Tehnologii de inginerie tisulară pentru regenerarea valvelor cardiace

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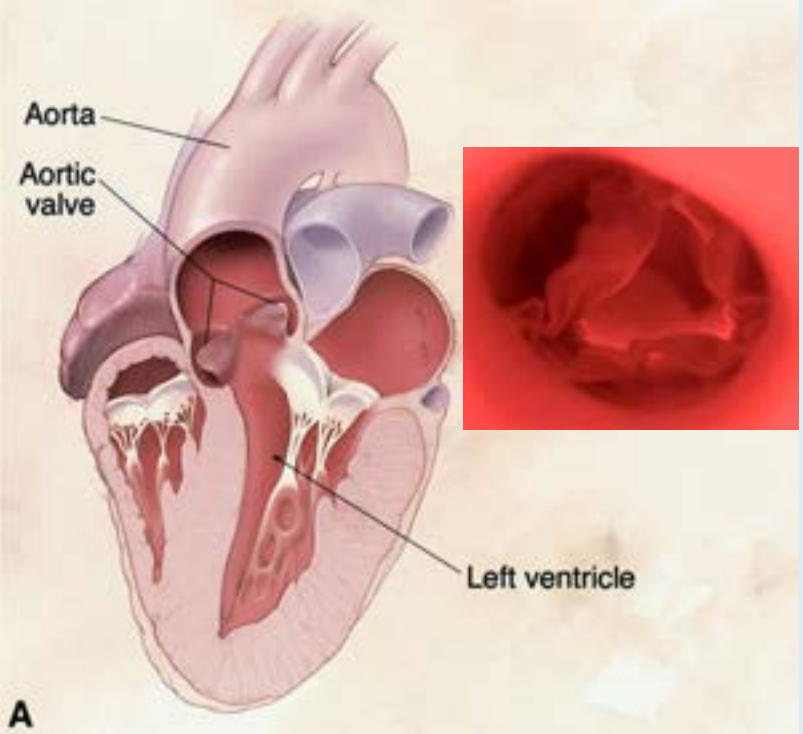
and Collaborators

Workshop: Medicina Personalizata

Research Goal: to regenerate cardiac valves



"...valves are the most mechanically stressed tissues in the body"[1]

- **Continuous performance**
- **Aggressive environment**

Q: Secret to valve durability?

A1: Unique 3D Structure

1. **Matrix components, collagen, elastin**
2. **3D distribution, architecture**

A2: Specific Cells

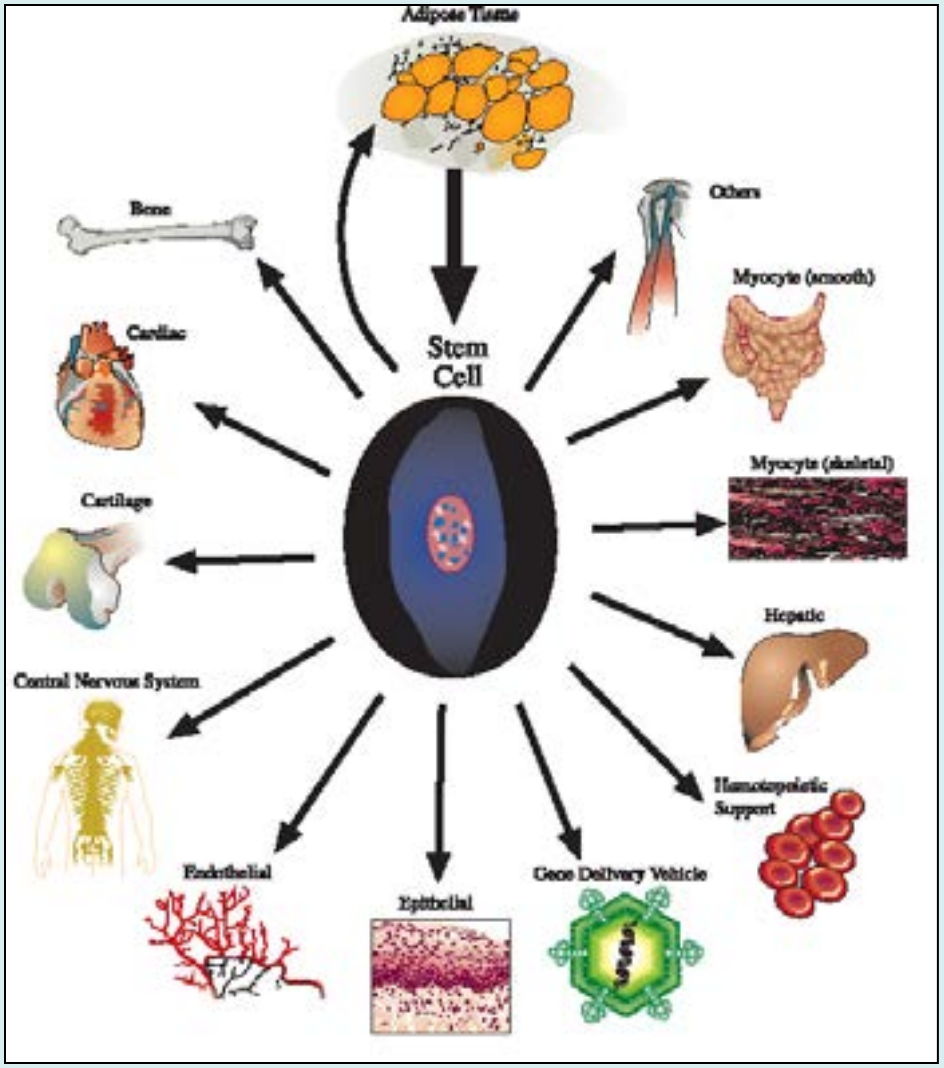
1. **Fibroblasts (VICs), Endothelial cells (VECs)**
2. **Active matrix homeostasis**

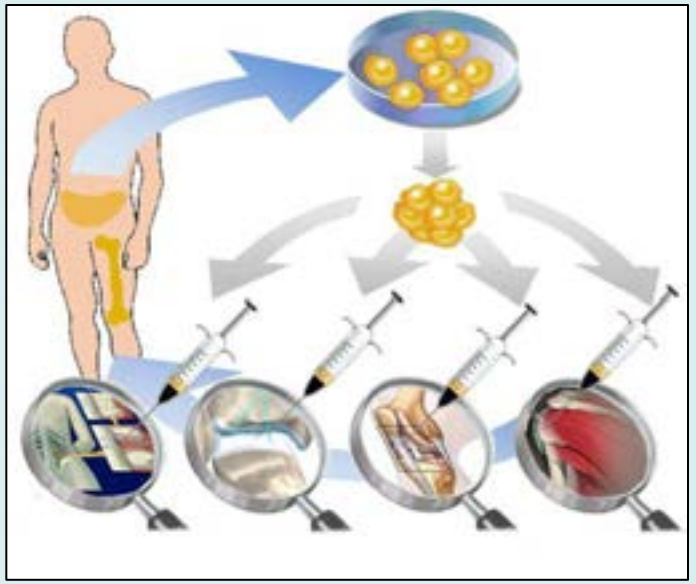
[1]R.A. Nishimura. Aortic Valve Disease. *Circulation 2002, 106:770-772*

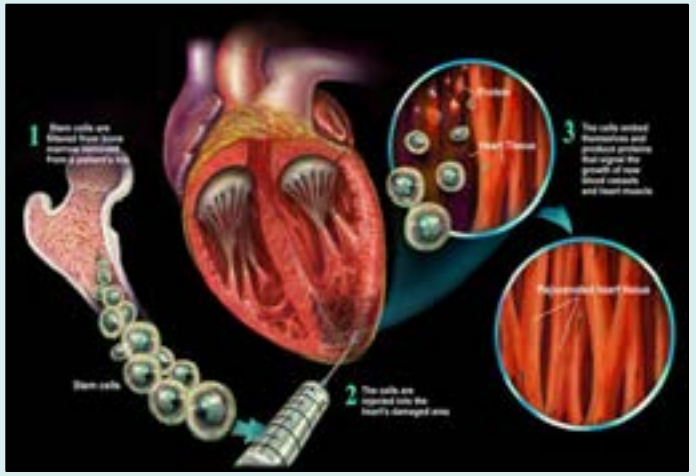
Our Approach

- **3D structure**: use decellularized valves
  - **Eliminates antigenicity, preserves structure**
- **Seed with cells**: use **autologous stem** cells
  - **Differentiated into valve cells**
- **Prepare for implantation**
  - **Dynamic conditioning in bioreactors**

