TISSUE ENGINEERING

Obtaining scaffolds through decellularization techniques

21 - COP-0017

Decellularization. Concept.

Decellularization is the complete removal of all cellular components from a tissue, while preserving the extracellular matrix, including vascularization.

Encyclopedia of Tissue Engineering and Regenerative Medicine, 2019

Decellularization is defined as the chemical or physical removal of the cellular phase from living tissues, creating an acellular scaffold of the original tissue, which can be subsequently used in the artificial regeneration of organs and tissues.

Organ Repair and Regeneration, 2021

The decellularization process aims to eliminate all cellular and nuclear matter, minimizing any adverse effects on the composition, biological activity, and mechanical integrity of the remaining ECM for the development of new tissue.

Encyclopedia of Biomedical Engineering, 2019

Decellularization is the process of harvesting an organ, either from a human donor or an animal model, and sterilizing it until all components outside the collagen network are removed; the cell-depleted tissue remaining is classified as a natural scaffold.

Nanoengineered Biomaterials for Regenerative Medicine, 2019

Decellularization. Concept..

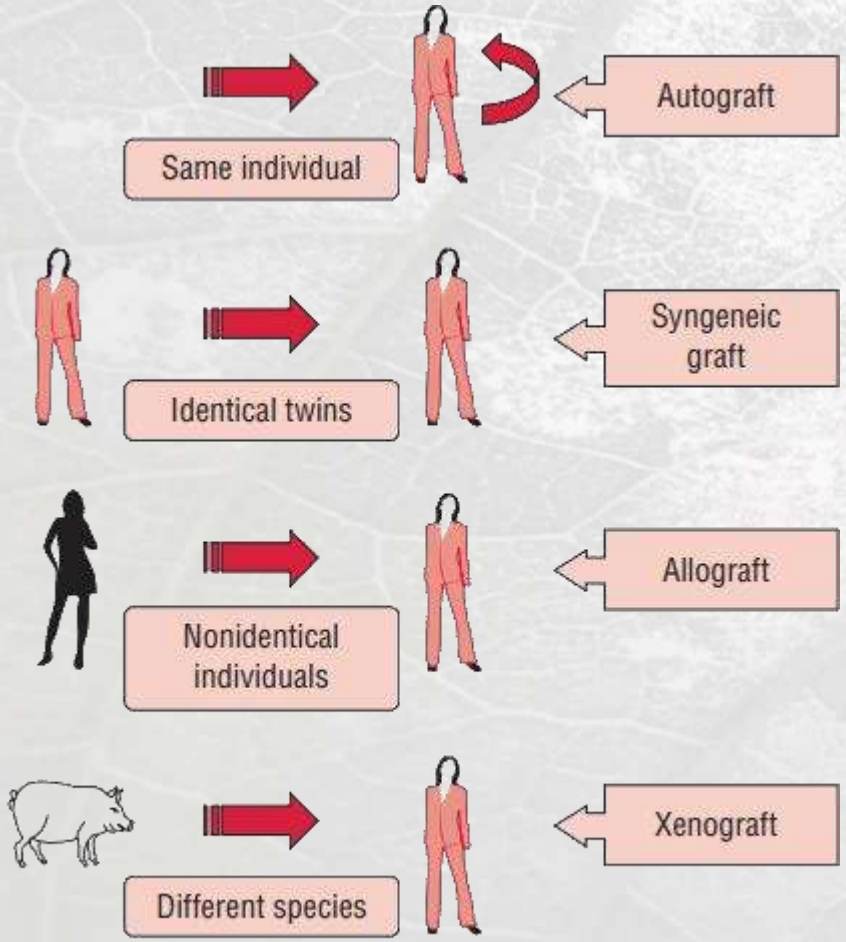


FIG. 21-1. Grafts in transplantation.

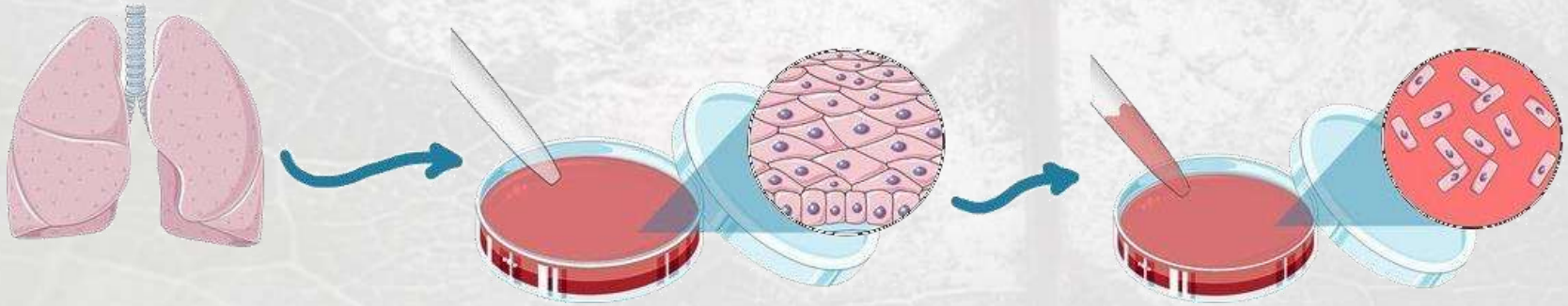
Decellularization is the process of removing allogeneic or xenogeneic cellular antigens from a tissue, leaving behind an intact ECM comprising a mixture of structural and functional molecules useful in supporting the repopulation of the three-dimensional network with cells from the transplant recipient.

Comprehensive Biomaterials II, 2017

https://www.brainkart.com/article/Transplant-Immunology-and-Types-of-Transplants_17989/

Decellularization is NOT a method for obtaining cell lines used in in vitro studies..

Primary cell culture is the first culture obtained directly from animal tissue through mechanical and chemical disintegration or enzymatic methods (proteolytic enzymes) of the ECM, consisting of slowly growing cells that retain all characteristics of the original tissue or cells.



Secondary cell cultures are obtained after primary cell cultures are subsequently subcultured over a period of time in fresh culture media. The cells in secondary cell cultures have a longer lifespan due to the availability of proper nutrients at regular intervals, allowing them to survive through numerous passages. Cell lines typically exhibit functional characteristics that are close to primary cells, but the genotype and phenotype of the cells may be altered.

