



POLITECNICO
MILANO 1863



2nd Centro 3R Annual Meeting

3Rs in Italian Universities

June 20 -21 2019

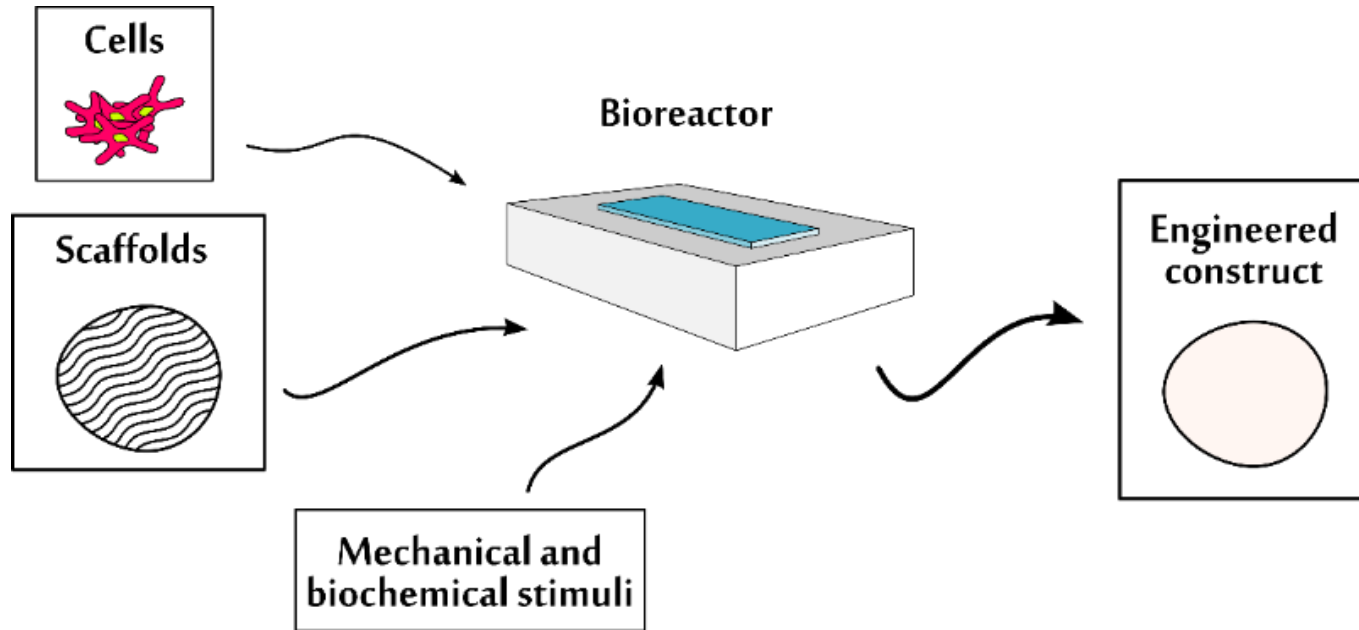
Gio Francesco Balbi Palace, Via Balbi 2, 16126 Genova, Italy.

Bioreactors as physiological-like in vitro models

Sara Mantero
Politecnico di Milano

June 20-21 2019

Background – Multifold potentials of bioreactors



to produce
functional biological tissue

Background – Multifold potentials of bioreactors

Bioreactors

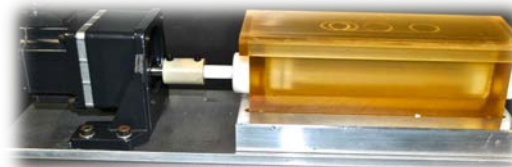
for tissue engineering

for decellularization of tissues

as tools to produce innovative scaffolds

as *in vitro* model

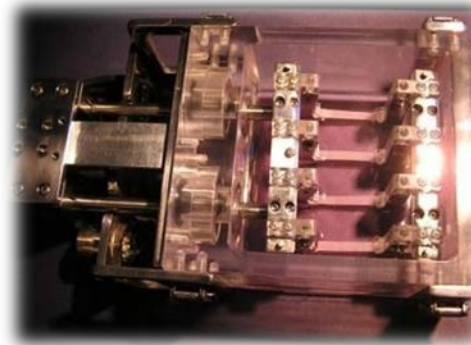
- To *in vitro* development of **engineerized** substitutes.
- Closely -controlled culture conditions (biological, physical, and mechanical)