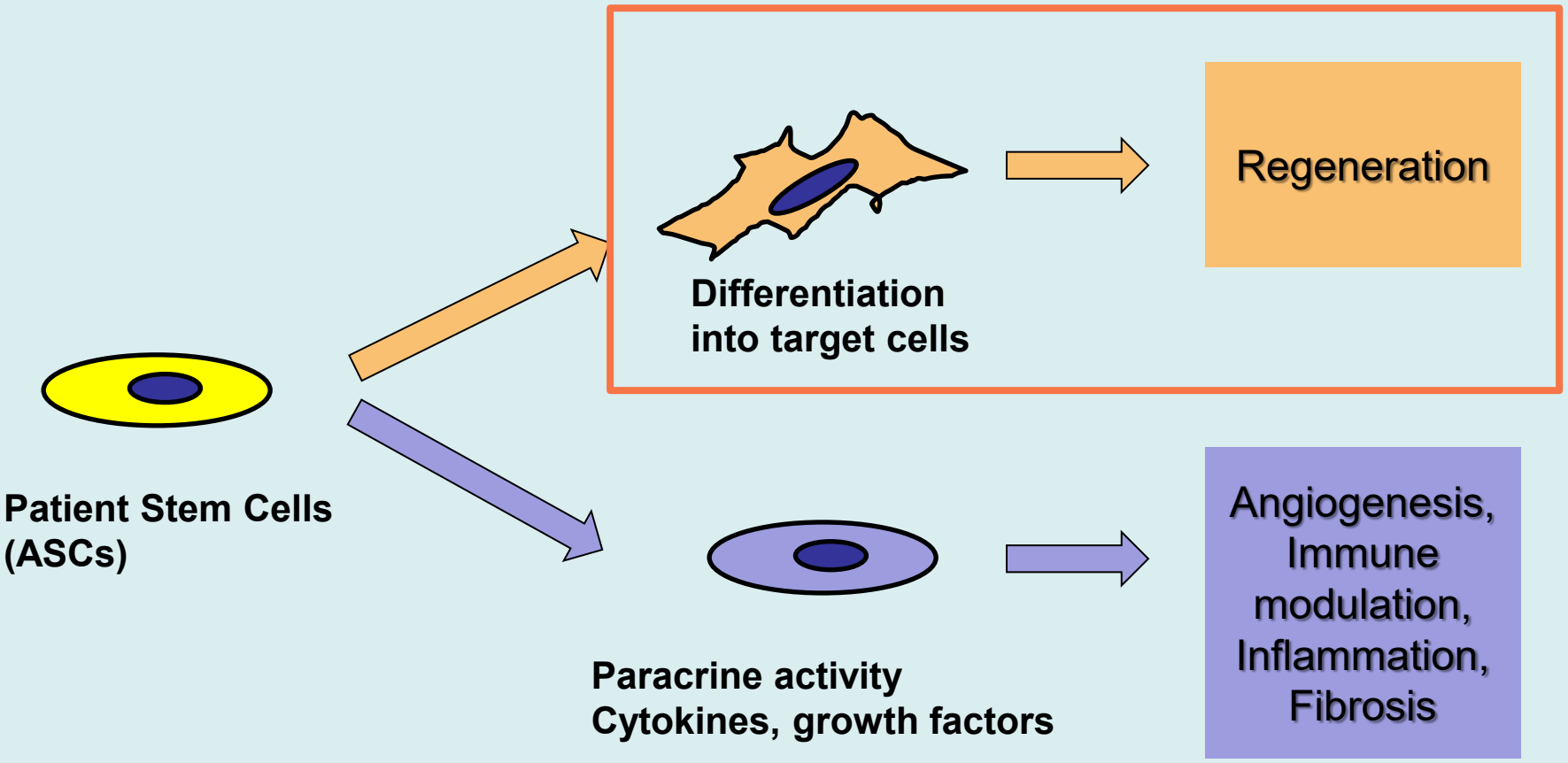
Adult Stem Cells Have Great Potential for Therapy and Regeneration







Adult Stem Cells



Decellularized tissues are safe for human use

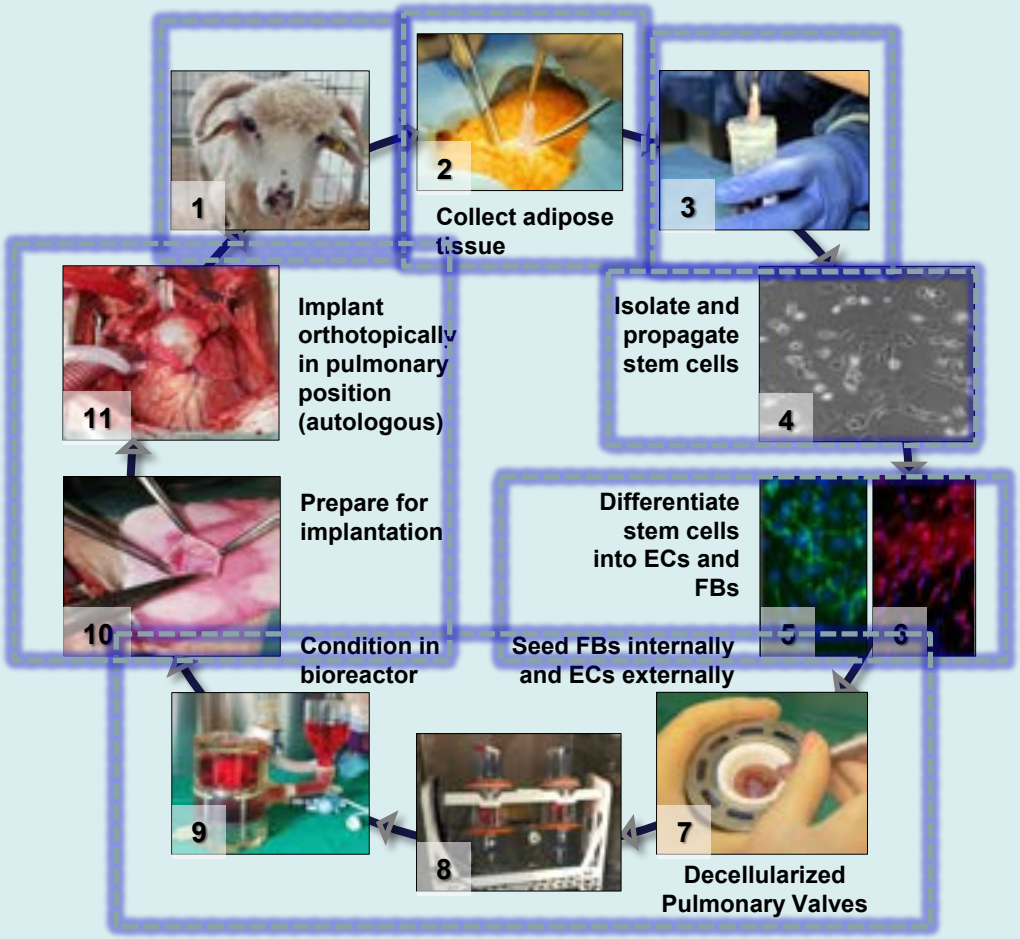
>3 million patients have been implanted with matrix-based, acellular tissues from human, bovine, porcine, equine sources (FDA approved)

Table 1
Source material and form of commercial ECM-based products available for therapeutic applications.

Product	Company	Material	Form
AlloDerm®	LifeCell™	Human skin	Natural
Axis™ dermis	Mentor	Human dermis	Natural
Bard Dermal Allograft	C R Bard	Cadaveric human dermis	Natural
CuffPatch™	Biomet Sports Medicine	Porcine small intestinal submucosa (SIS)	Cross-linked
DuraADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked
Dura-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked
Durasis®	Cook® Medical	Porcine small intestinal submucosa (SIS)	Natural
Durepair®	TEI Biosciences/Medtronic	Fetal bovine skin	Natural
FasLata®	C R Bard	Cadaveric fascia lata	Natural
Graft Jacket®	Wright Medical Tech	Human skin	Natural
Oasis®	Cook® Biotech/Healthpoint	Porcine small intestinal submucosa (SIS)	Natural
OrthADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked
Pelvicol™	C R Bard	Porcine dermis	Cross-linked
Peri-Guard®	Synovis® Surgical Innovations	Bovine pericardium	Cross-linked
Permacol™	Covidien	Porcine skin	Cross-linked
PriMatrix™	TEI Biosciences	Fetal bovine skin	Natural
Restore®	DePuy	Porcine small intestinal submucosa (SIS)	Natural
SurgiMend®	TEI Biosciences	Fetal bovine skin	Natural
Surgisis®	Cook® Medical	Porcine small intestinal submucosa (SIS)	Natural
Suspend™	Mentor	Human fascia lata	Natural
TissueMend®	TEI Biosciences	Fetal bovine skin	Natural
Veritas®	Synovis® Surgical Innovations	Bovine pericardium	Cross-linked
Xenform®	TEI Biosciences/Boston Scientific	Fetal bovine skin	Natural

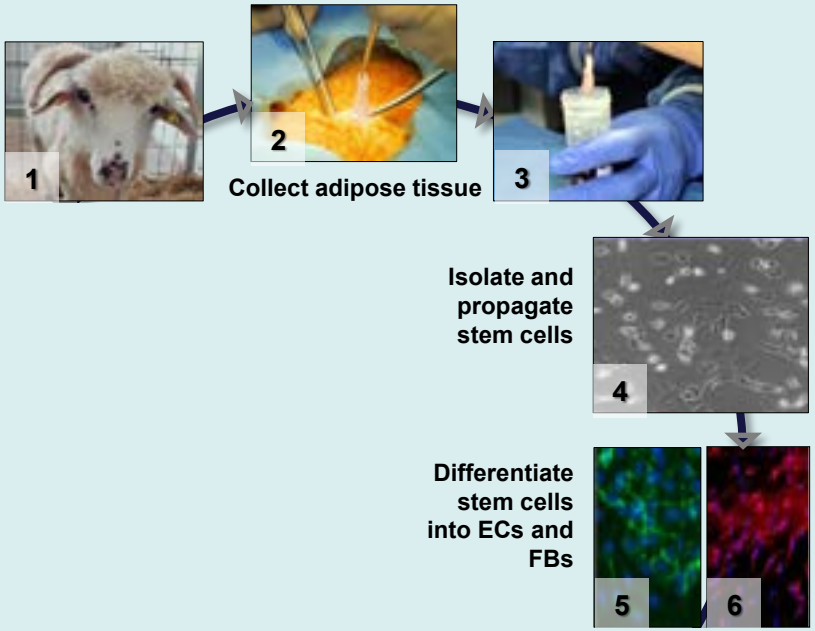
Badylak, 2013; Simionescu et al. Biomaterials, 2009, 2011.