THE OBJECTIVES OF THE METHOD

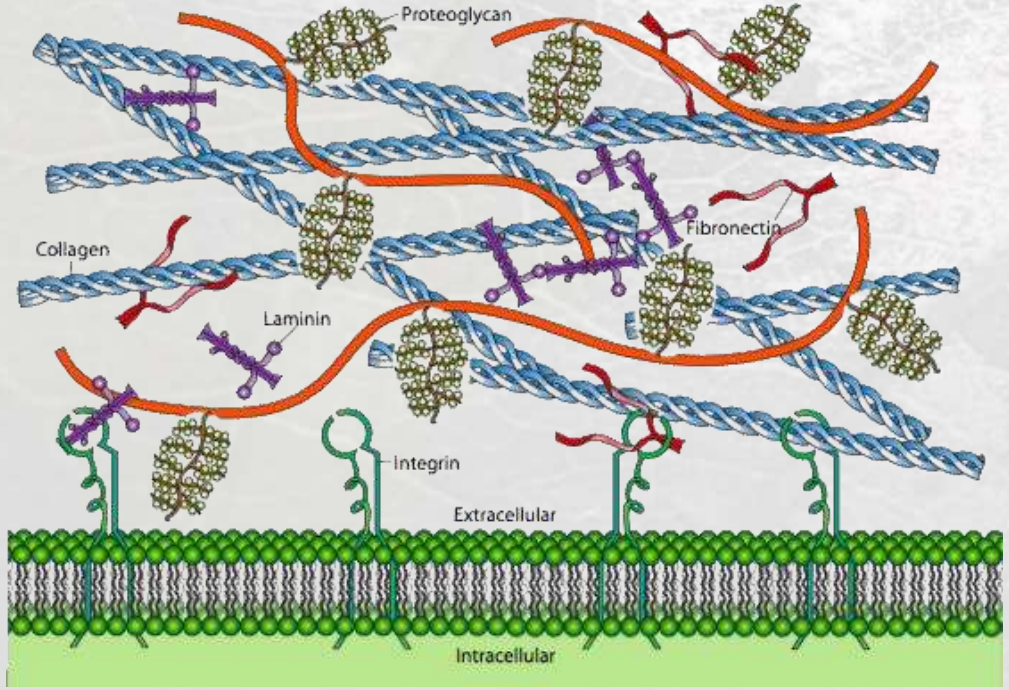
Organ/tissue

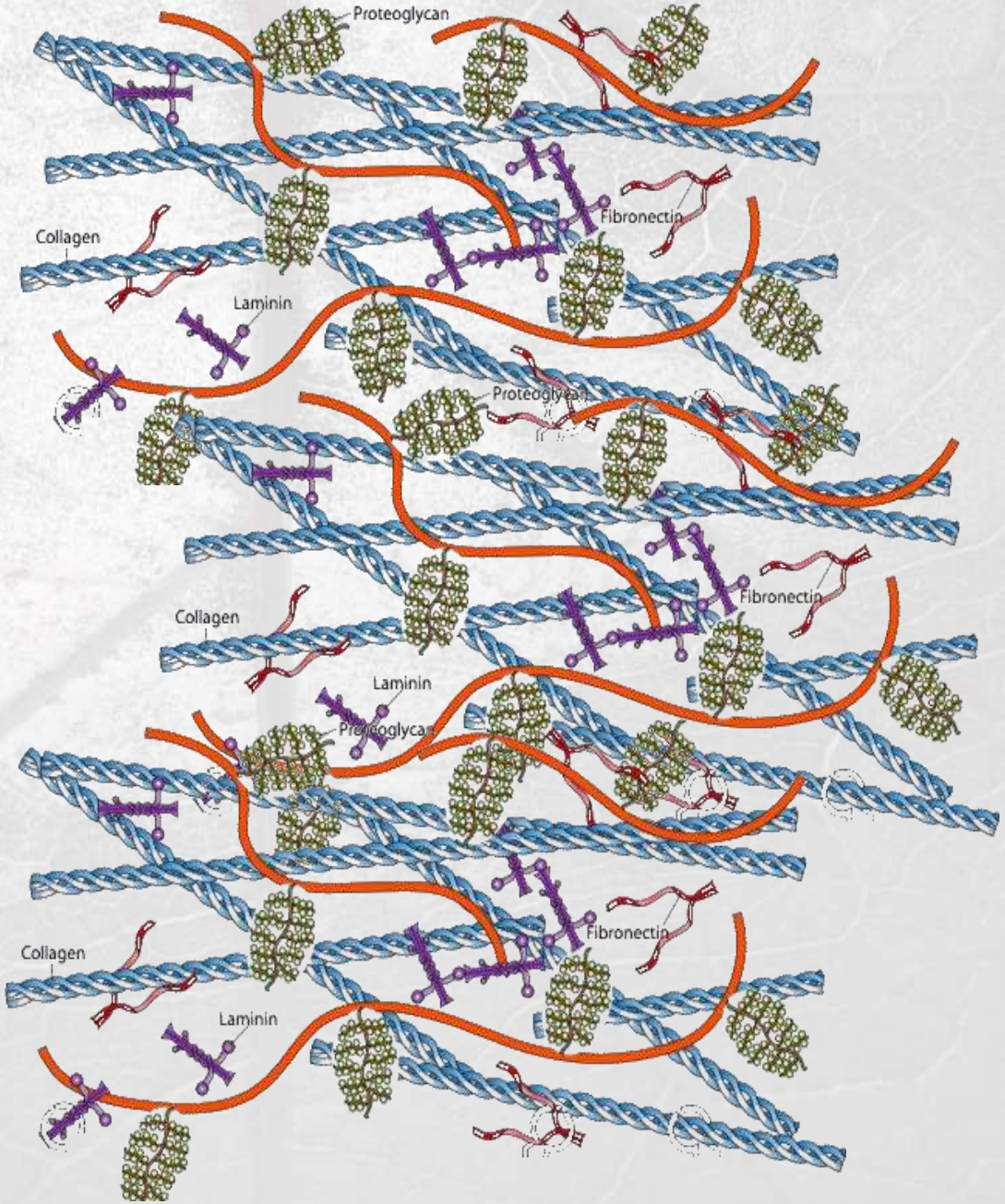
Total organ substitutes

Partial graft substitutes

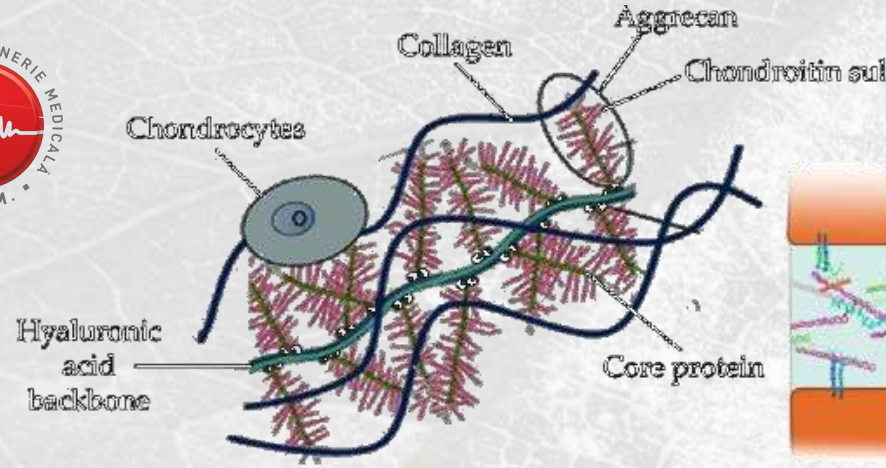
Raw materials for gels

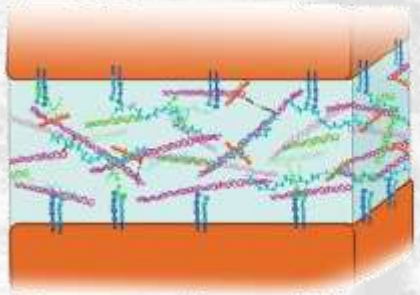
Obtaining a complex material with the structural characteristics of ECM is one of the main advantages of decellularization.





