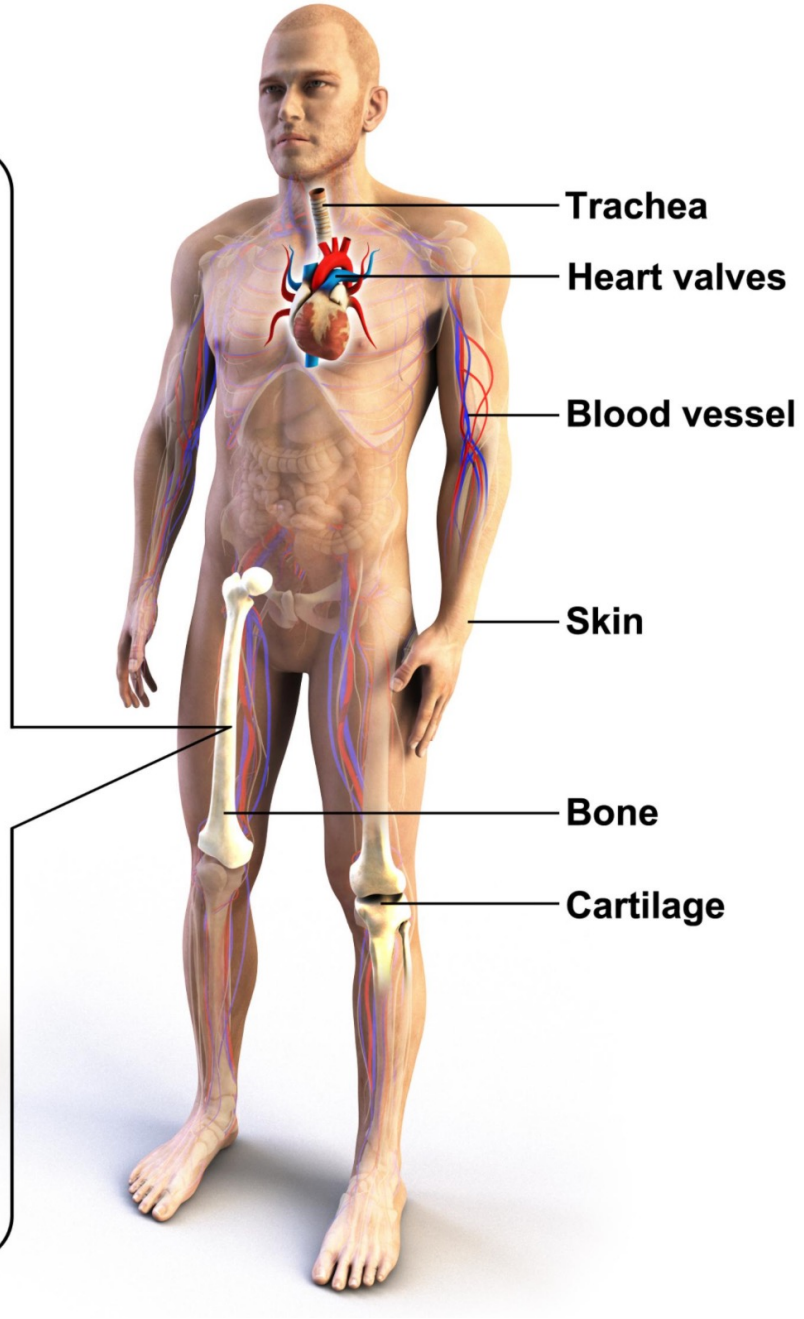
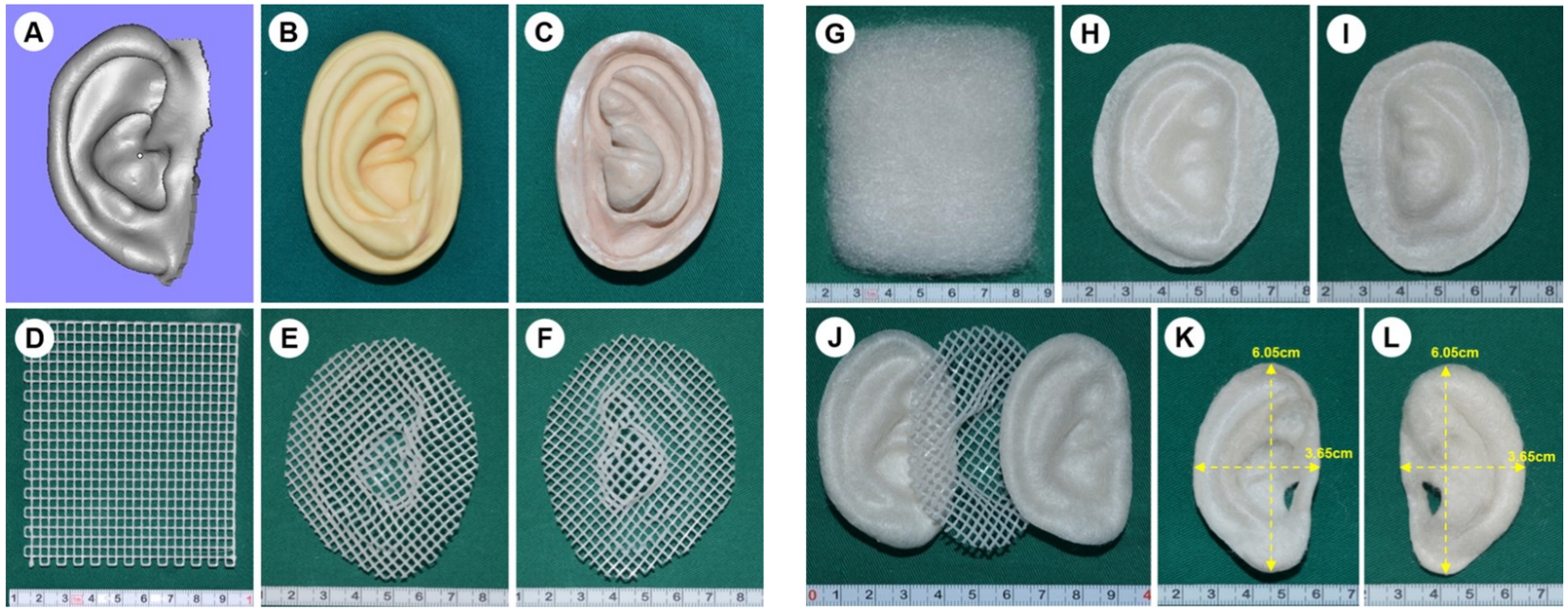
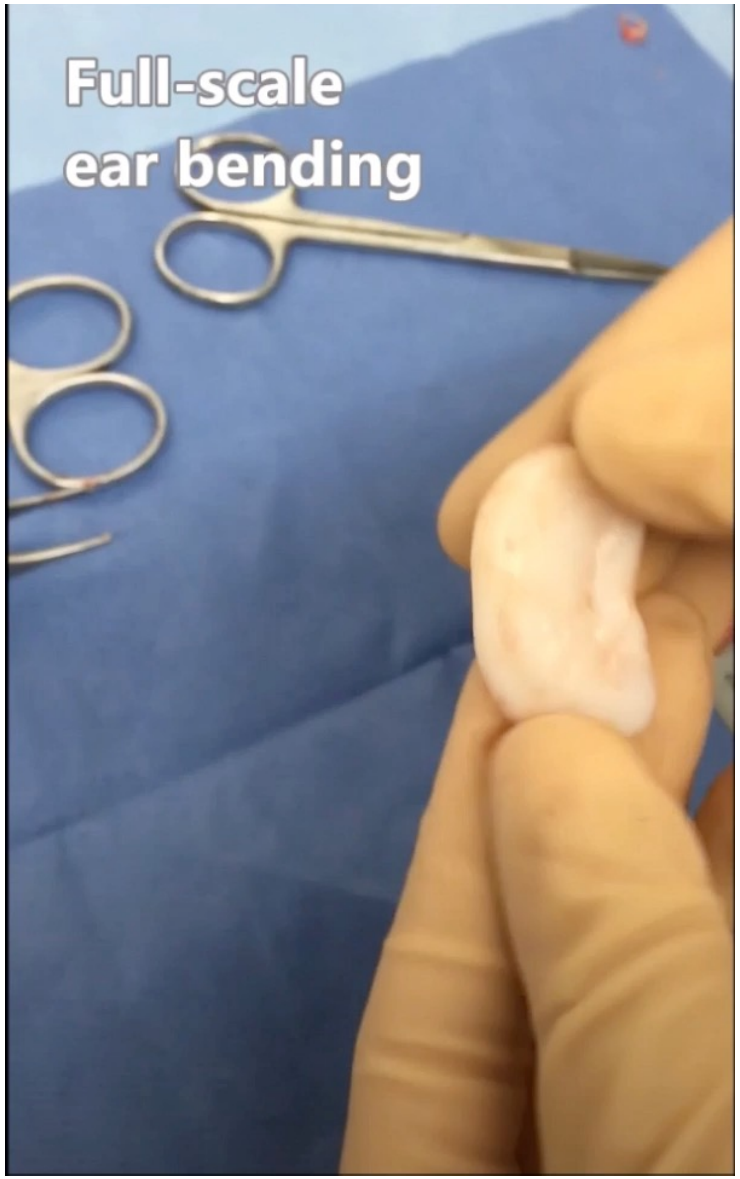