Preclinical Testing of a Proposed *Translational Scenario*



Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015; Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Autologous Adipose-Derived Stem Cells (ADSCs)

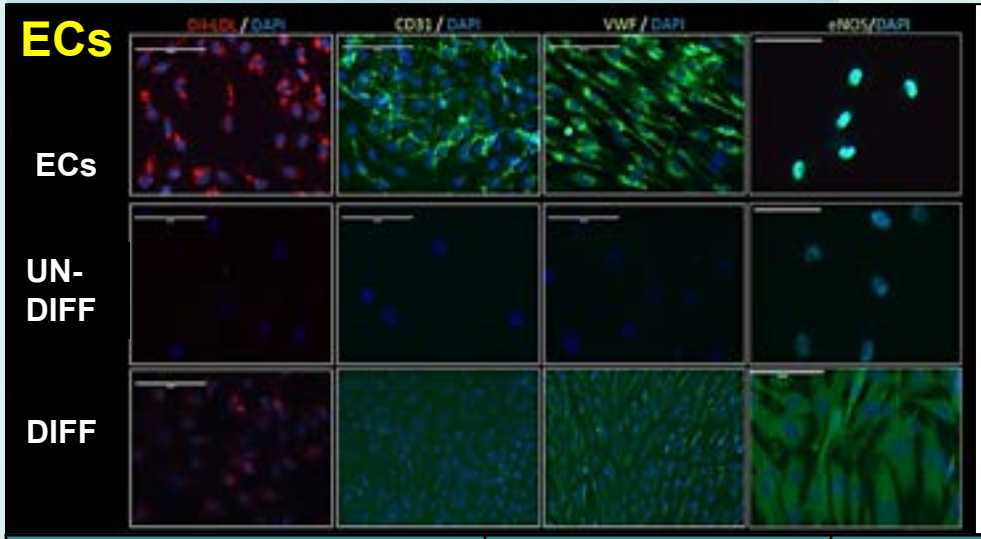


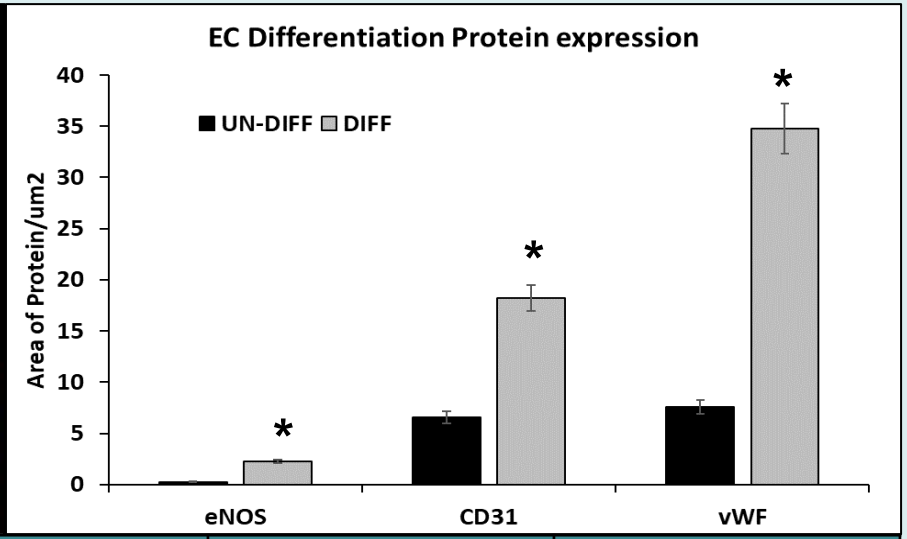
Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015; Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

ADSC differentiation into Endothelial Cells

Method:

- ***ECGS – growth factors + shear strain***
- ***3 weeks***





Human Cells	Ac-LDL uptake	eNOS	CD31	vWF
ECs (HUVECs)	+++	+++	++	+++
UN-DIFF	-	+	+/-	+
DIFF	++	+++	++	+++

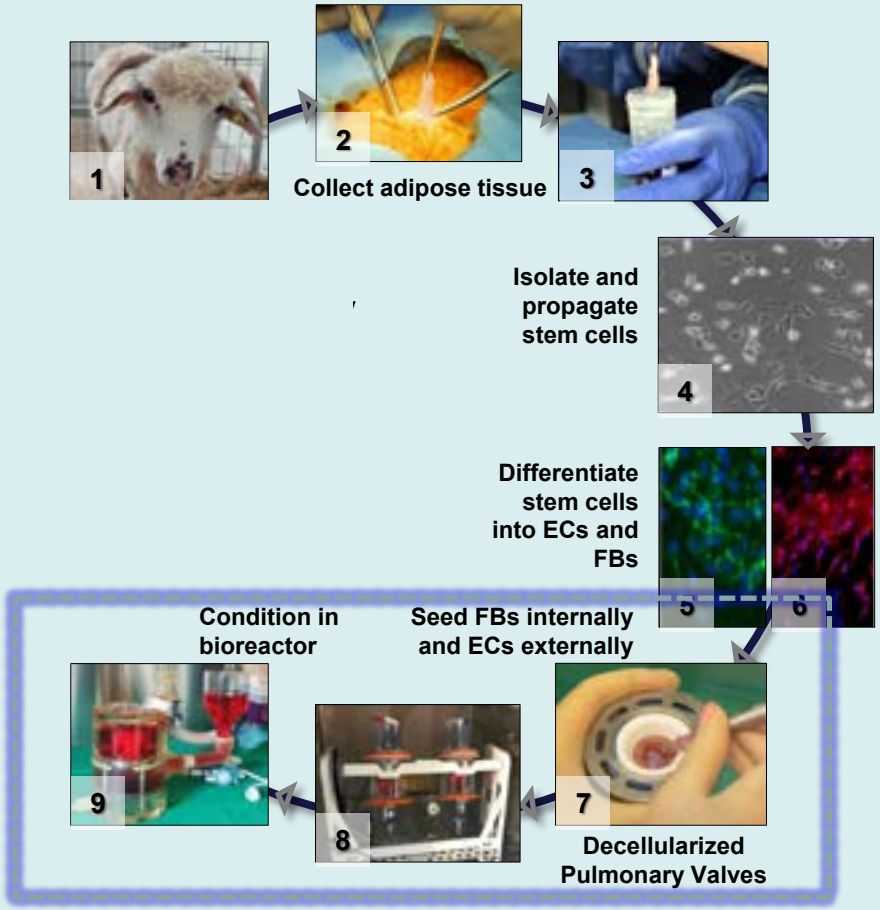
UN-DIFF = undifferentiated ADSCs
DIFF = differentiated ADSCs

Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Take home message #1

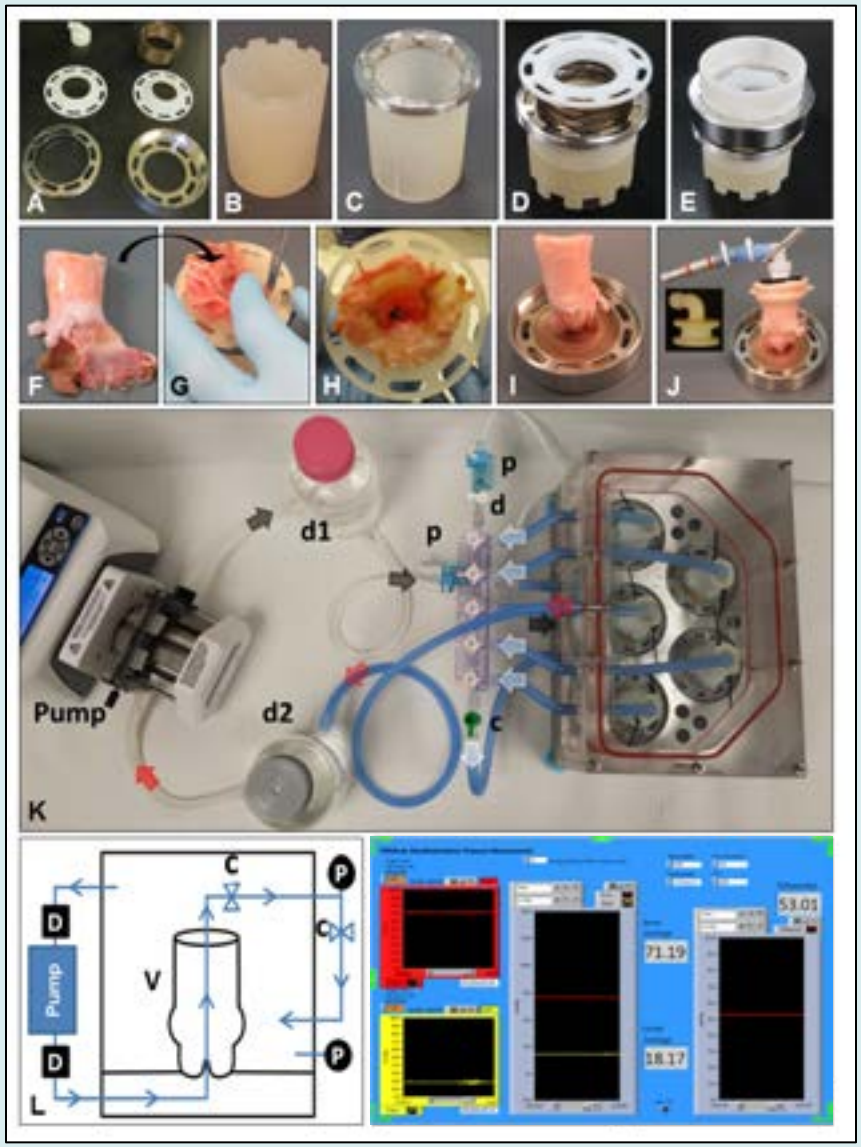
- **Adipose Derived Stem Cells (ADSCs) can be pre-differentiated towards valve cell phenotypes**
- **ADSCs could serve as cell sources for Valve Regeneration**

Valve Decellularization, Seeding, and Bioreactor Conditioning



Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015; Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Perfusion Decellularization (decell) System*



Features:

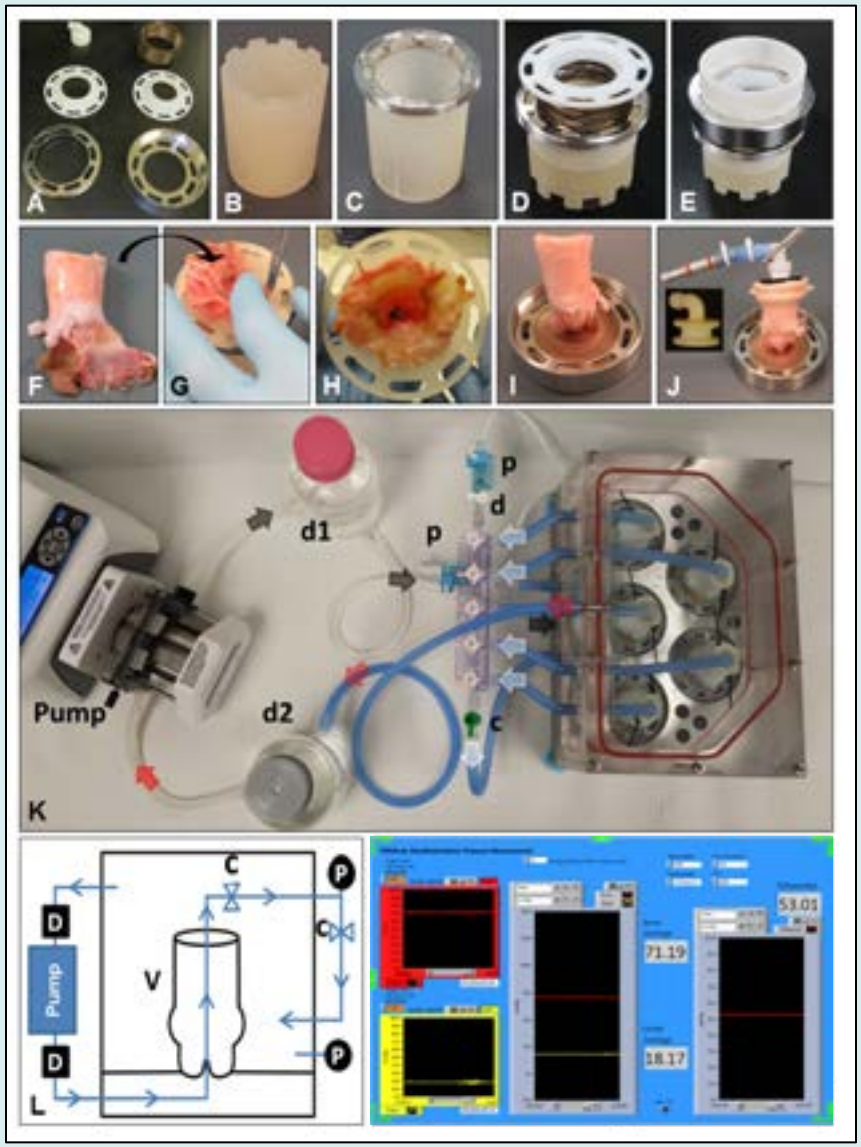
- ***Mounting* system for roots (A-J)**
- **Computerized cyclic *perfusion* system (K) with pressure control**
- **Decell reagents (detergents, nucleases) *flow* through *interior* and *exterior* of root (L)**
- **Entire root *dilates* to ensure wall decell (M)**

Patented; Licensed to Aptus Bioreactors LLC

Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015

Perfusion Decellularization (decell) System

(PDCell, Aptus Bioreactors, LLC)



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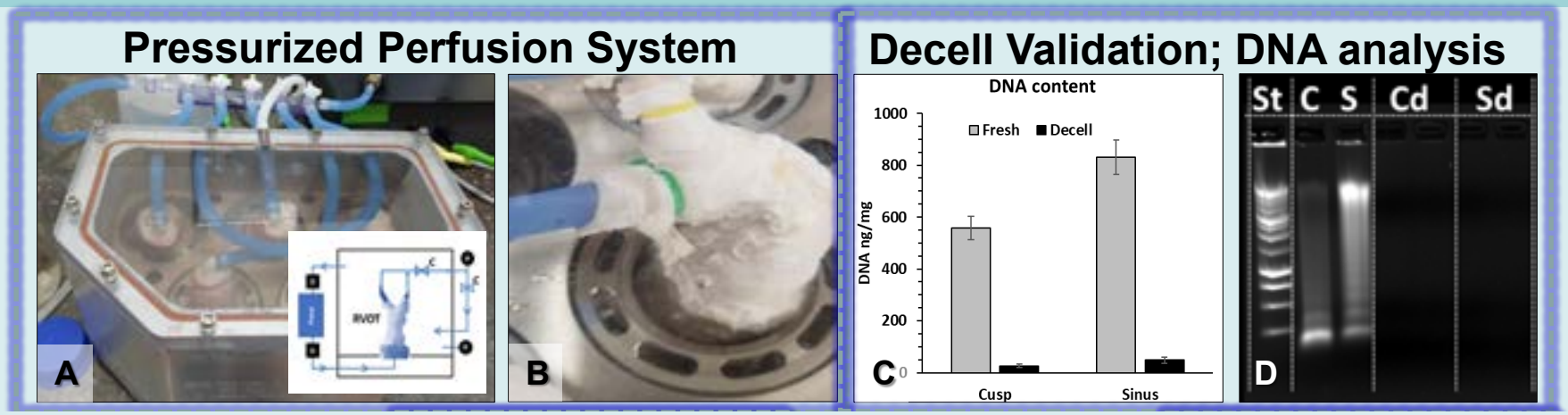
M

Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015

Decellularization of Ovine Pulmonary Valves

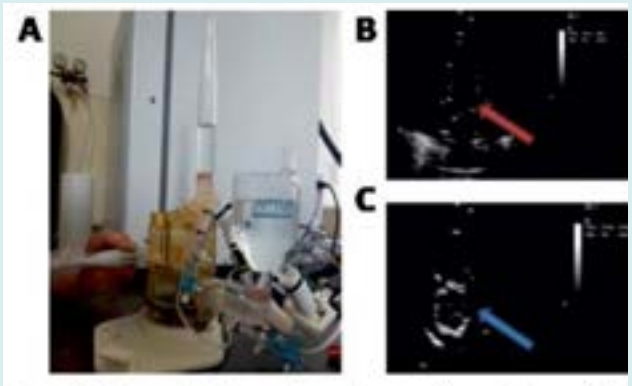


Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

St: DNA standard ladder
C: cusp; S: sinus; d: decell

Hemodynamic and Functional Evaluation of Decellularized Pulmonary Valves

- **Compared** Decellularized PuValves, Fresh PuValves *(Mechanical Valves, BHVs)*
- **Mounted in Aptus BR**, Pu conditions: 20/5 mmHg, stroke volume 60 mL, 70 cycles/min.
- **Ultrasound** - Logiq E, GE, Boston, MA, USA, 4.0 MHz phased array transducer.
- **Top highspeed video camera**, imaging software for GOA.



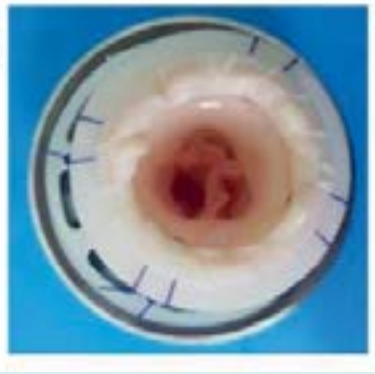


Table 1. Ultrasound examination

Pulmonary conditions	Decelled PuValve	Fresh Pulmonary valve
V max (cm/sec)	328 ± 85	355 ± 25
V mean (cm/sec)	127 ± 28	134 ± 10
P max (mmHg)	55.12 (19.00-60.78)	50.86 (45.98-57.63)
P mean (mmHg)	11.82 ± 5.42	12.26 ± 1.58
VTI (cm)	45.54 ± 9.83	47.46 ± 2.09
Functional area (cm^2)	1.14 (1.07-1.92)	1.24 (1.17-1.29)

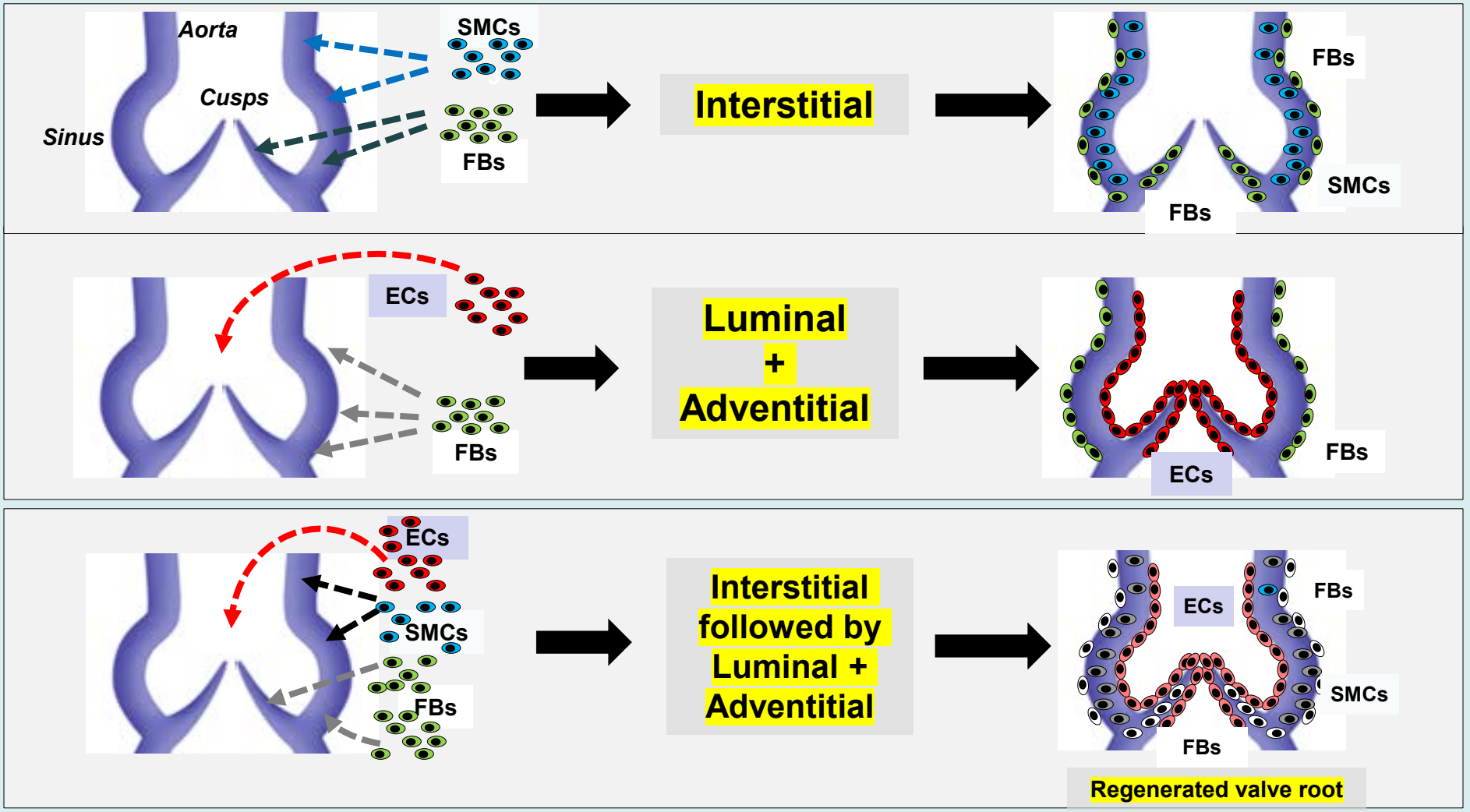
Table 2. Video analysis

	Decelled PuValve	Fresh valve
Normalized pulmonary conditions peak opening area (cm^2)	2.916±0.102	3.459±0.099