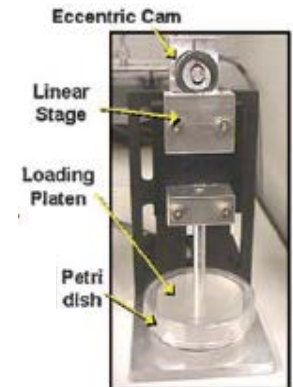
for **tracheas**¹



for **vessels**²



for **skeletal muscles**³



for **bone**⁴ and **cartilage**⁵

¹M. A. Asnaghi et al., *Biomaterials*. 2009 Oct;30(29):5260-9

²I. Stefani et al., *J Tissue Eng Regen Med*. 2016

³G. Candiani et al, *J Appl Biomater Biomech*. 2010 May-Aug;8(2):68-75

⁴F. K. Kasper et al., *StemBook* [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008

⁵J. A. Burdick et al *Tissue Eng Part A*. 2009 Feb;15(2):205-19



Background – Bioreactors Applications

Bioreactors

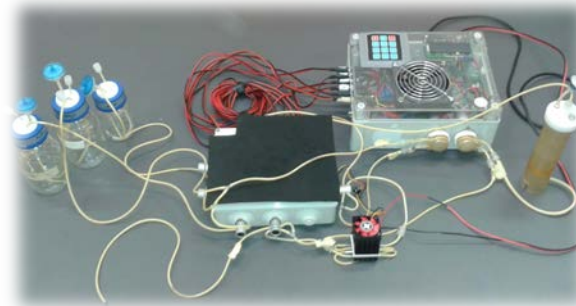
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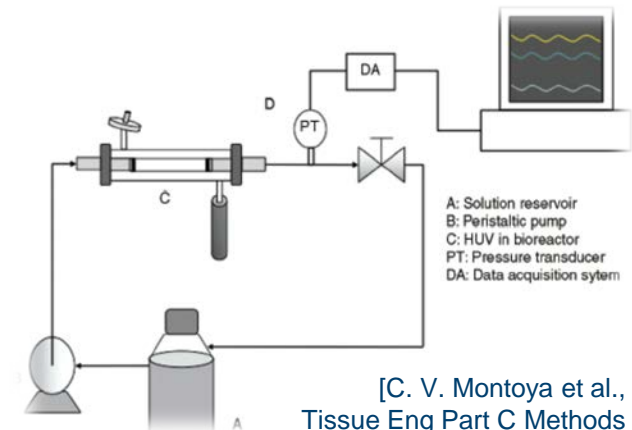
as *in vitro* model

as tools to produce innovative scaffolds

- Remove cellular components of tissues or whole organs, without damage or disruption of extracellular matrix (ECM)
- Apply hydrostatic pressure and convective flow using bioreactors



[A. F. Pellegata et al., Biomed Res Int. 2013;2013:918753]



[C. V. Montoya et al., Tissue Eng Part C Methods 15(2), 191-200 (2009)]

Background – Bioreactors Applications

Bioreactors

for tissue engineering

for decellularization
of tissues

as *in vitro* model

as tools to produce
innovative scaffolds

- *In vitro* 3D models as bridge between 2D models and *in vivo* models to determine mechanisms of functioning of human systems
- Allow for the application of **principle of 3R** (replacement, reduction, refinement) of animal usage
- Closer *in vitro* replication of native tissues through bioreactor technologies

[A. J. Ryan et al., Towards 3D *in vitro* models for the study of cardiovascular tissues and disease, Drug Discovery Today, 2016]