Cell
Molecules
Polymers
3D Printer
3D Printer


Trachea
Heart valves
Blood vessel
Skin
Bone
Cartilage





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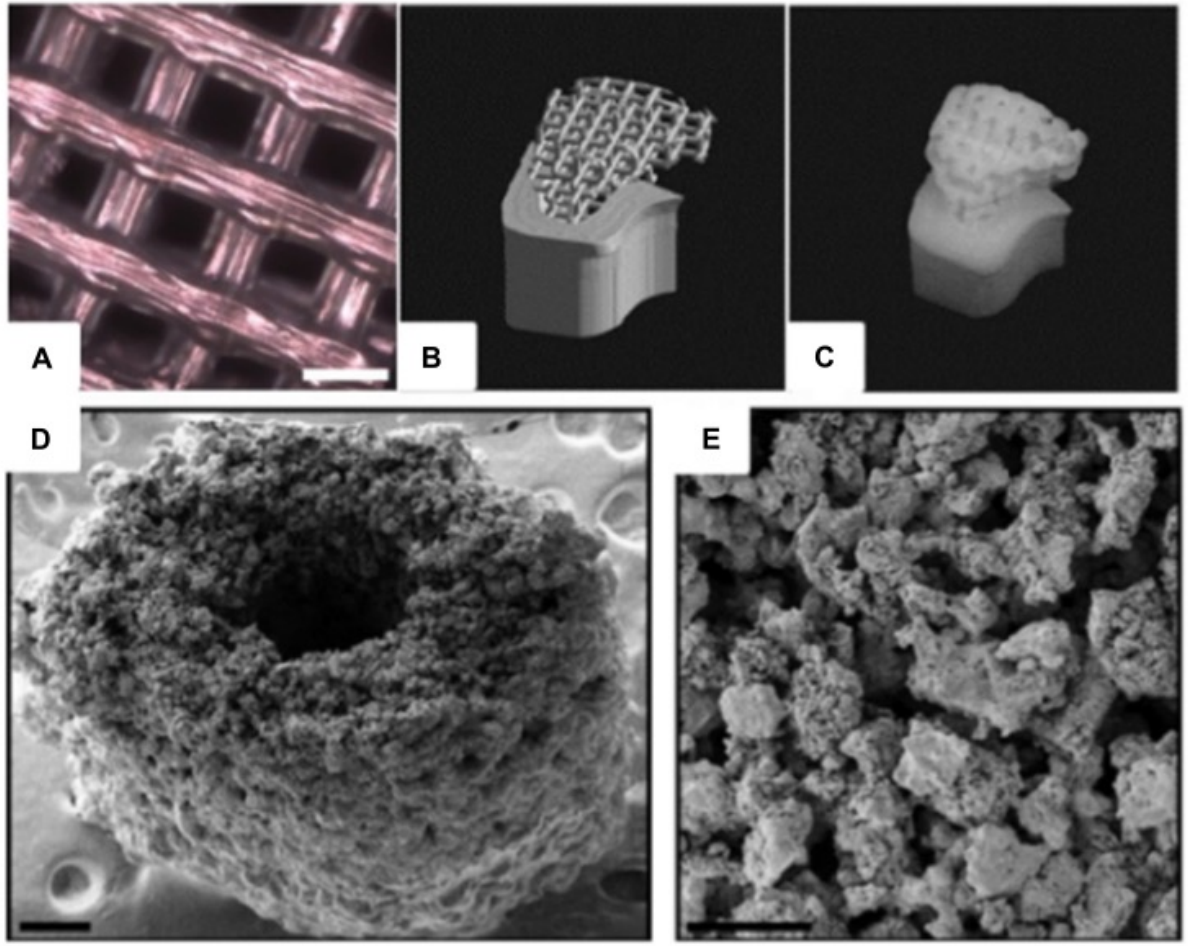