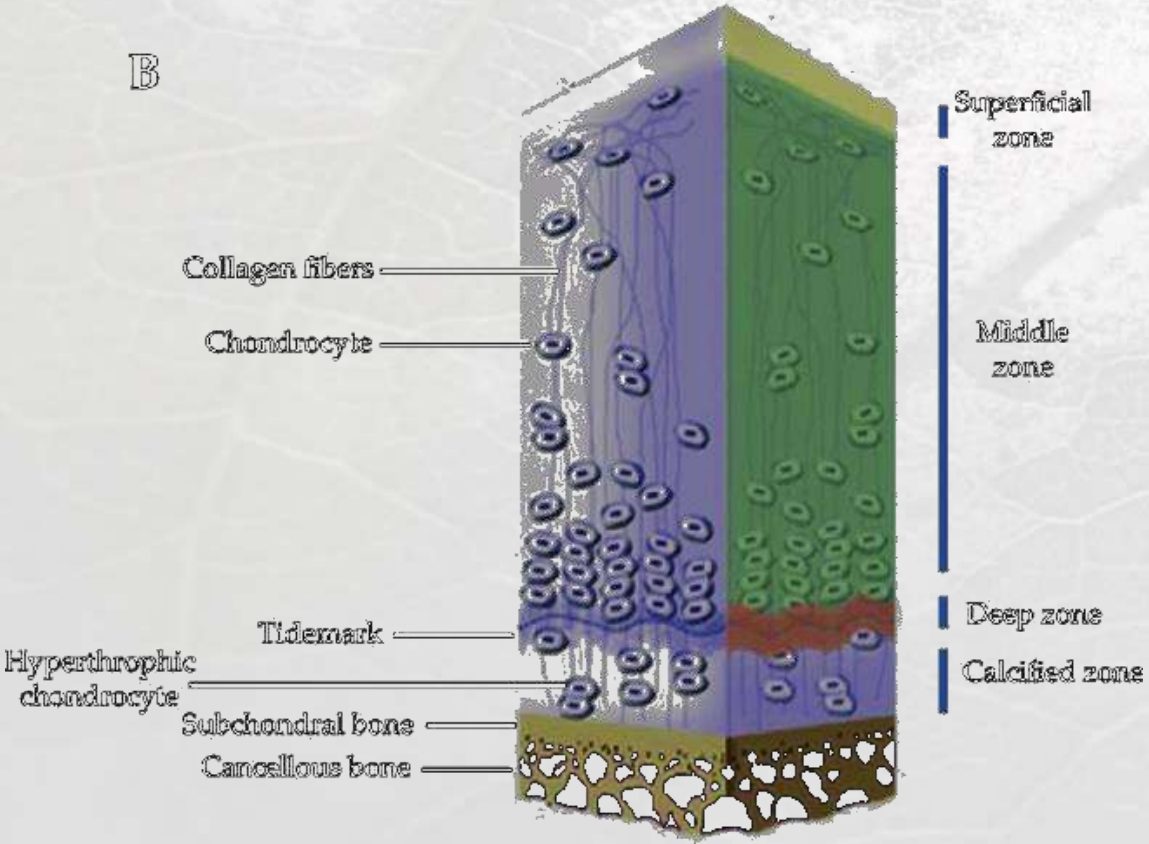
Wong, Eric. Cells: molecules and mechanisms. Axolotl Academic Publishing, 2009.

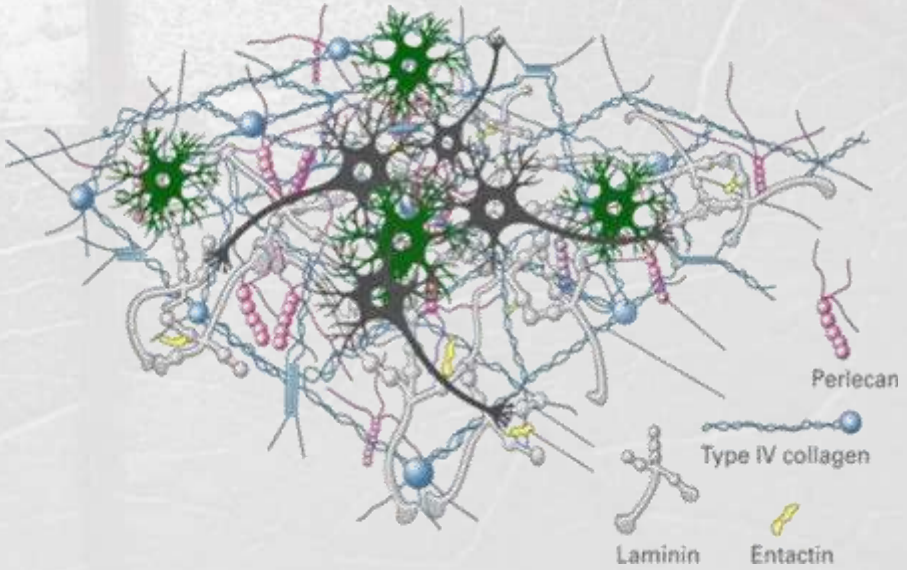




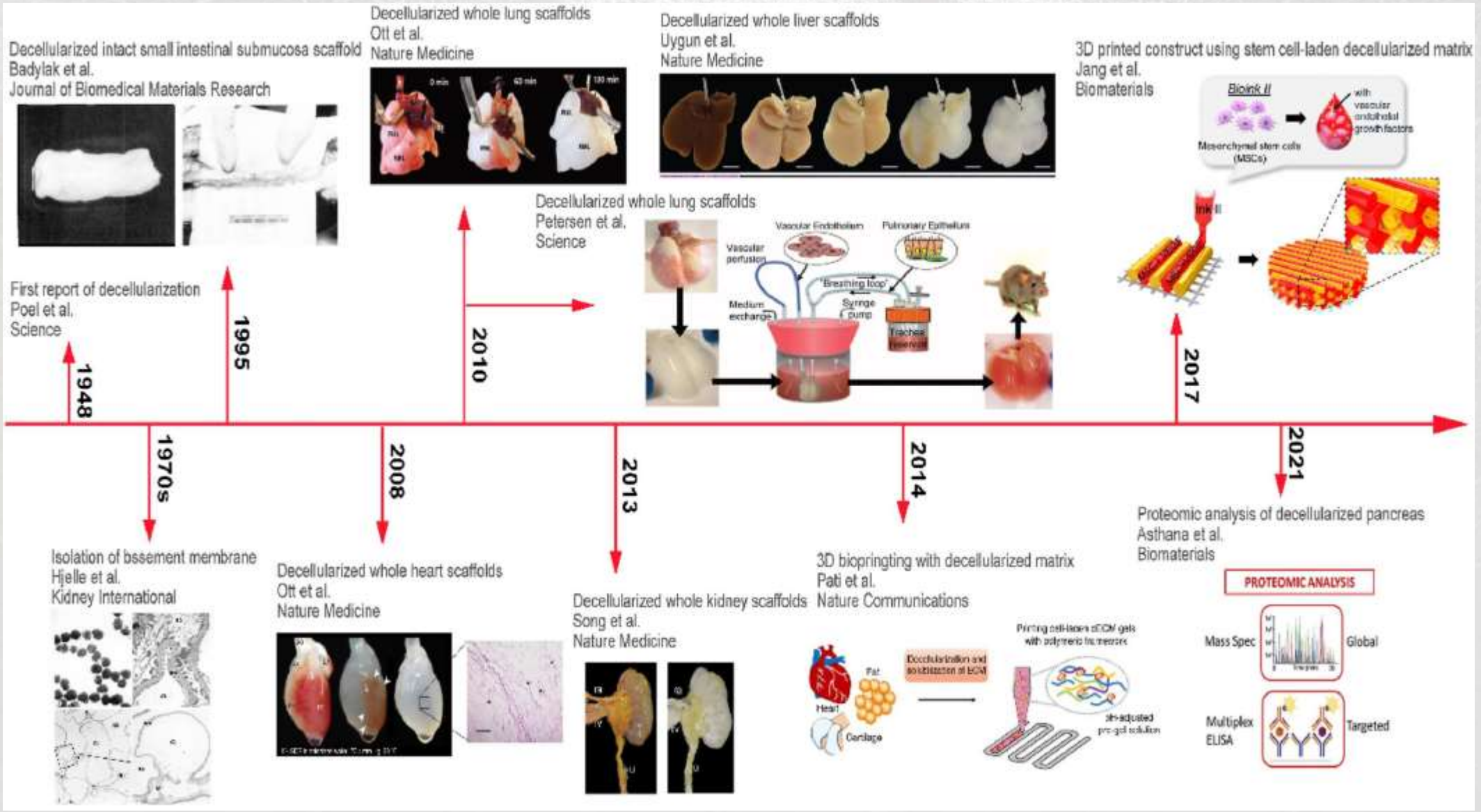
https://journals.sagepub.com/doi/full/10.1177/20417314221105305