Background – Bioreactors Applications

Bioreactors

for tissue engineering

for decellularization of tissues

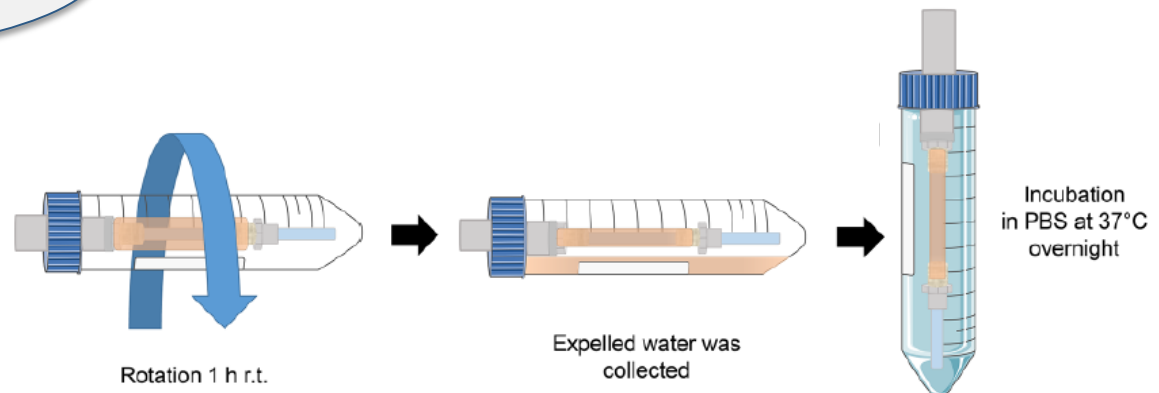
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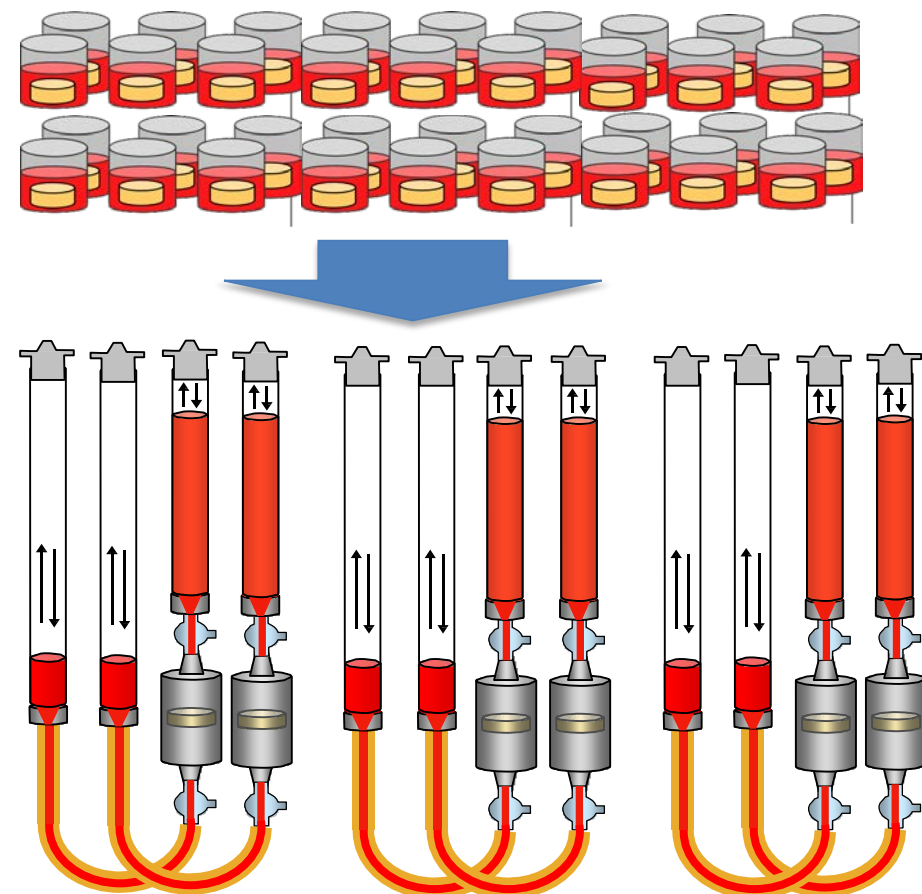
- To tailor scaffold structures
- To produce 3D scaffolds
- Specifically, to produce **tubular** and **multilayered** scaffolds for **Vascular Tissue Engineering**