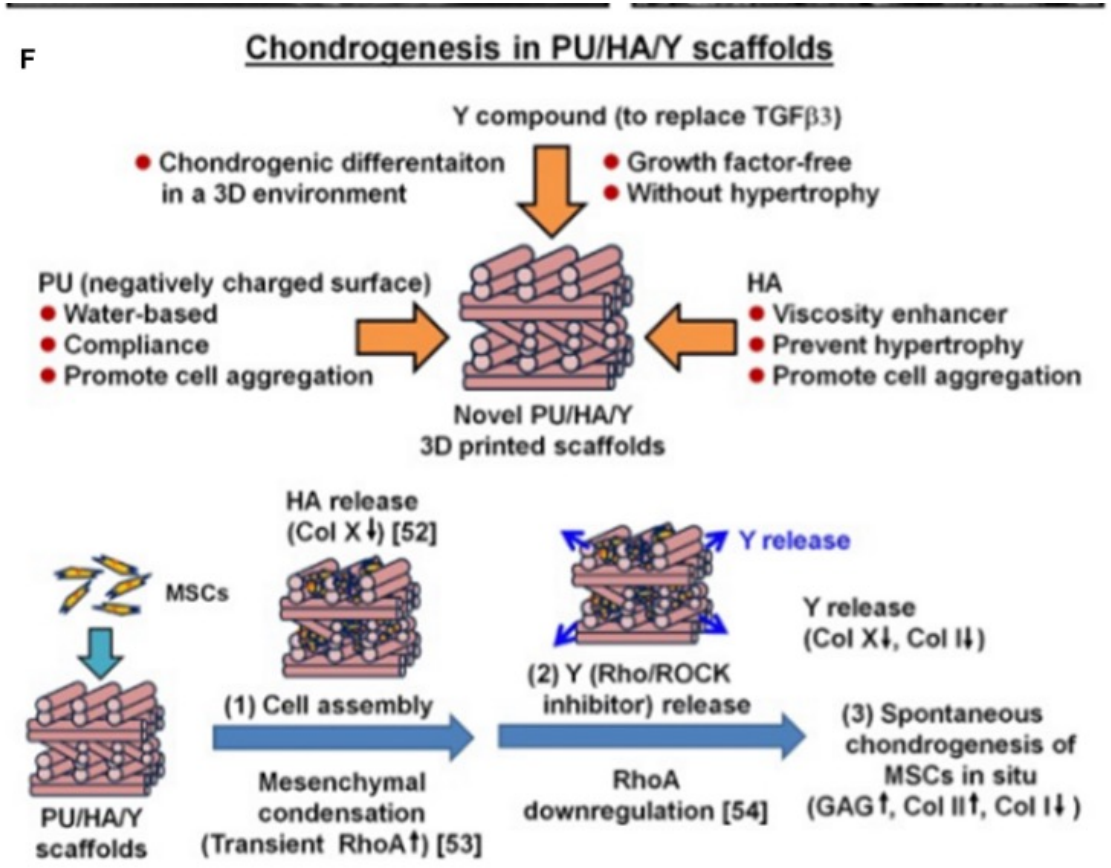


FIGURE 2 | (A) Optical microscope image of a 3D printed poly(l-lactide-co-ε-caprolactone) scaffold for adipose tissue engineering (courtesy of YJ, scale bar = 2 mm). **(B)** STL image of a pig condyle scaffold. **(C)** Front view of the 3D printed PCL scaffold (Williams et al., 2005). **(D)** SEM image of a 3D printed murine-sized scaffold for femoral mid-diaphysis regeneration (scale bar = 250 μm). **(E)** Micro-porosity of the calcium phosphate-collage composite scaffold with pore sizes of 20–50 μm (scale bar = 100 μm) (Inzana et al., 2014). **(F)** 3D printed PU/HA-based scaffold design, and possible mechanism of spontaneous chondrogenesis *in situ* (Hung et al., 2016) (Reproduced with permission from Williams et al., 2005; Inzana et al., 2014; Hung et al., 2016).