B

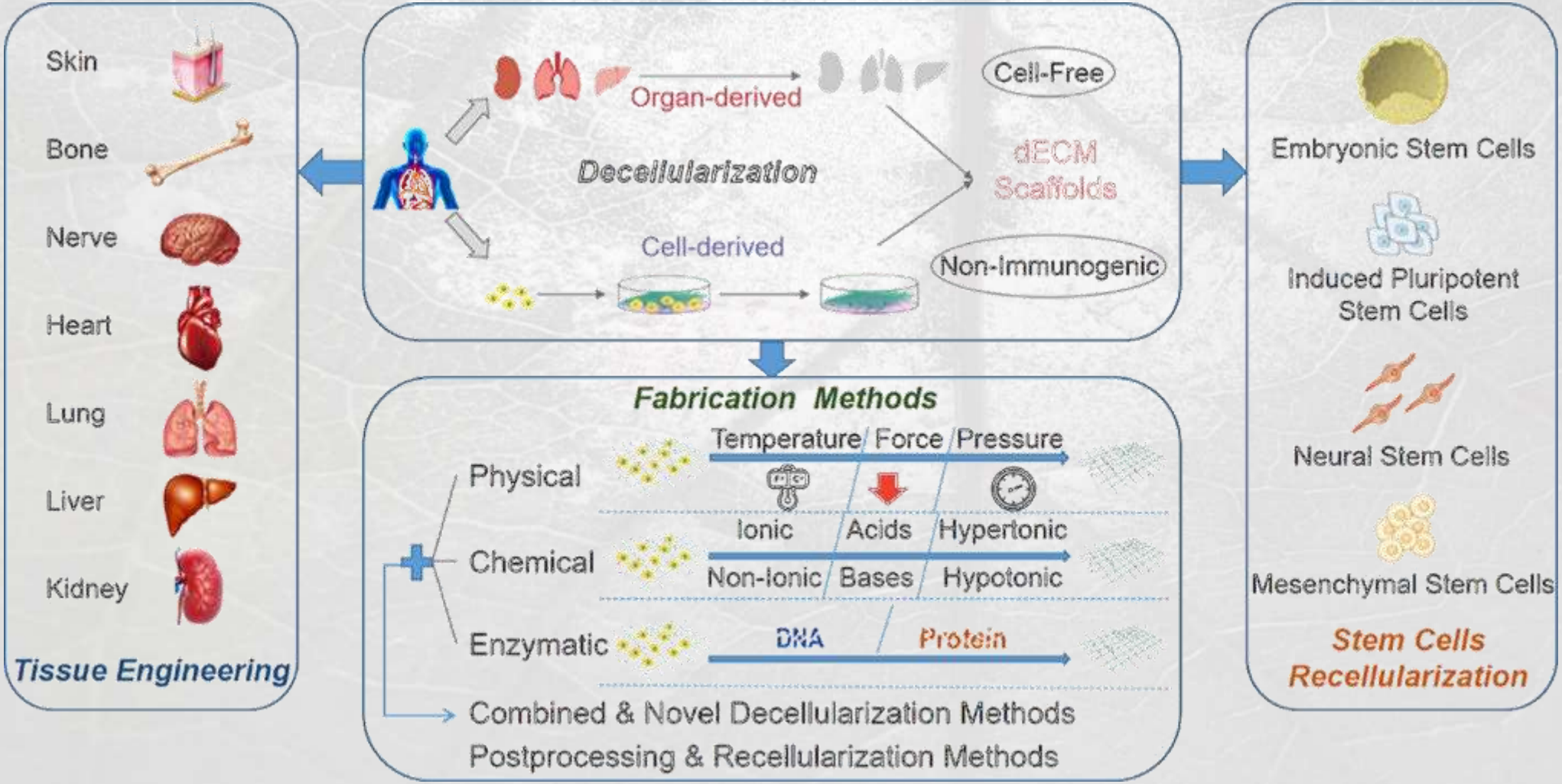




https://onlinelibrary.wiley.com/doi/abs/10.1002/ijch.201900052

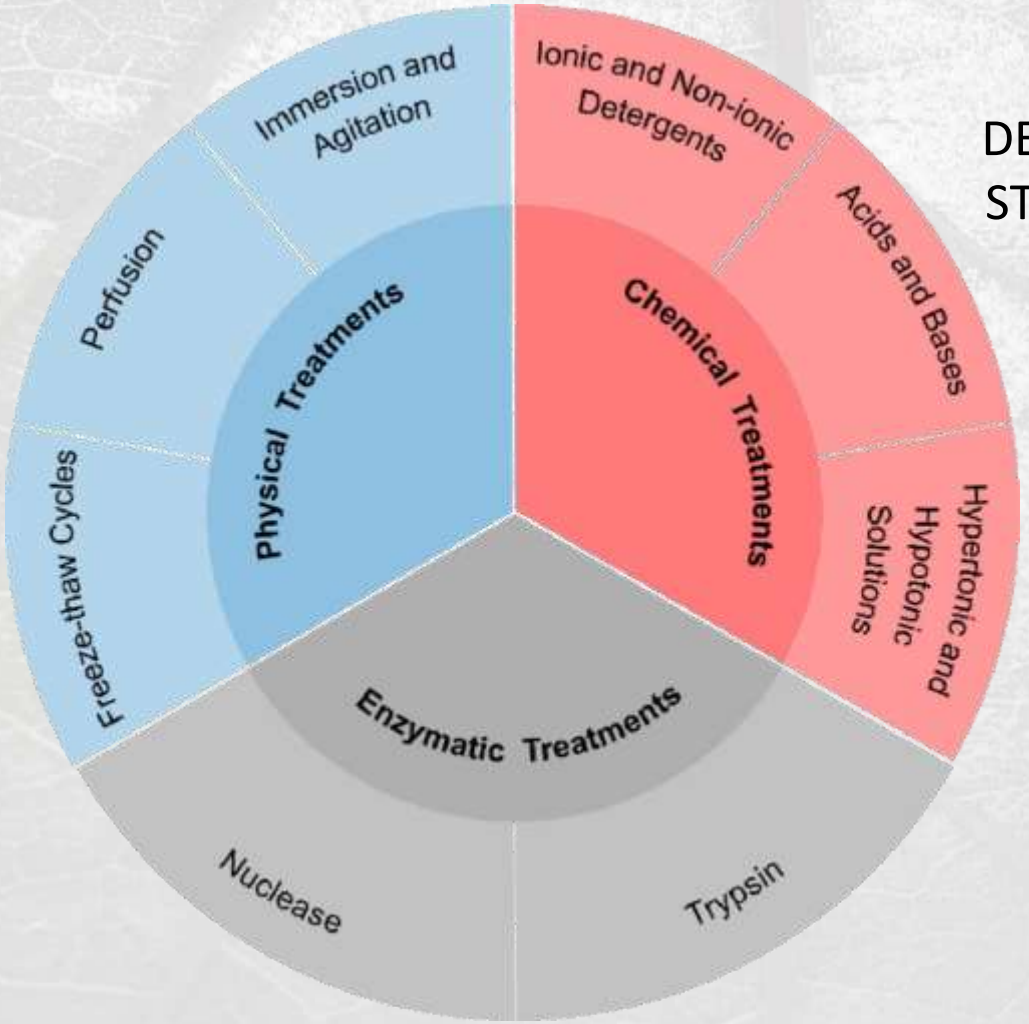

Decellularized intact small intestinal submucosa scaffold
Badylak et al.
Journal of Biomedical Materials Research
Decellularized whole lung scaffolds
Ott et al.
Nature Medicine
Decellularized whole liver scaffolds
Uygun et al.
Nature Medicine
3D printed construct using stem cell-laden decellularized matrix
Jang et al.
Biomaterials
Bioink II
Mesenchymal stem cells (MSCs)
Decellularized whole lung scaffolds
Petersen et al.
Science
Vascular Endothelium
Pulmonary Epithelium
Vascular perfusion
Breathing loop
Medium exchange
Syringe pump
Trachea
First report of decellularization
Poel et al.
Science
1948
1995
2010
2017
Isolation of bssement membrane
Hjelle et al.
Kidney International
1970s
Decellularized whole heart scaffolds
Ott et al.
Nature Medicine
2008
Decellularized whole kidney scaffolds
Song et al.
Nature Medicine
2013
3D biopringting with decellularized matrix