e.g. dense collagen matrix for 3D tubular scaffolds using a bioreactor

[C. Loy et al, Biotechnol. J. 2016, 11, 1673–1679]



Bioreactors as tools to create physiological construct models



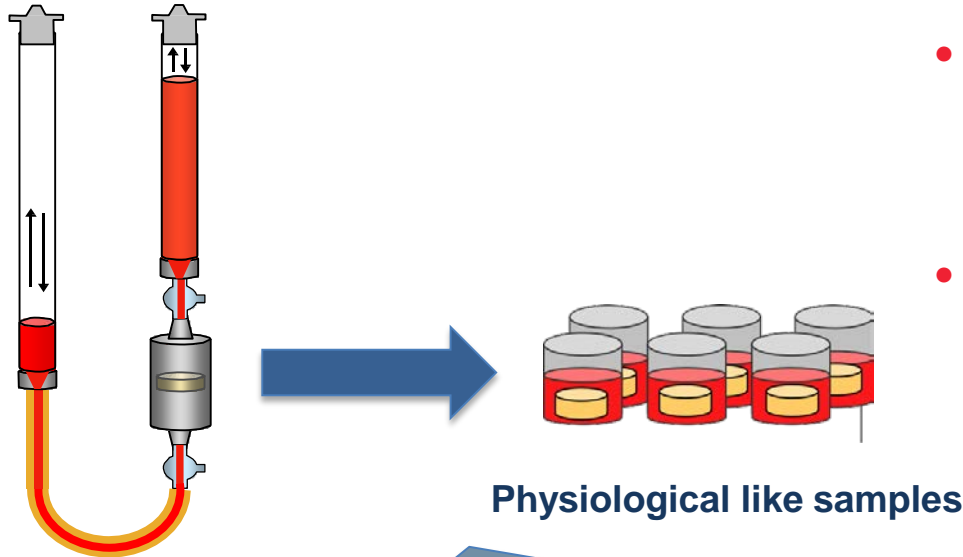
Translation: Static → dynamic

Physiological conditions: τ , v , P , $1/t$, σ , ϵ ..
Sensing and Control over the experiment
Tunable samples (tissue engineerable)

Wendt D et al., Biotech Bioeng (2003)

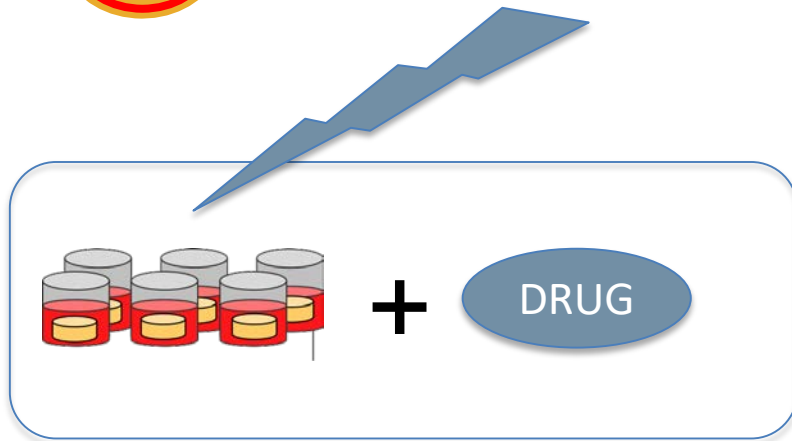


Bioreactors as tools to create physiological construct models



- Monitoring and control of culture parameters
(T, pH, pO₂, pCO₂, RH, glucose, lactate, others)
- Feedback control on conditioning and environmental cues