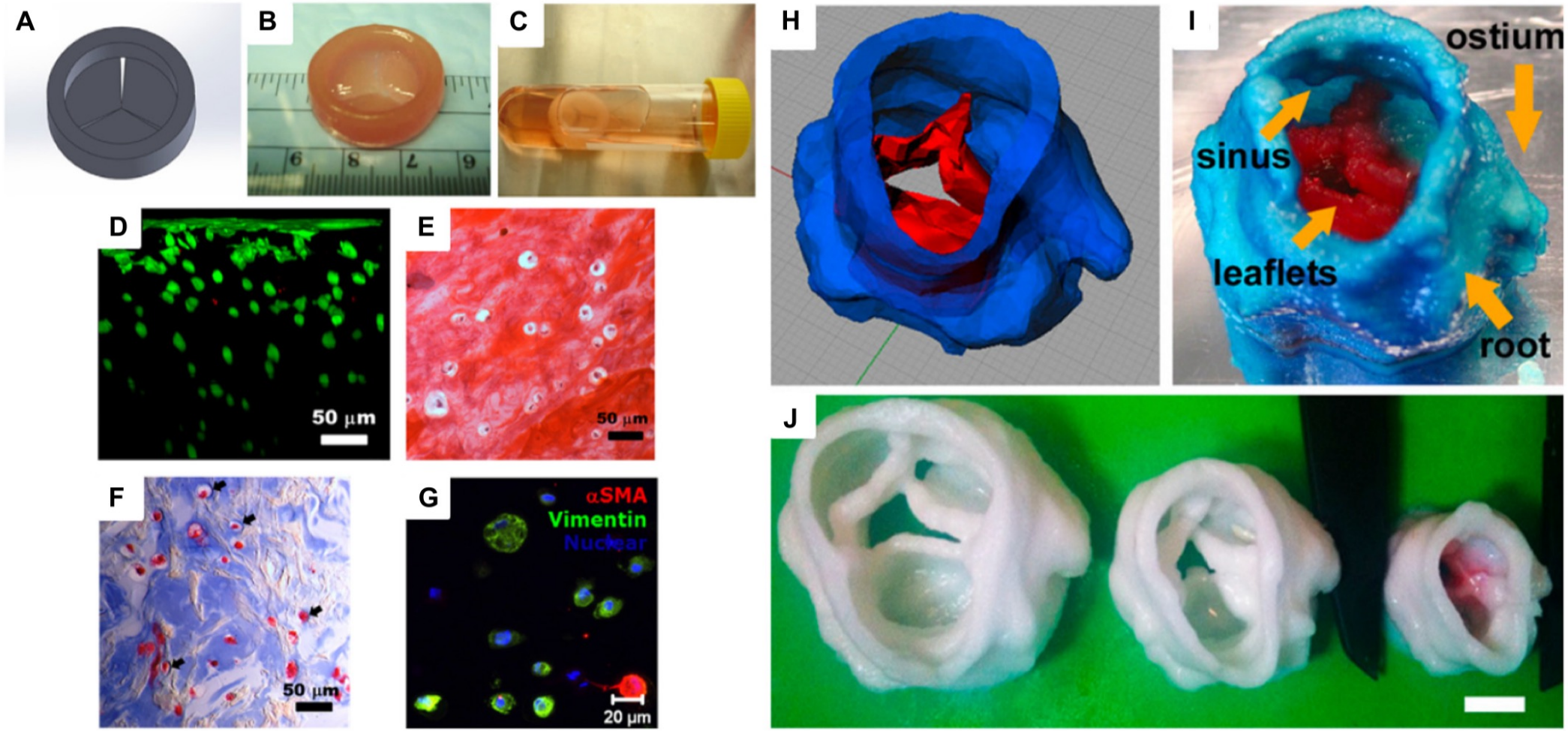


FIGURE 3 | (A) Computer-aided design (CAD) model of a heart valve. **(B)** Bioprinted methacrylated hyaluronic acid/gelatin heart valve conduit. **(C)** The hydrogel hybrid conduit after 7 days of static culture, and **(D)** cross-sectional view of a live/dead cell viability assay. **(E)** Safranin-O staining image and **(F)** Masson's Trichrome staining images showed that the heart valve conduit was composed of collagen type II and GAG. **(G)** Representative immunohistochemical staining image of αSMA, vimentin, and nuclei (Duan et al., 2014). **(H)** Porcine aortic valve model and **(I)** 3D printed scaffold with two types of PEG-DA inks [root: 700 molecular weight (MW) PEG-DA and leaflets: 700/8000 MW PEG-DA]. **(J)** Scaffolds were printed with 700 MW PEG-DA at different scales for fidelity analysis. The inner diameters (ID) were 22, 17, and 12 mm. Scale bar = 1 cm (Hockaday et al., 2012) (Reproduced with permission from Hockaday et al., 2012; Duan et al., 2014).