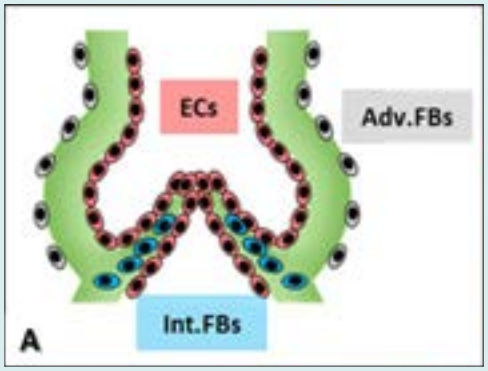
No statistically significant differences in functional valve parameters after decell

Movileanu I., Simionescu A., Simionescu D., et al . (2021). Developing the Tissue Engineered Heart Valve – a Descriptive Hemodynamic and Ultrasound in Vitro Characterization Study of Heart Valves in a Bioreactor. *Romanian Journal of Cardiology*. 31. 555-563. 10.47803/rjc.2021.31.3.555.

Seeding Workplan



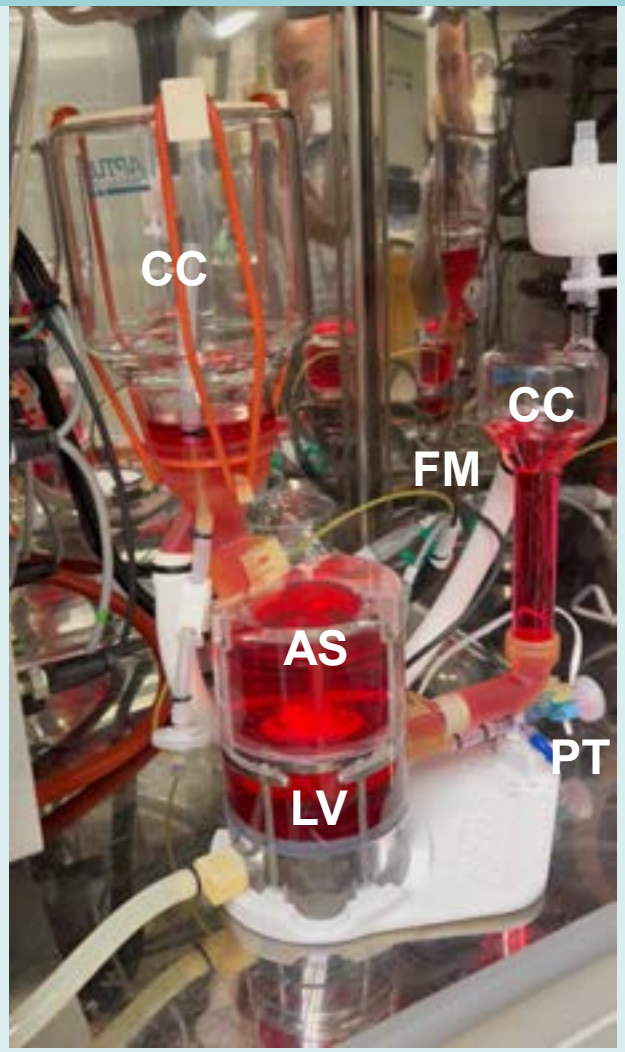
Seeding and Conditioning in Bioreactors

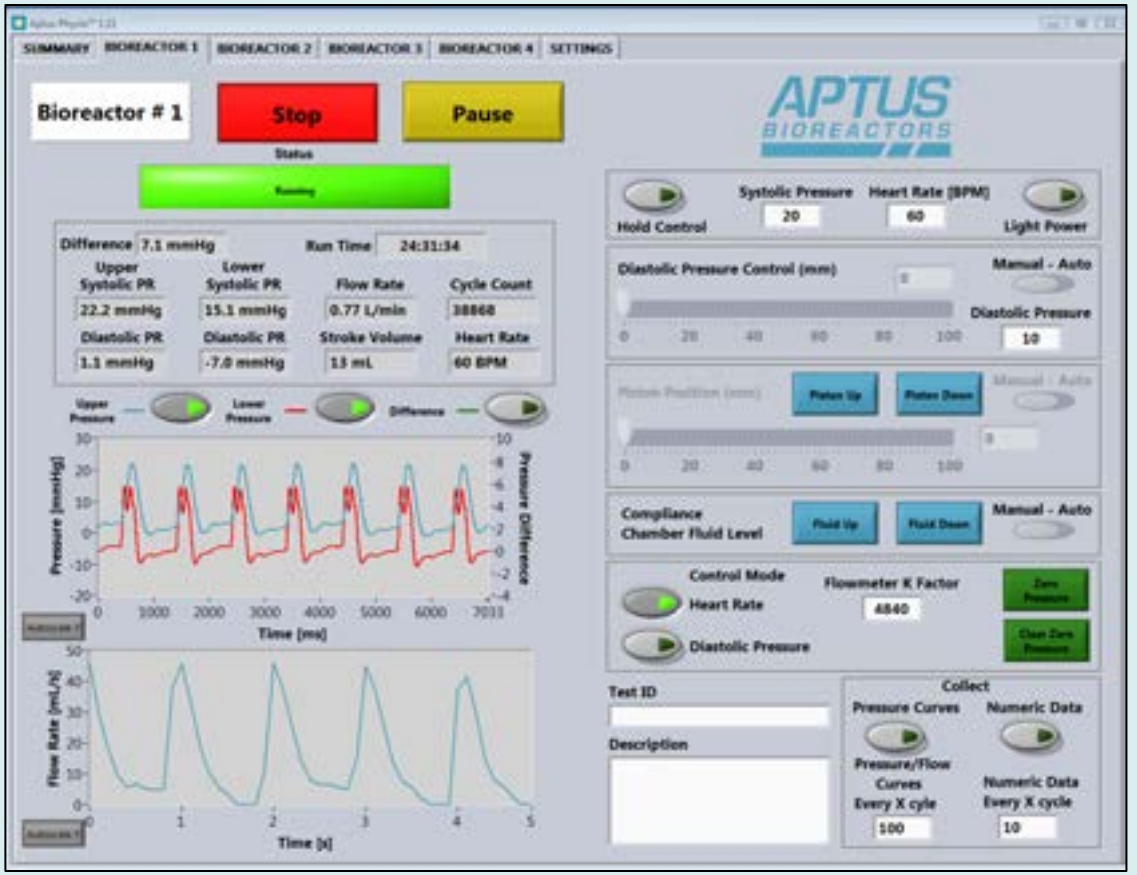


- **Interstitial seeding (FBs)**
- **Adventitial seeding (FBs)**
- **Luminal seeding (ECs)**
- **Pre-conditioning in a rotator device**
- **Progressive adaptation to pulmonary conditions in the heart valve bioreactor for 5 days)**

Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Living Valve in the Sterile Aptus Heart Valve Bioreactor



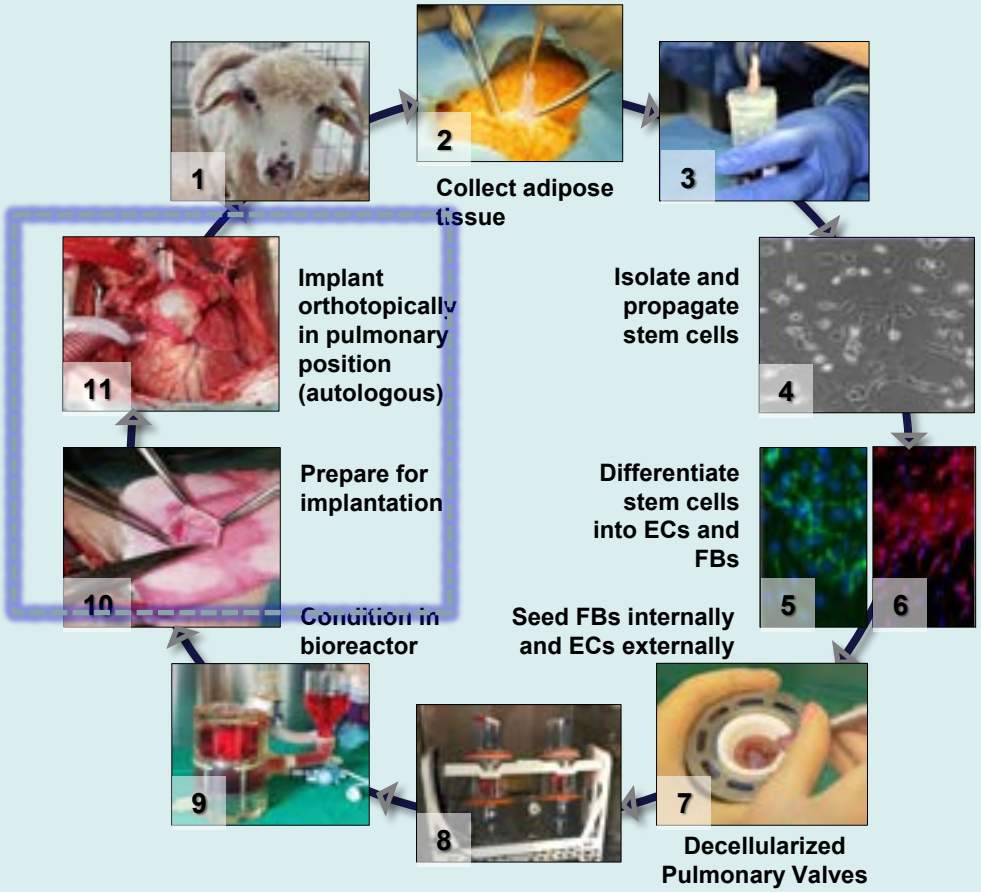


Left **V**entricle, **A**ortic **S**egment, **C**ompliance **C**hamber, **P**ressure **T**ransducers, **F**low **M**eter. (red fluid = sterile cell culture medium)