Physiological like samples

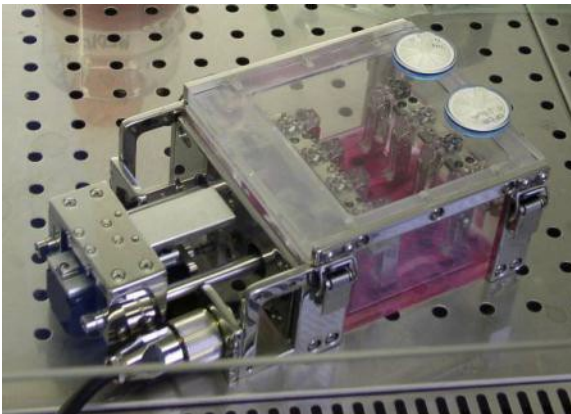


= 3R TEST SAMPLE

Hybrid samples from bioreactors

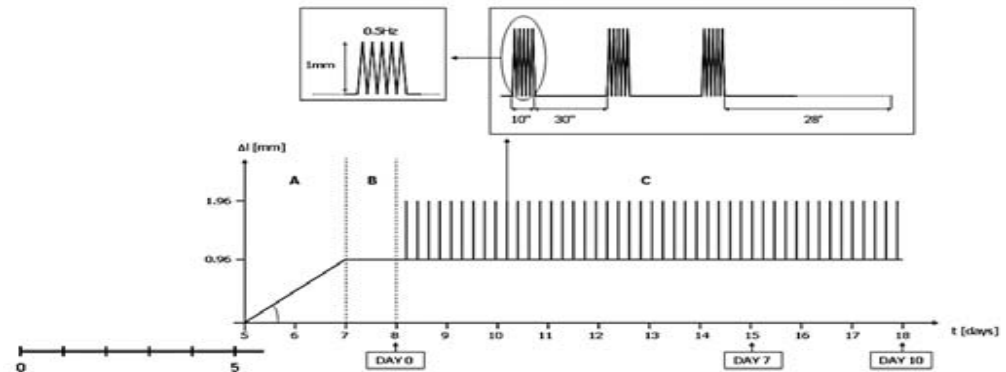
- Drive tissue development by mimicking the native physical environment
- Functional Tissue Engineered to be used as embryonic muscle model