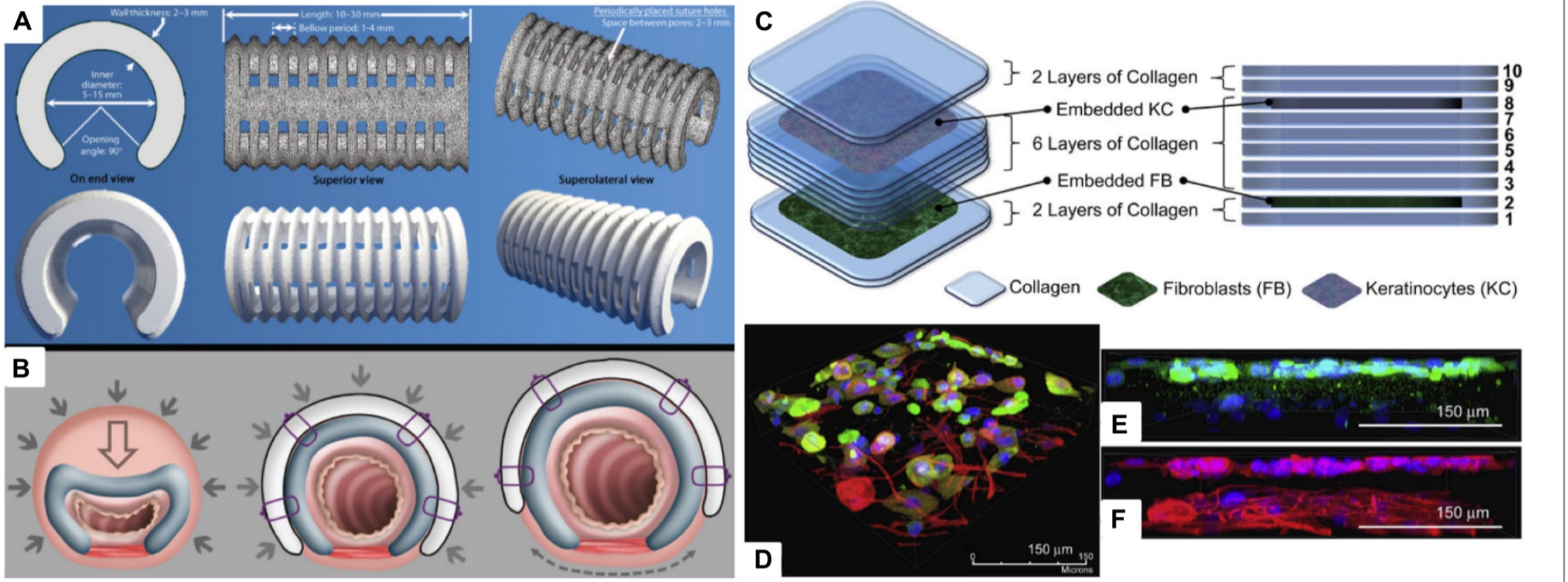


FIGURE 4 | (A) Virtual rendering of tracheobronchial splint STL file in top, bottom, and side views. Inner diameter, length, thickness, and suture hole spacing were patient-specifically designed, and then it was placed over an airway through the 90° opening angle. **(B)** The mechanism of the tracheobronchial splint. Filled arrows signify intrathoracic pressure when breathing out, and empty arrows represent reducing vector values. Dashed arrow indicates the vector movement of a splint according to the airway growth (Morrison et al., 2015). **(C)** Representative scheme of a multi-layered collagen scaffold for tissue regeneration. Primary adult human dermal fibroblast-seeded collagen is printed in the 2nd layer, and primary adult human epidermal keratinocyte embedded collagen layer is deposited in the 8th layer. **(D)** Immunofluorescent image of the 3D printed multi-layered scaffold with fibroblast and keratinocyte on a tissue culture dish. **(E)** Keratinocyte layer with keratin, and **(F)** keratinocyte and fibroblast layer with β-tubulin (Lee et al., 2009) (Reproduced with permission from Lee et al., 2009; Morrison et al., 2015).

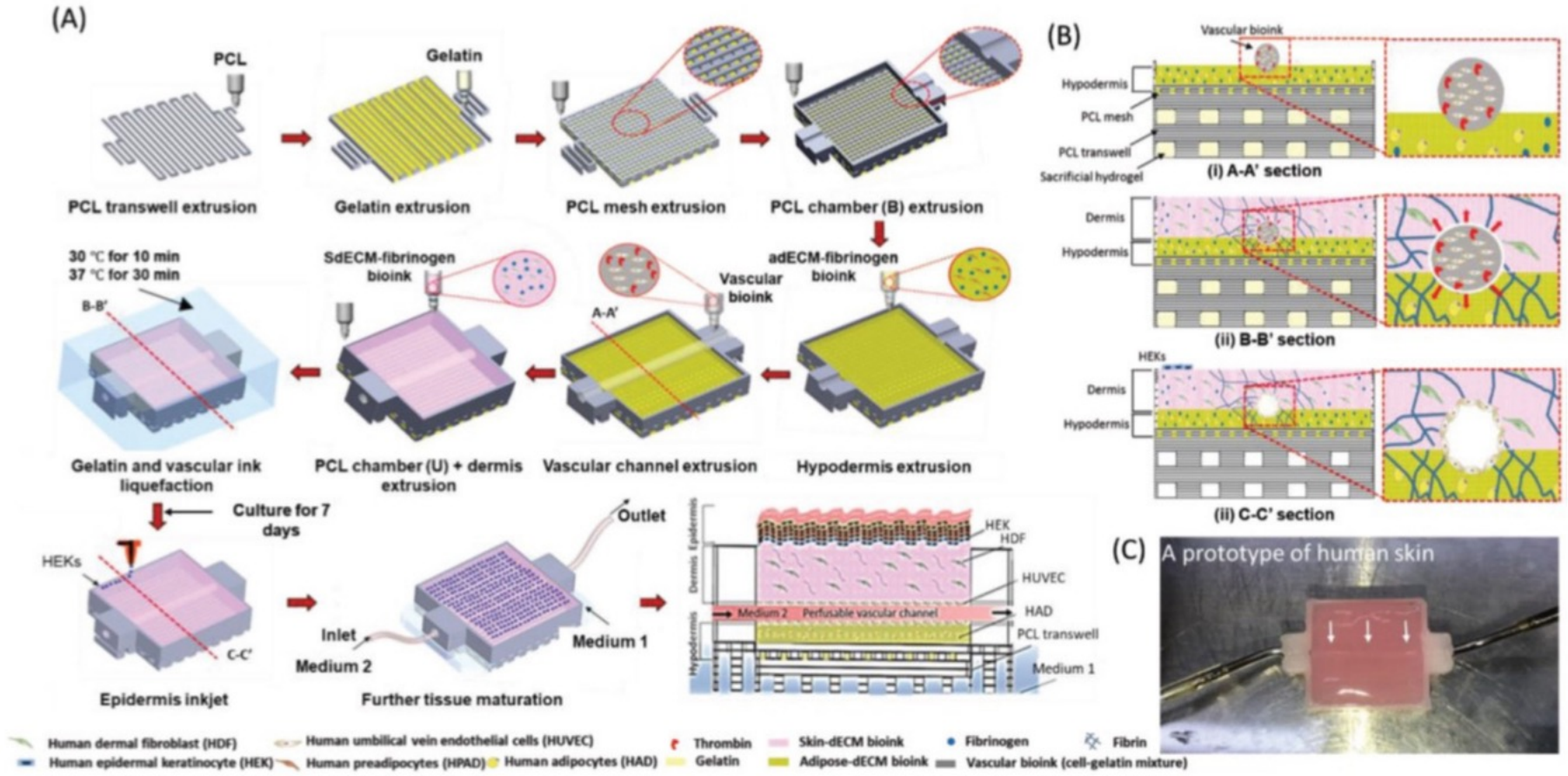


Figure 17. The 3D cell printing process for fabrication of 3D P/V full-thickness skin model. (**A**) Schematic diagram exhibiting the step-by-step fabrication process. (**B**) Sectional views provided from the aforementioned fabrication process. (**C**) A prototype of the fabricated skin construct. Reproduced from [249] with permission from John Wiley and Sons, 2018.