Take home message #2

- Complete decell of aortic roots = feasible
- ECM integrity = maintained
- Biomechanics/hemodynamics = preserved
- Re-cell is feasible = but challenging
- Rotators and Bioreactors facilitate construct conditioning

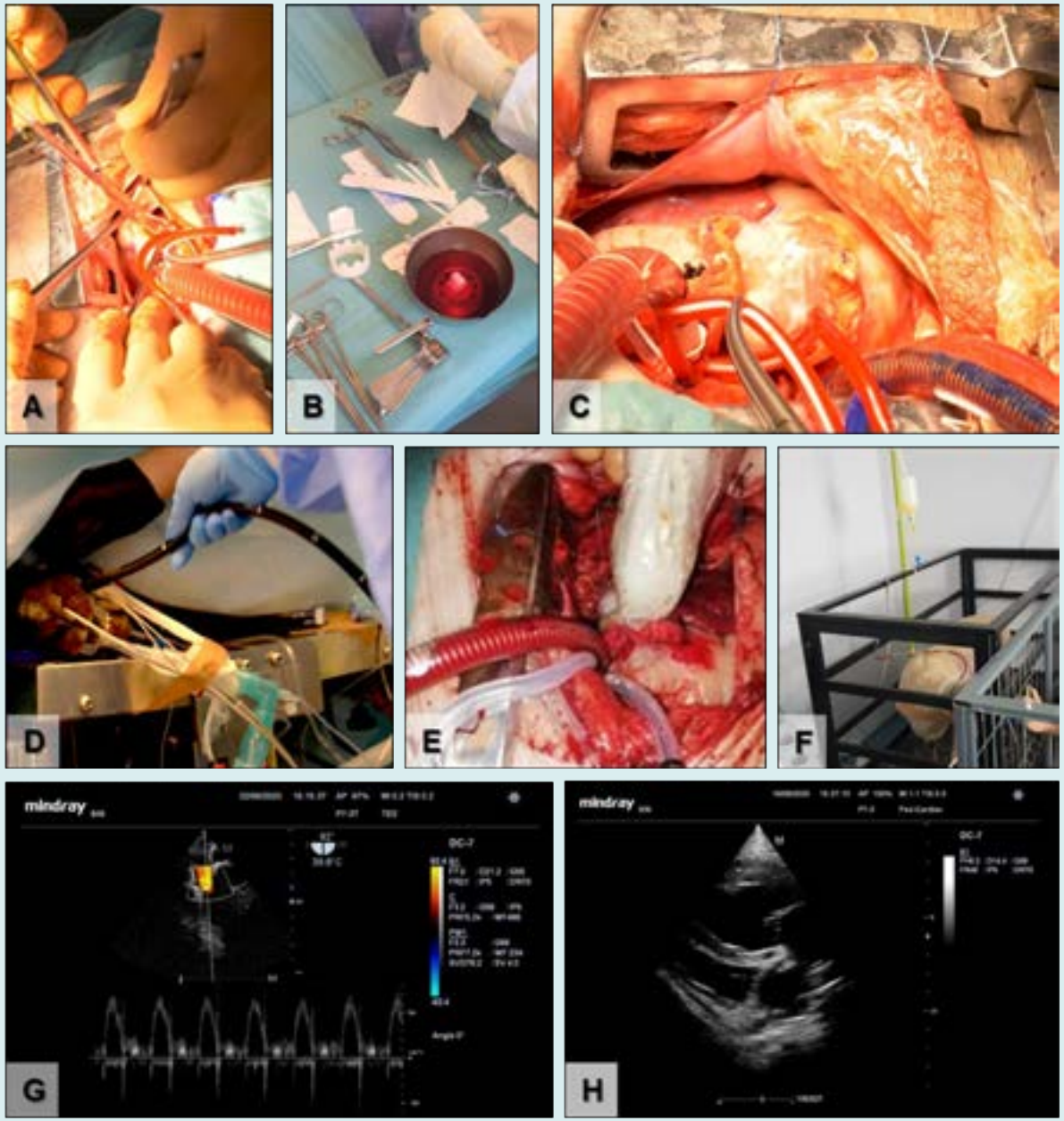
Preclinical Testing of a Proposed *Translational Scenario*



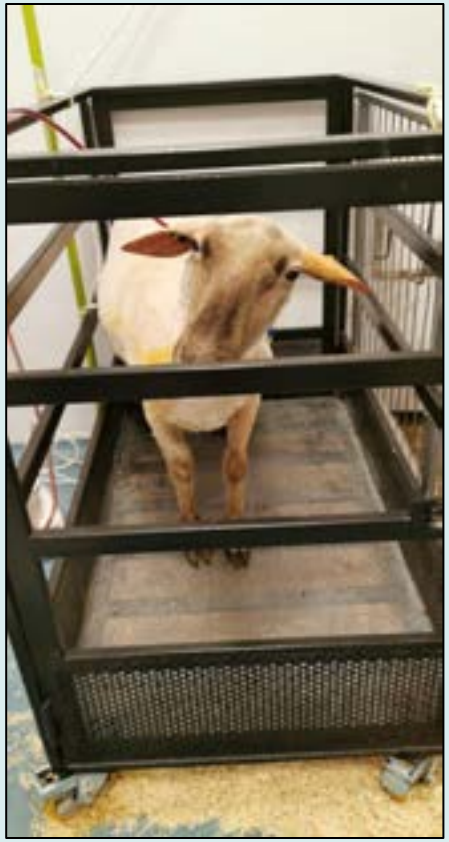
Harpa, Simionescu et al., RRML, 2015; Sierad, Simionescu et al., TE Part C, 2015; Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Implantation

- Sheep ~18 months
- Randomized to unseeded acellular valve controls n=6
- Cell-seeded acellular valves n=6 (with autologous cells)
- Cardiopulmonary Bypass (CBP), orthotopic implant pulmonary position (RVOT)
- 3.5 hrs. surgery
- Average 70 min CPB
- Intra-op epicardial echocardiography
- Post-op trans-thoracic echo



Simionescu, et al. 2021, Frontiers Cardiovasc. Med.



Post-op recovery, stabilization

Follow-up for 6 months
Weight gain ~28 kg

Monitored by echo

[1] Al Hussein, H., Simionescu, D. *et al.* Challenges in Perioperative Animal Care for Orthotopic Implantation of Tissue-Engineered Pulmonary Valves in the Ovine Model. *Tissue Eng Regen Med* **17**, 847–862 (2020). https://doi.org/10.1007/s13770-020-00285-1

TABLE 1 | Echocardiographic evaluation of the TEHVs.

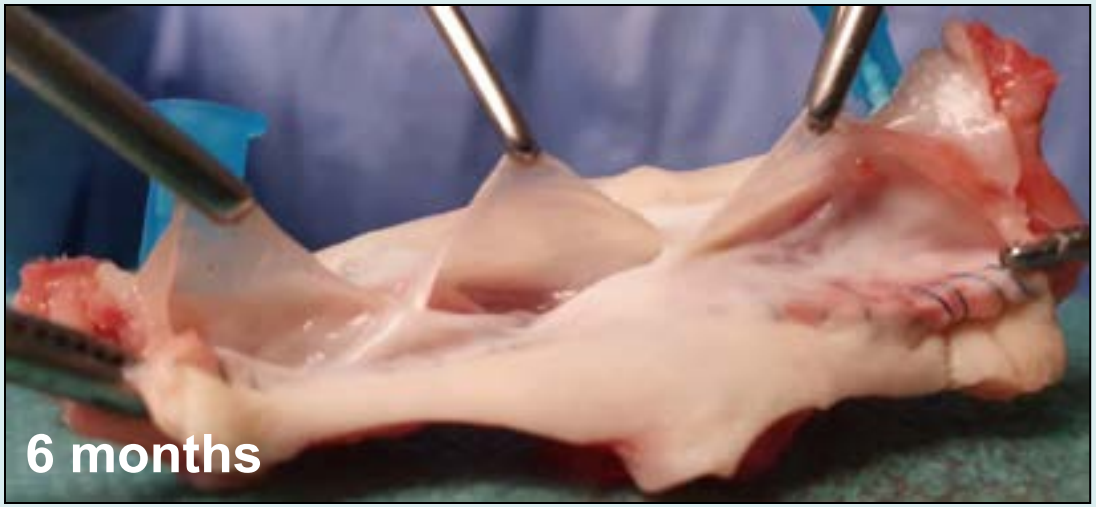
Timeline	Initial evaluation—at implantation			End of the follow-up evaluation		
Animal #	**Right and left heart morphology and function**	**TEHV morphology and function**	**Trans- TEHV maximum velocity (m/s)**	**Right and left heart morphology and function**	**TEHV morphology and function**	**Trans- TEHV maximum velocity (m/s)**
Group 1 - control TEHVs						
#1	Normal size and function	Normal function	0.5	Normal size and function	Trivial regurgitation	0.7
#2	Normal size and function	Normal function	0.8	Normal size and function	Moderate regurgitation	0.5
#3	Normal size and function	Normal function	0.7	Dilatation of right ventricle	Important regurgitation	0.7
#4	Normal size and function	Normal function	0.6	Normal size and function	Normal function	0.6
#5	Normal size and function	Normal function	0.5	Normal size and function	Normal function	0.7
#6	Normal size and function	Normal function	0.8	Normal size and function	Normal function	0.7
	Mean +/- SEM		**0.65+/- 0.13**	**Mean +/- SEM**		**0.65+/−0.08**
Group 2 - cell seeded TEHVs						
#1	Normal size and function	Normal function	0.7	Dilated right ventricle	Important regurgitation	0.5
#2	Normal size and function	Normal function	0.8	Normal size and function	Normal function	0.7
#3	Normal size and function	Normal function	0.5	Normal size and function	Moderate regurgitation	0.6
#4	Normal size and function	Normal function	0.6	Normal size and function	Normal function	0.6
#5	Normal size and function	Normal function	0.7	Dilated right ventricle and pulmonary artery trunk	Hyper-echogenic aspect of the TEHV with impaired opening of the cusps	2.4
#6	Normal size and function	Mild regurgitation	0.7	Normal size and function	Mild regurgitation	0.7
	Mean +/- SEM		**0.66 +/- 0.10**	**Mean +/- SEM**		**0.91 +/−0.73**

Statistical analysis revealed no significant difference between the mean maximum trans-TEHV velocity between the two groups at all time points ($p = 0.06$).