Pati et al.
Nature Communications
Decellularization and solubilization of ECM
Fat
Heart
Cartilage
2014
Proteomic analysis of decellularized pancreas
Asthana et al.
Biomaterials
2021
PROTEOMIC ANALYSIS
Mass Spec
Global
Multiplex ELISA
Targeted

TRIAD: INITIAL ECM MATRIX - TECHNIQUE - CELL LINE



There is no standardized protocol for any type of tissue/organ, however, certain techniques have been observed to be more suitable for specific geometries of the initial samples.

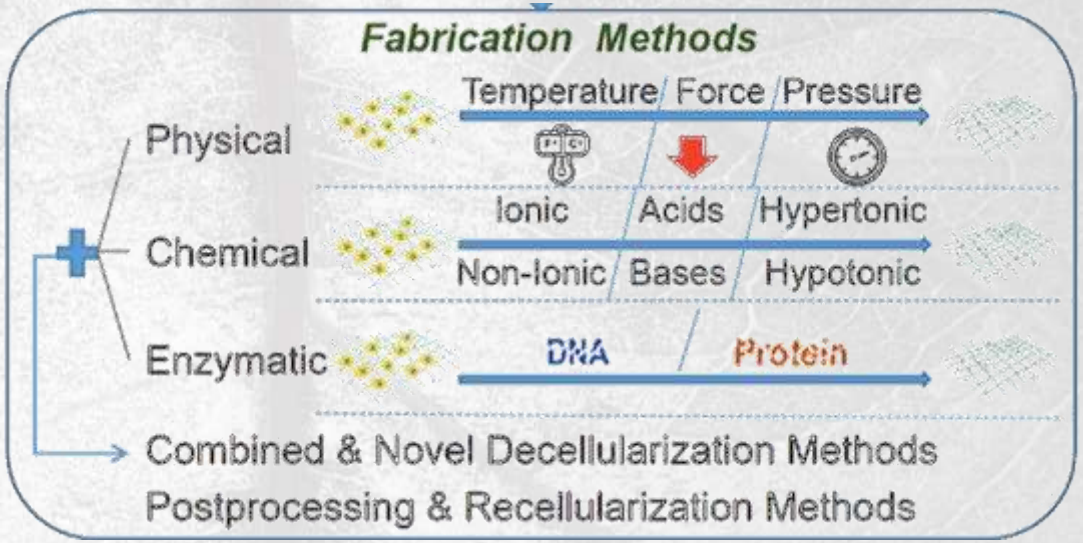
DECELLULARIZATION FLAT STRUCTURES (epithelia, endothelia)



DECELLULARIZATION OF 3D STRUCTURES WITH NATIVE VASCULARIZATION (lungs, liver, kidneys...)

MANDATORY FINAL STEP FOR ANY DECELLULARIZED STRUCTURE

DECELLULARIZATION TECHNIQUES AND AGENTS



1. CHEMICAL METHODS

surfactants

*anionic, cationic, zwitterionic

acidic/basic treatment

2.PHYSICAL METHODS

freeze/thaw cycles

ultrasonication

mechanical stress

3. ENZYMATIC METHODS

trypsin

lipase

nucleases (DNase, RNase)