Edmundo Antezana, *Pharmaceutics* **2022**, *14*, 464

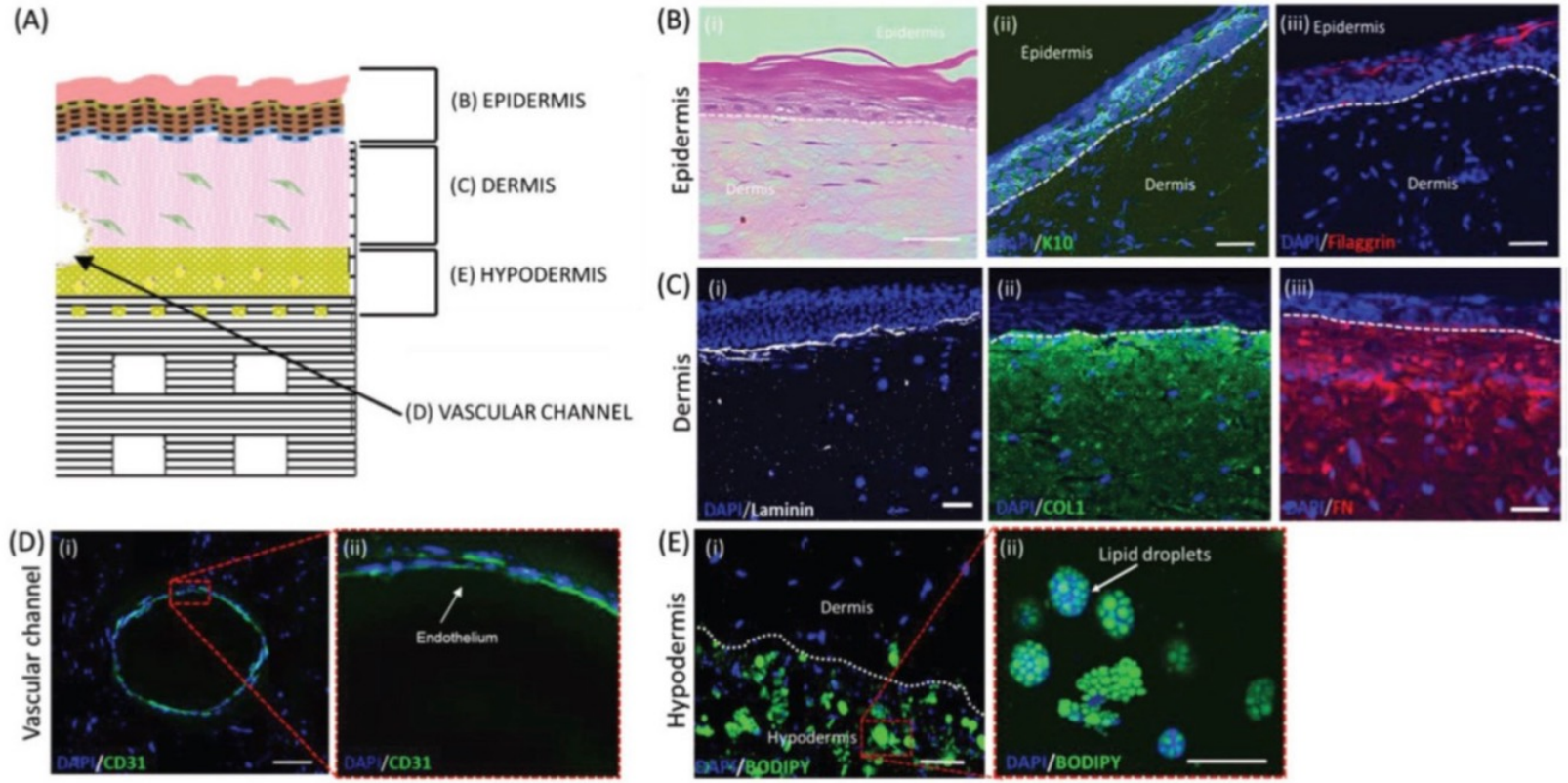
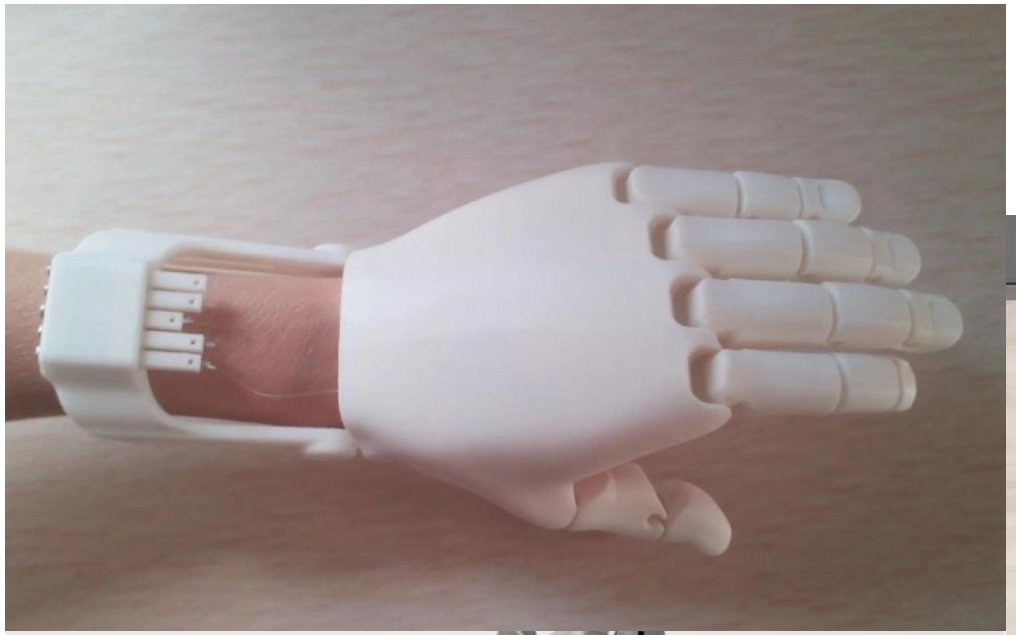