No statistically significant differences in functional valve parameters after 6 months

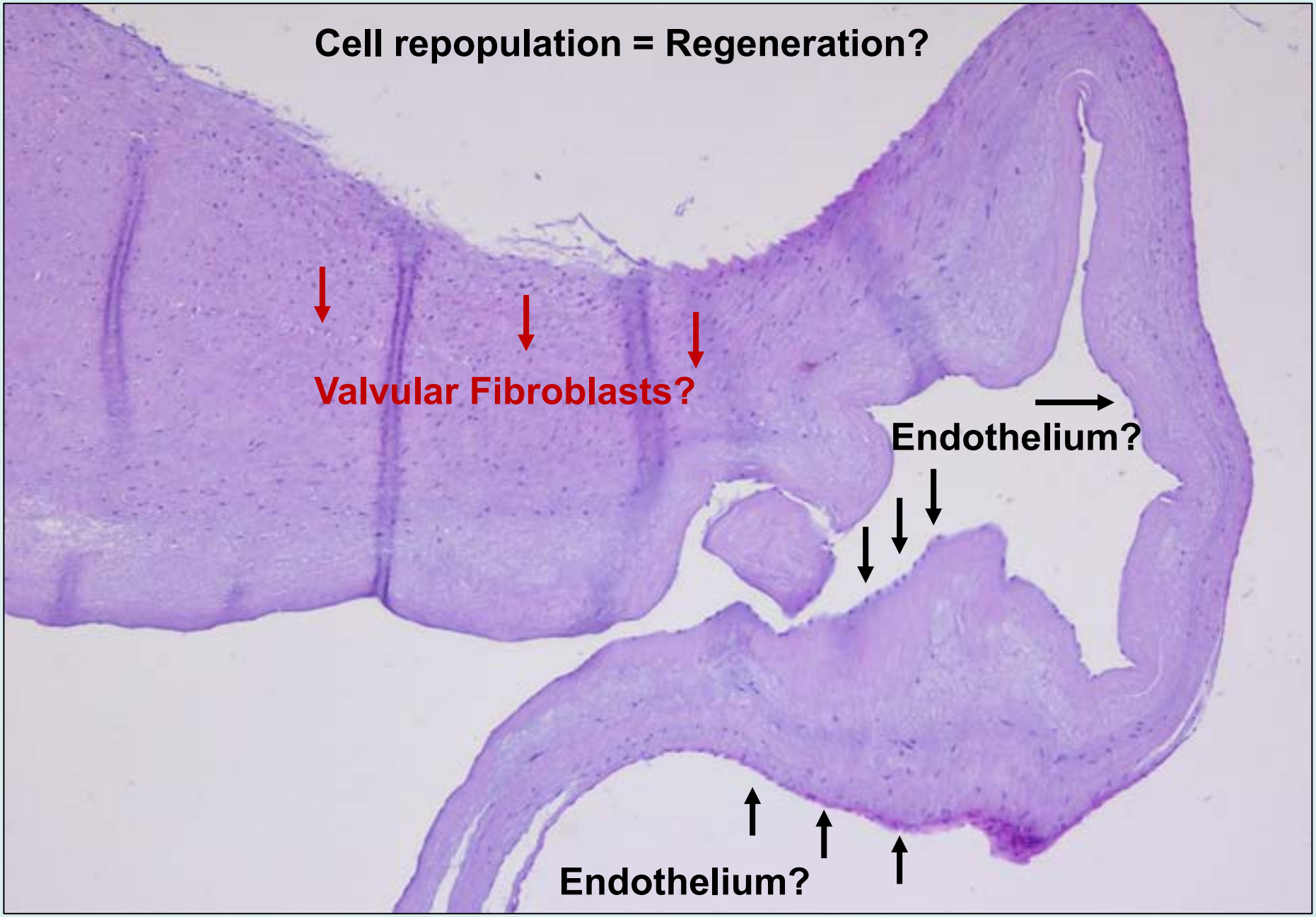
Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Explant Analysis

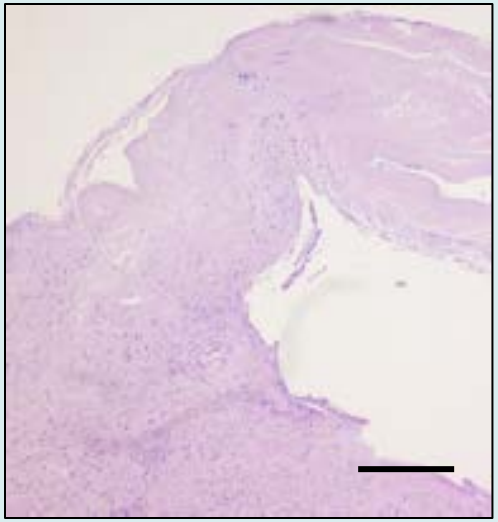


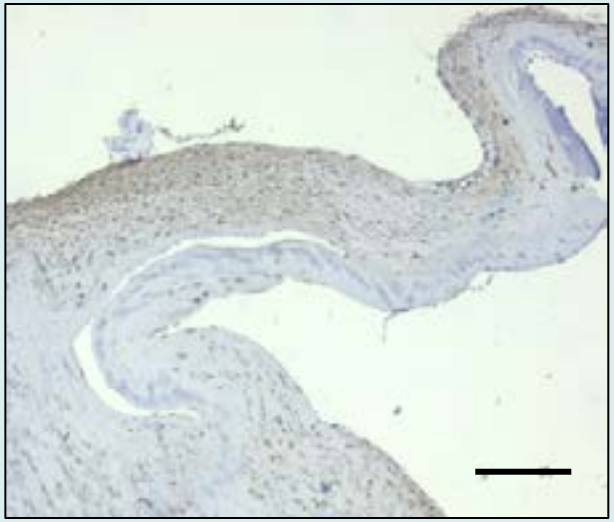
- Anastomoses intact
- No thrombus
- No pannus overgrowth
- Leaflets – supple, thin
- No leaflet fibrosis
- No calcification
- No inflammation
- No signs of immune rejection

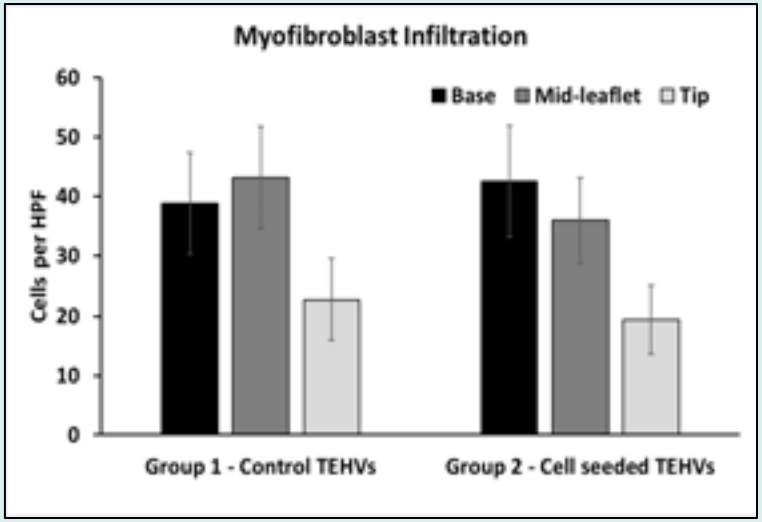
Simionescu, et al. 2021, Frontiers Cardiovasc. Med.


Cell repopulation = Regeneration?
Valvular Fibroblasts?
Endothelium?
Endothelium?

Histology Results







- **H&E shows cell infiltration, mostly in cusp base, fibrosa, spongiosa**
- **Most cells were positive for a-SMC actin by IHC**

- *More IHC staining needed*

Simionescu, et al. 2021, Frontiers Cardiovasc. Med.

Conclusions

Heart valve regeneration is possible by combining:

- ✓ **Acellular valve scaffolds –** *non-immunogenic, preserved structure and hemodynamics*

with

- ✓ **Autologous stem cells:** *differentiated into endothelial cells, fibroblasts*