HYBRID METHODS: combinations of 1/2/3

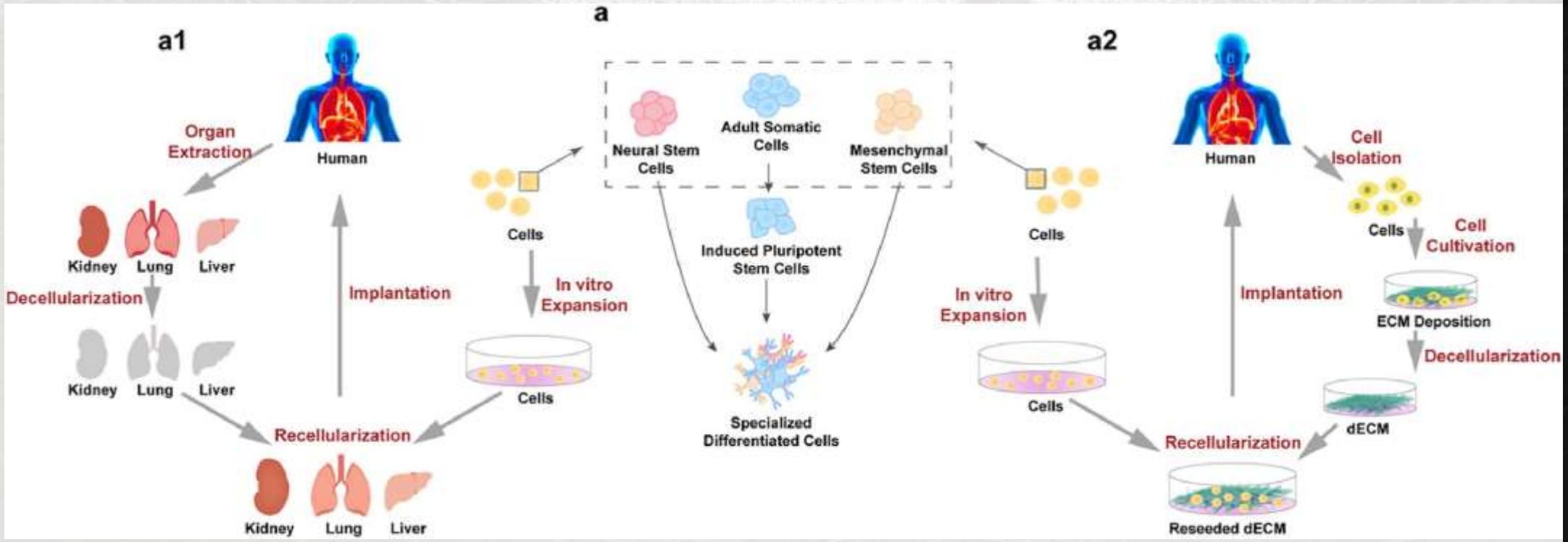
(Very) VARIABLE PARAMETERS

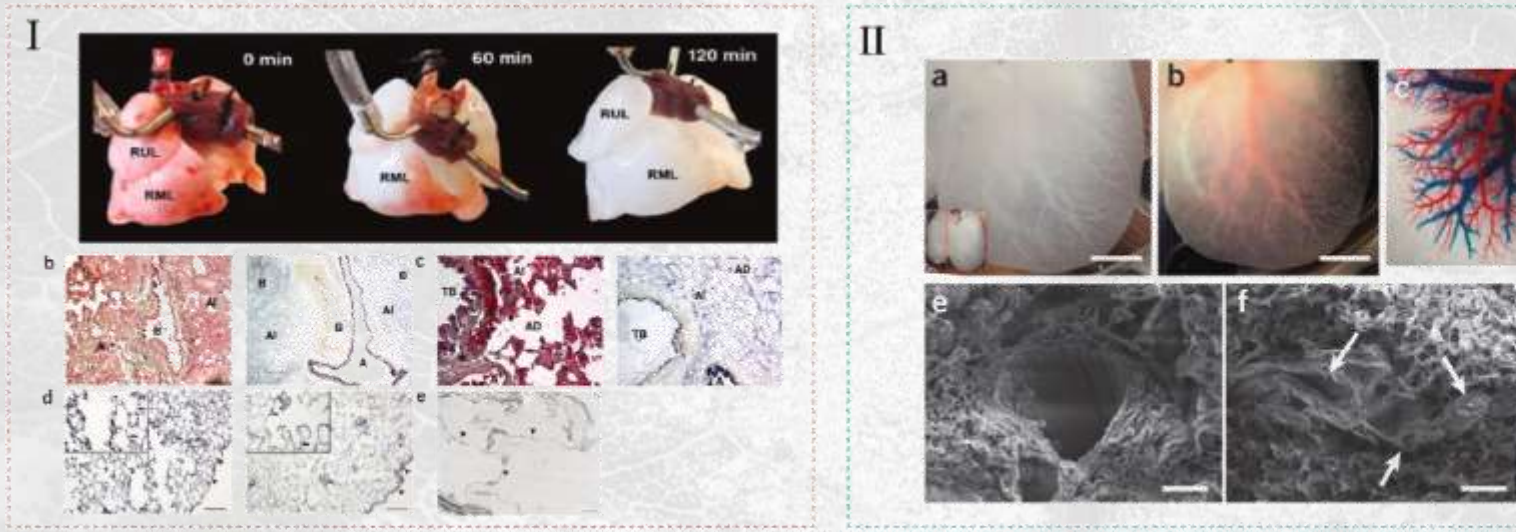
a. working temperature

b. concentration of active agent or intensity of physical stimuli

c. exposure duration

DECELLULARIZATION OF ORGANS / DECELLULARIZATION OF CELL CULTURES



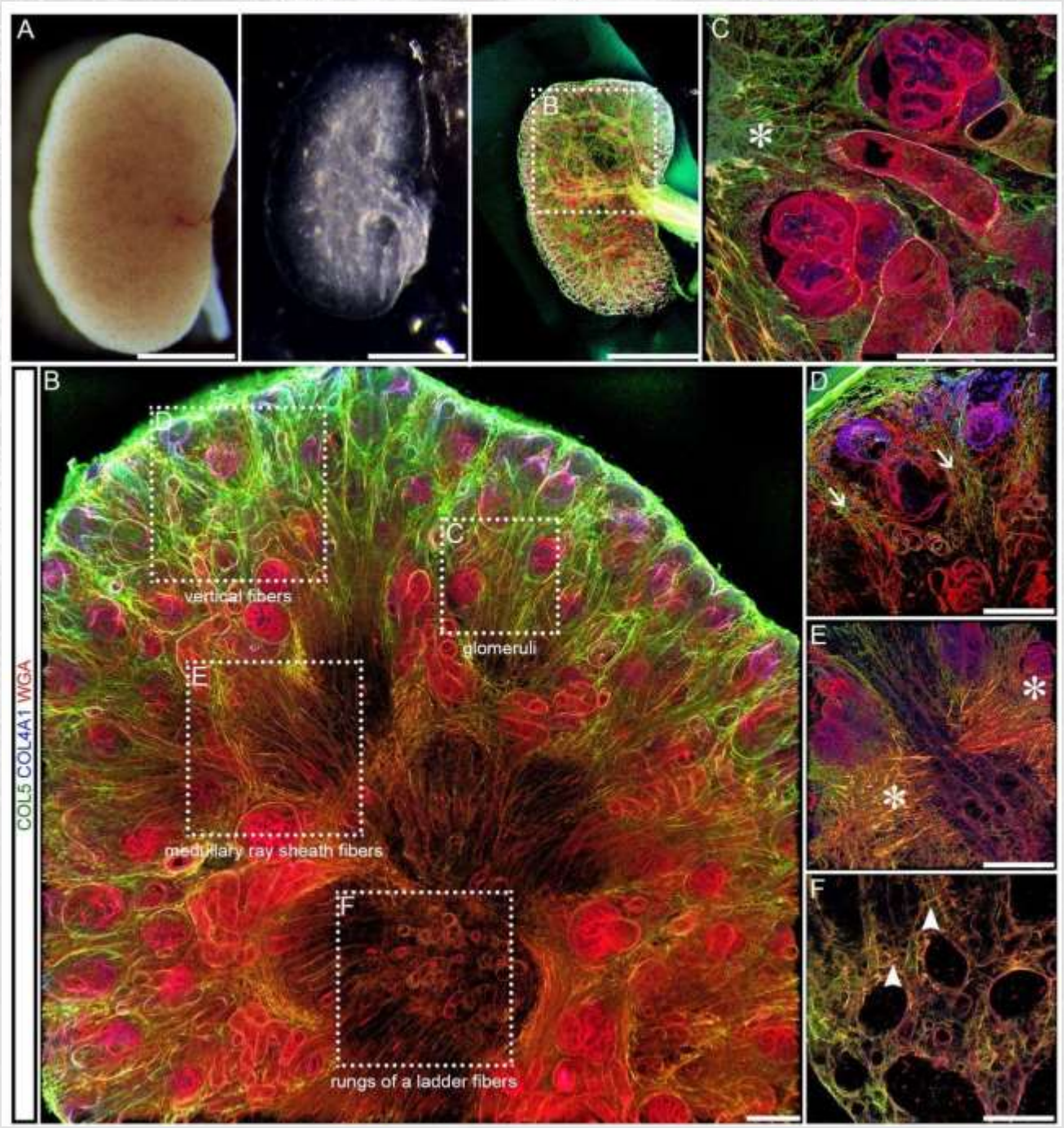


Decellularized ECM for pulmonary, hepatic, and renal tissue engineering

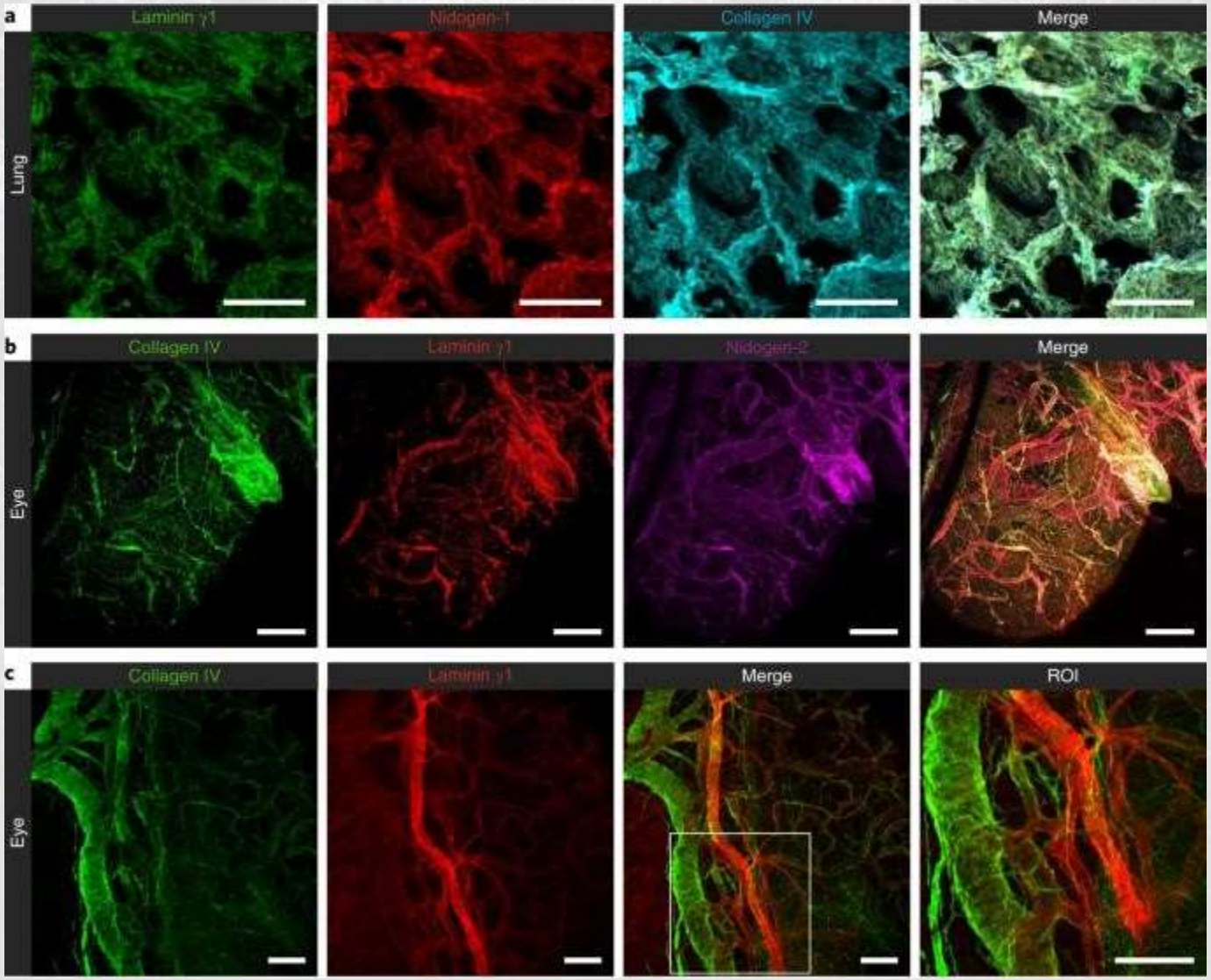
Decellularization helps highlight the ECM structure (kidney model).

(A) Kidneys decellularized in DSS. Colorimetrically labeled matrix for various ECM components: green = COL5; blue = COL4A1; red = WGA (proteoglycans).
(B) Fibers from glomeruli, cortex, and corticomedullary junction are visualized in 3D.
(C-F) Representative confocal images from different kidney areas.

Scale bar: 100µm.



VALIDATION OF THE PROTOCOL THROUGH STAINING TECHNIQUES



https://www.nature.com/articles/s41596-019-0225-8

DECELLULARIZATION CLINICAL STUDIES

Implanted Tissue	Pathology	Type of Scaffolds	Recipients	Follow-up	Findings/Complications	Reference
Trachea	Long-segment congenital tracheal stenosis and pulmonary sling	Decellularized trachea by detergent-enzymatic method + bone marrow mesenchymal stem cells + patches of autologous epithelium	n = 1 (age 10 years)	7 years	- Tracheal stenosis close to anastomoses by 12 months; repeated endobronchial stenting was required - the tissue-engineered trachea remained open, well vascularised, completely recellularised with functional respiratory epithelium - no stem cell-related hematopoietic/bone marrow disease attributable - normal lung function/cough reflex and social/working life	Gonfiotti et al., 2014 [37]
	Long-segment congenital tracheal stenosis and pulmonary sling	Decellularized trachea by detergent-enzymatic method + bone marrow mesenchymal stem cells + patches of autologous epithelium	n = 1 (age 10 years)	2 years	- Functional airways - revascularisation - mild strong local neutrophil response - no evident cytological evidence of epithelium maturation for 1 year - no local biomechanical strength - normal chest CT scan and ventilation-perfusion scan - growth of 11 cm in height - the patient returned to school	Elliott et al., 2012 [38]
					- Restriction of growth within an area of recurrent stenosis	