Figure 18. Histological analyses representing skin tissue maturation in in vitro environment. (**A**) Illustration of each zone of epidermis, dermis, hypodermis, and vascular channel. (**B**) Epidermis stratified (H&E staining) and stained with keratin 10 (K10) and filaggrin representing early differentiation and late differentiation of epidermis, respectively. (**C**) Dermis imaged with protein markers representing epidermal–dermal junction (Laminin) and secreted ECM components (COL1: collagen type I and FN: fibronectin). (**D**) Vascular channel in the mature 3D human skin equivalent stained with CD31 demonstrating the presence of endothelial cells. (**E**) Hypodermis stained with BODIPY representing lipid droplets of adipocytes (Scale bars: 50 μm). Reproduced from [249] with permission from John Wiley and Sons, 2018.



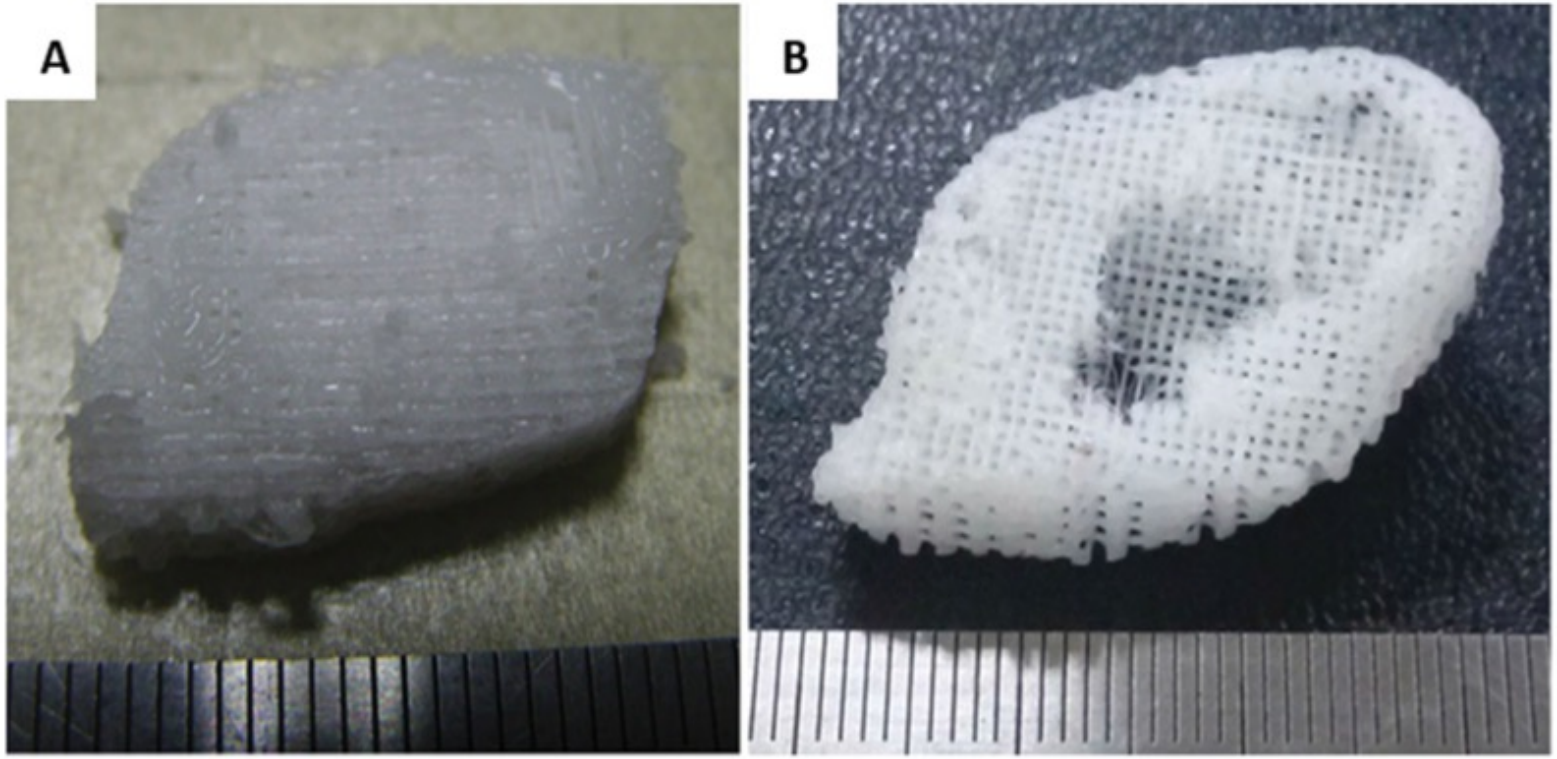


Figure 7. Ear-shaped scaffolds: Photographs of ear-shaped structures made from PCL using a PEG sacrificial layer (not shown) through a hybrid 3D printing system combining both inkjet printing and fused deposition modeling. A) back of ear scaffold, B) front of ear scaffold. Reproduced with permission.[27] Copyright 2014, IOP Publishing.