and with

- ✓ **In vitro seeding and conditioning** *within rotators and bioreactors*

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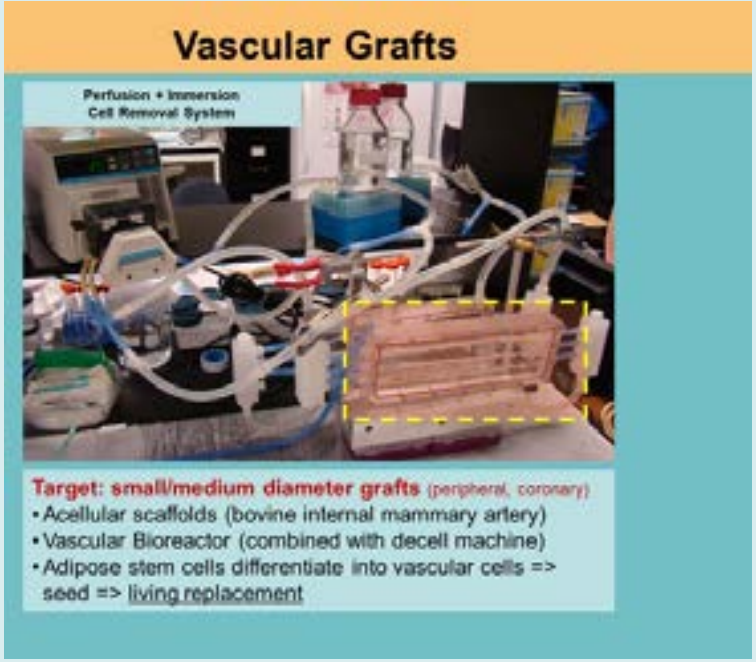
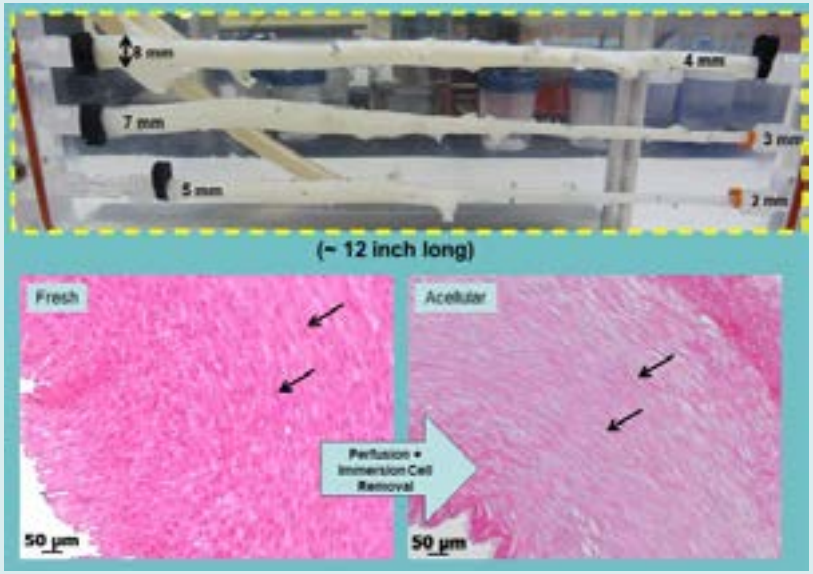
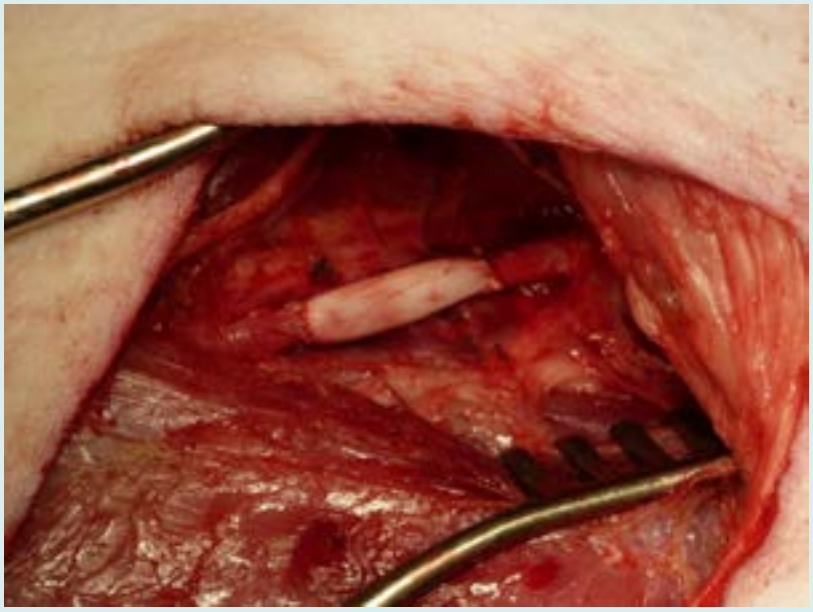
- ✓ **Validation by implantation of autologous cell-seeded valves as orthotopic implants**

Other projects using a similar approach

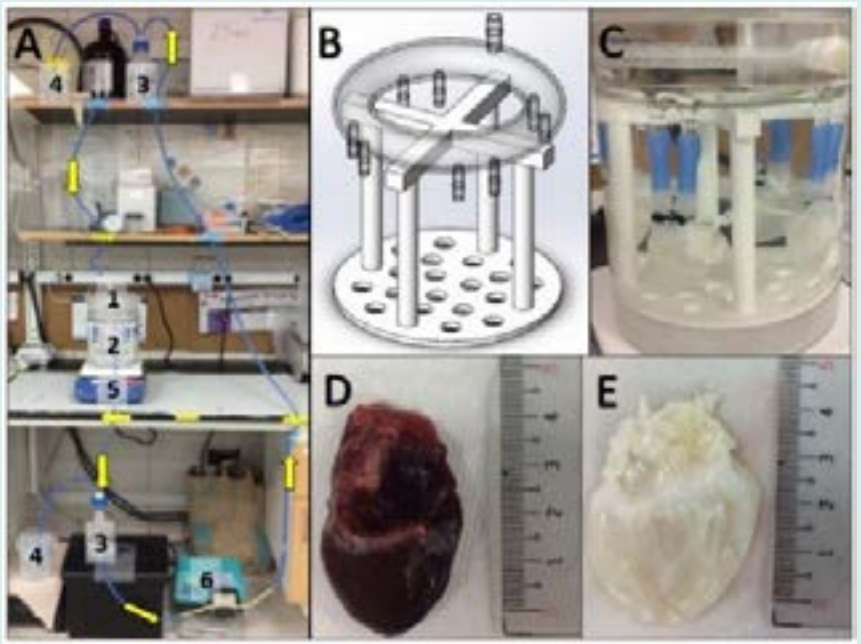
Vascular Grafts

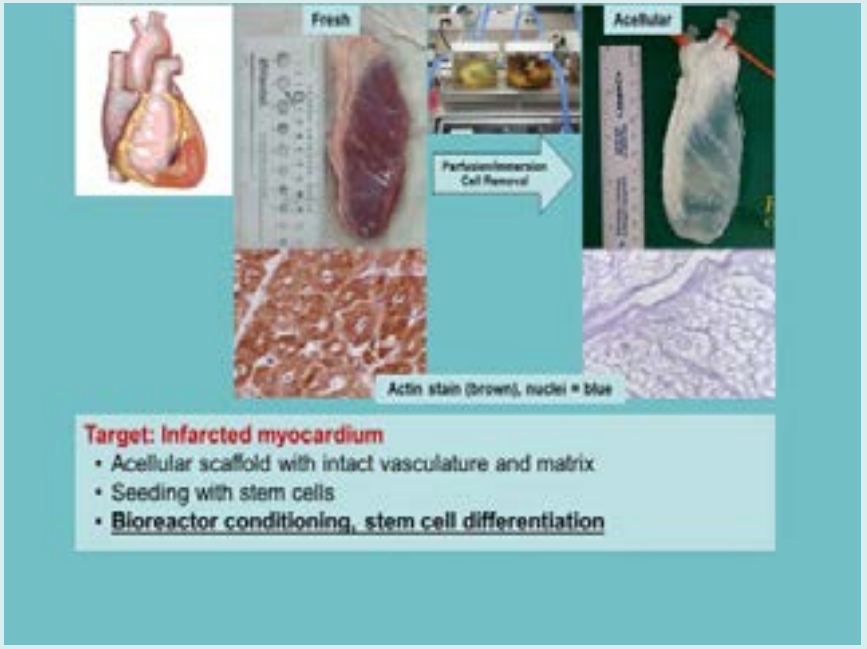


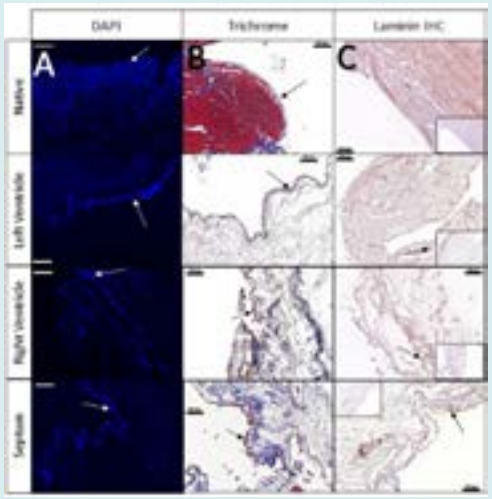




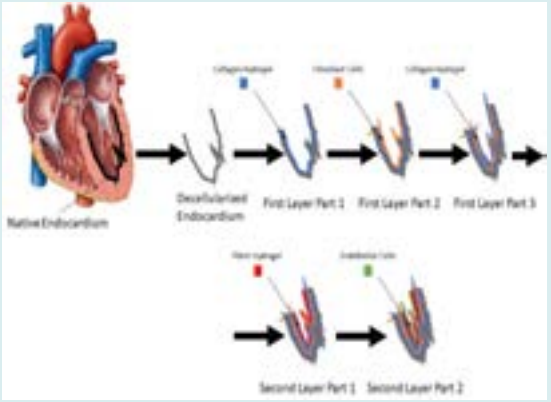
Myocardial Regeneration

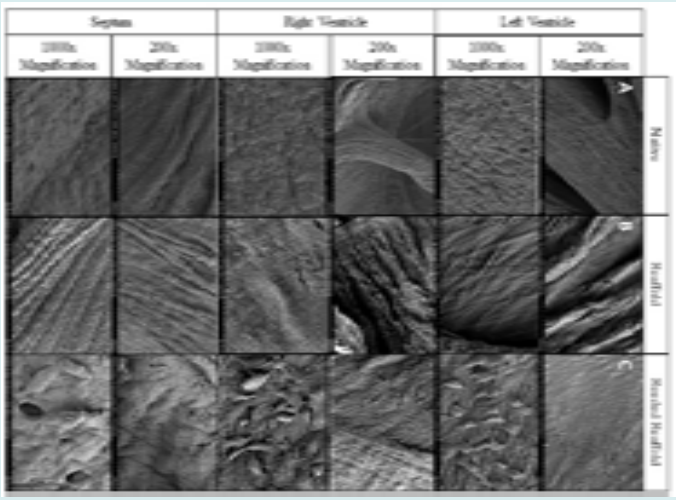






Endocardial Regeneration





Compton, C., Simionescu, D. *et al.* Reconstitution of the Ventricular Endocardium Within Acellular Hearts. *Regen. Eng. Transl. Med.* **6**, 90–100 (2020).

Acknowledgements

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