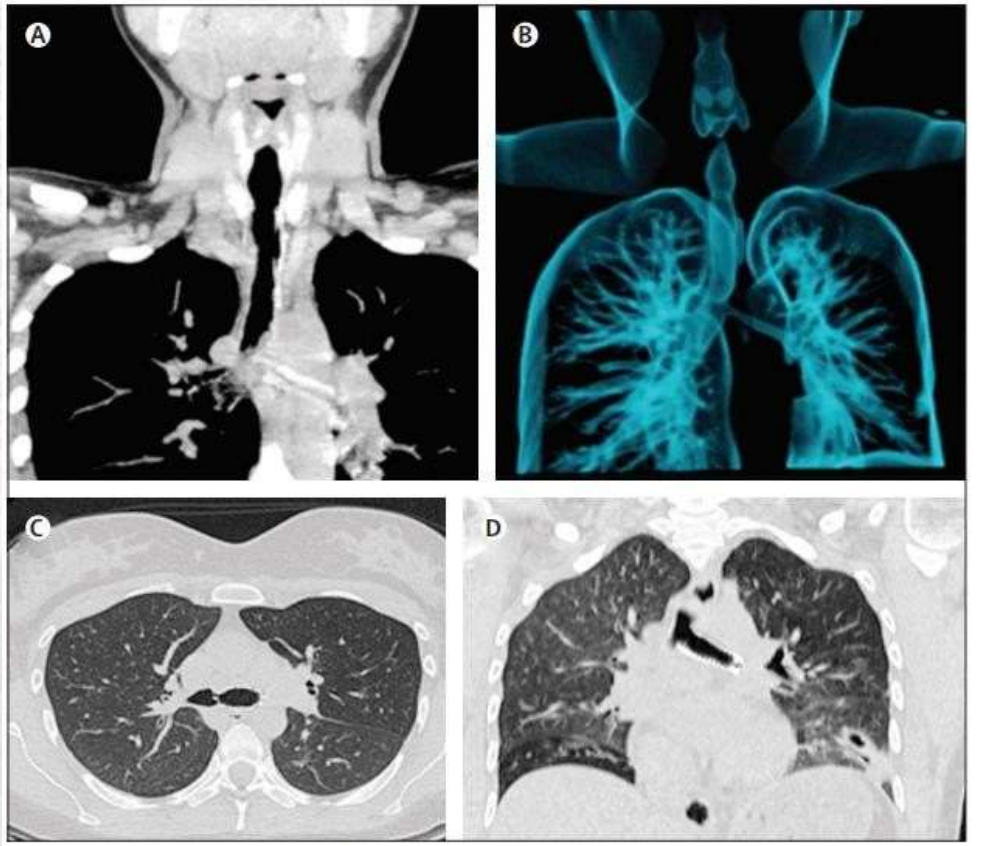
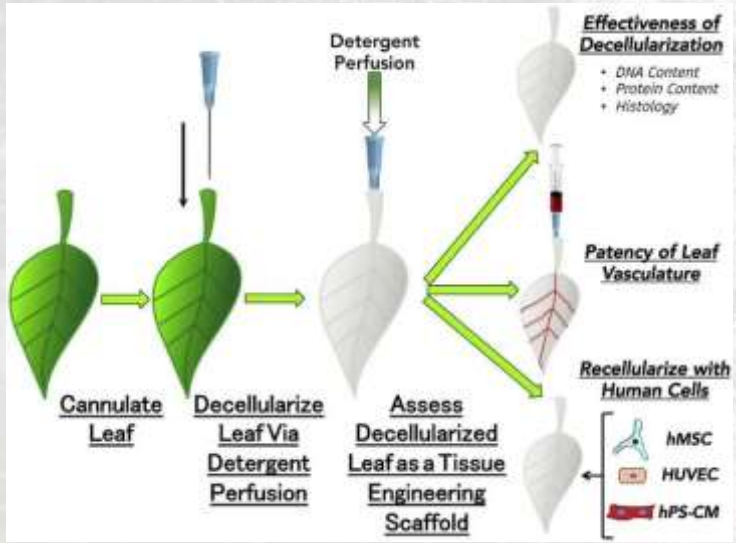
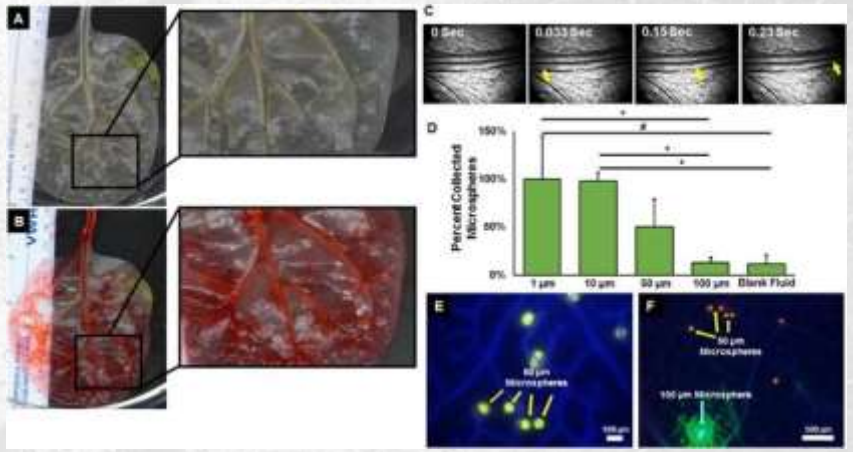


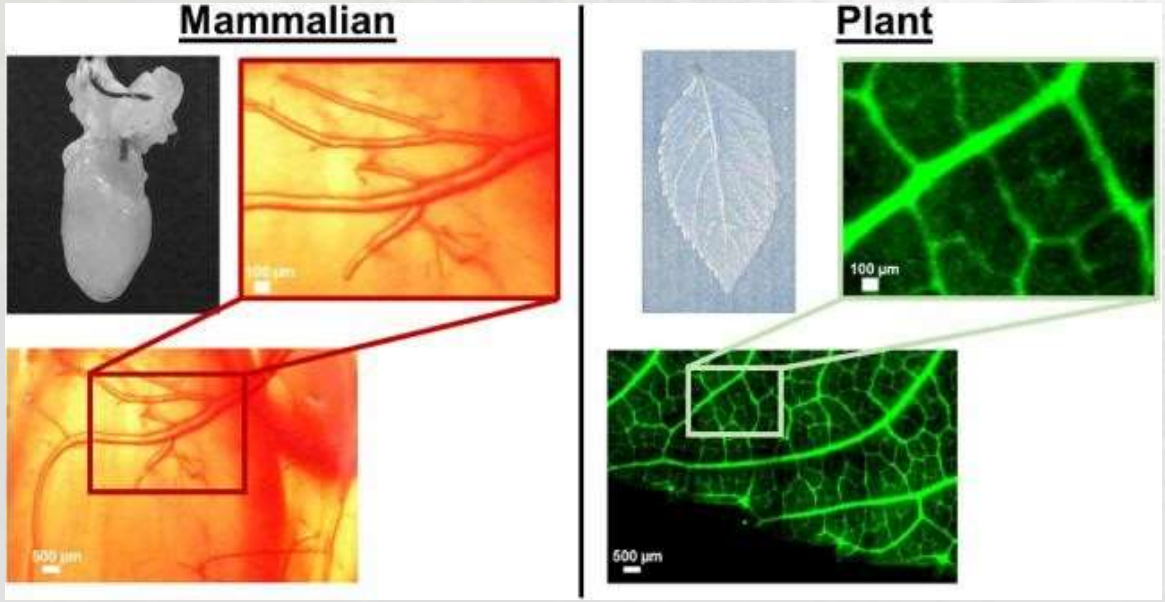
Figure 1: **Imaging findings at 3 years after transplantation**
(A) Multidetector CT scan (June 2011) showing a subtotal cicatricial stenosis of the origin of the left main bronchus, at the level of the proximal anastomosis. (B) 3D reconstruction of the whole graft; distal to the proximal anastomosis, the graft and the distal anastomosis are viable. Axial view (C) and coronal view (D) multidetector CT scan (November, 2011) showing the graft with a metallic Ultraflex stent.

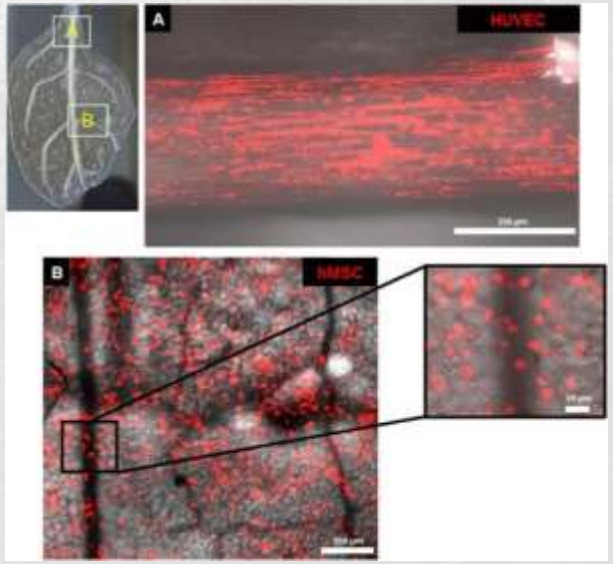
Gonfiotti, Alessandro, et al. "The first tissue-engineered airway transplantation: 5-year follow-up results." *The Lancet* 383.9913 (2014): 238-244.

Decellularization. Plant models









Gershlak, Joshua R., et al. "**Crossing kingdoms:** Using decellularized plants as perfusable tissue engineering scaffolds." *Biomaterials* 125 (2017): 13-22.

[Click here for video // Spinach Leaf Hearts | The Henry Ford's Innovation Nation]

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