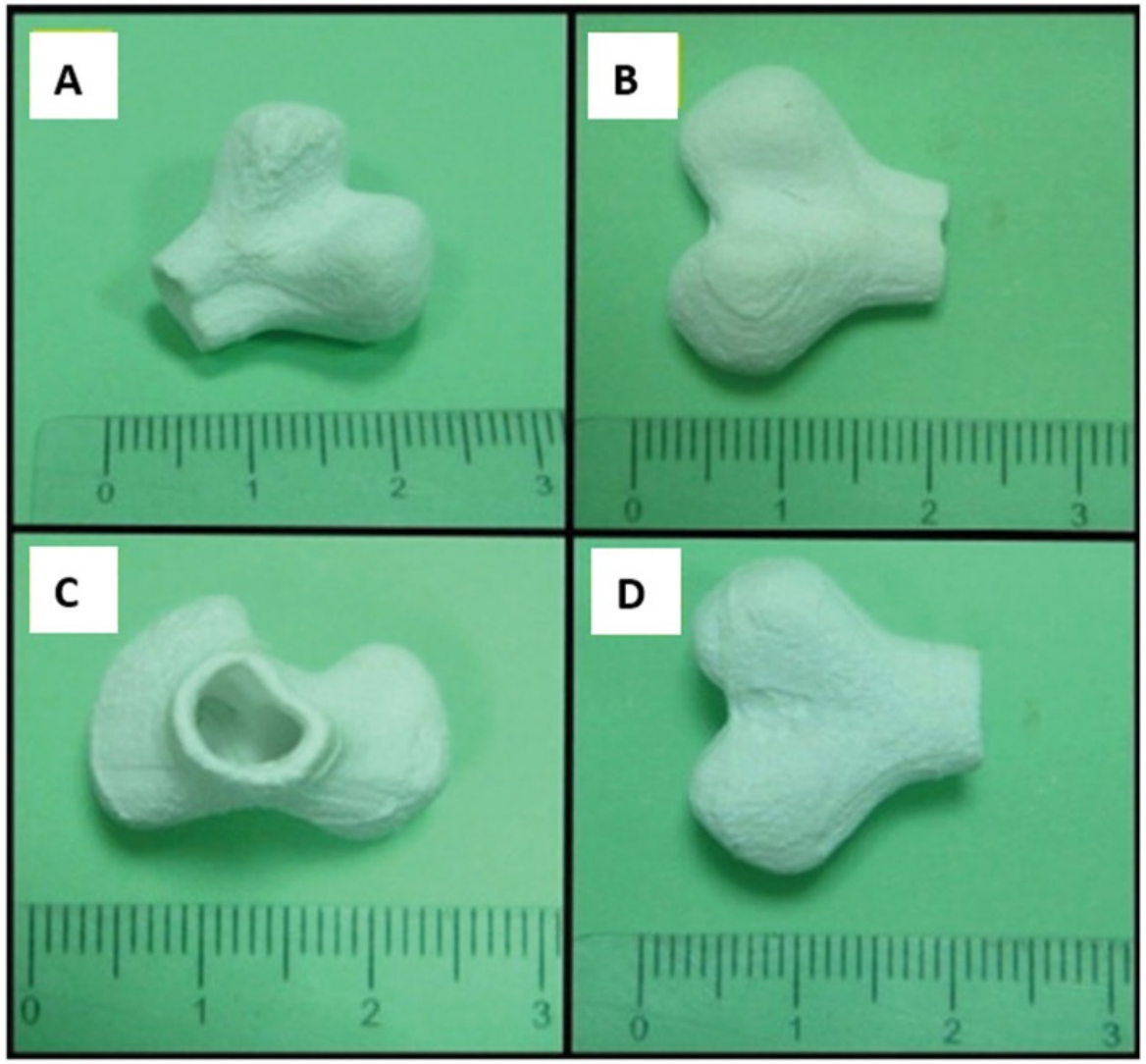
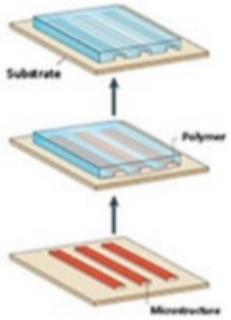


Figure 12. Bone scaffolds generated by SLS: A) Image of the scaffold, B) front view, C) top view, and D) back view of bone scaffold parts. Size is based on a centimeter ruler, with hatch marks indicating per millimeter. Reproduced with permission.[115] Copyright 2014, Elsevier.

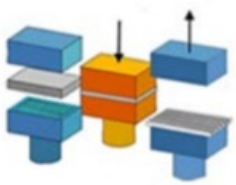
Do et al., Adv. Healthcare Mater. **2015**, *4*, 1742–1762

Organ-On-Chip



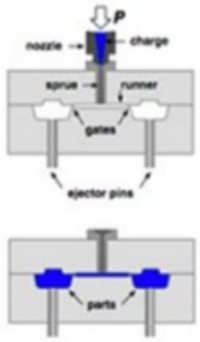
Soft-lithography

- Combination of photolithography and molding
- Suitable for elastomeric materials



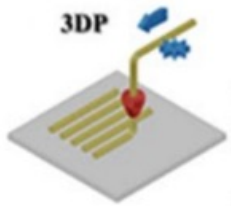
Hot Embossing

- Requires master mold fabrication
- Suitable for polymeric materials



Injection Molding

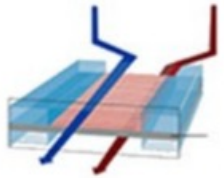
- Requires master mold fabrication
- Low-cost high precision microfabrication suitable for batch production



3D Printing

- Supports both additive and subtractive manufacturring
- Used for master preparation

Creative Methods



- Easy implementation methods without high cost facilities
- Suitable for preliminary experiments

Tajeddine et al., Micromachines 2021, 12, 1443