SKELETAL MUSCLE TISSUE ENGINEERING via mechanical stimulation

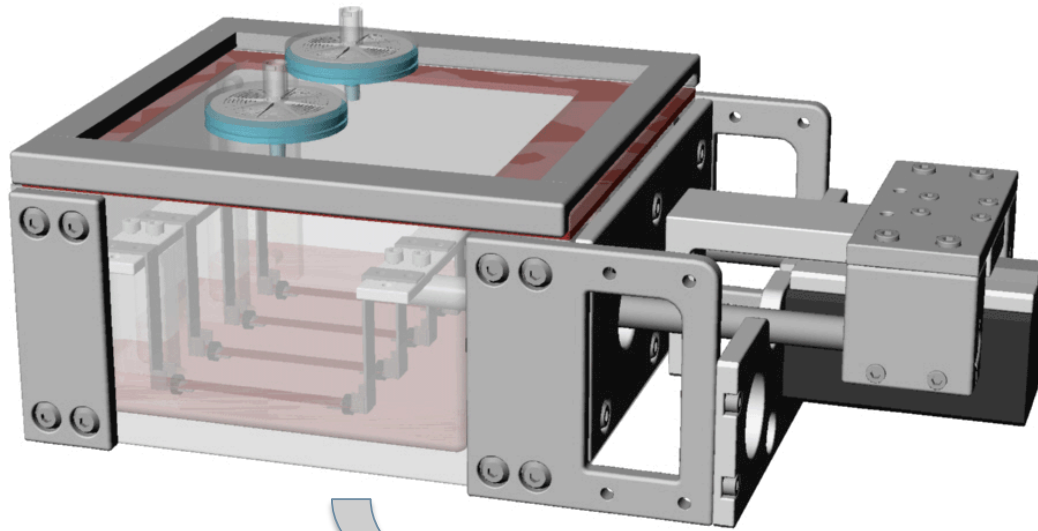


- Stretching bioreactor
- **Polyesterurethane scaffold**
- **Skeletal muscle line cells**
- Stretching pattern derived from in vivo data

- adapted from [Vandenburg 1991]
- resembling murine muscle development
- unidirectional stretching: $\epsilon \cong 3.3\%$
- burst amplitude $\epsilon \cong 3.4\%$



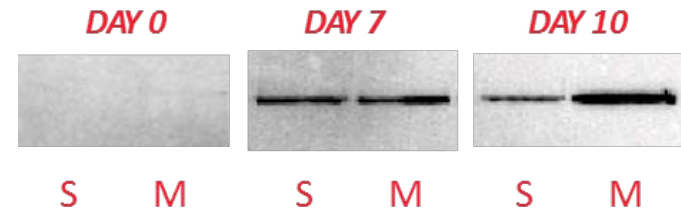
Hybrid samples from bioreactors



HYBRID TEST SAMPLE TO BE USED OR IN THE DEVICE OR OUTSIDE

- Western Blot for Myosin Heavy Chain
- *Mechanical stimulation favours MHC accumulation*

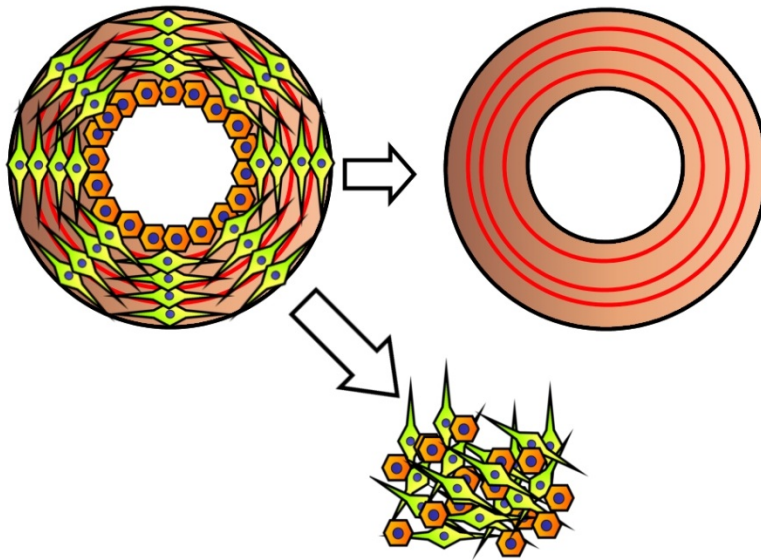
Riboldi SA et al., (2006)



Decellularized biological samples for testing

Decellularization

Decellularization is a process that is performed using chemical, biological or physical agents acting on the tissue, these are aimed at breaking cells and washing out of the tissue the cellular and nuclear remnants.



Parametri limite

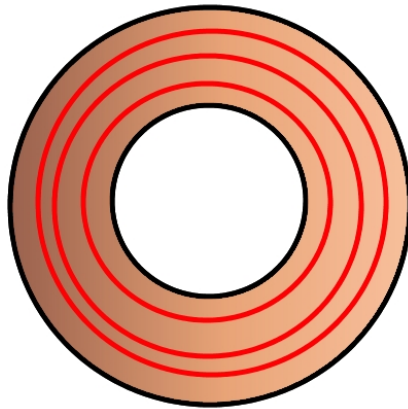
<50ng DNA per mg di tessuto disidratato

Lunghezza dei frammenti residui di DNA <200bp

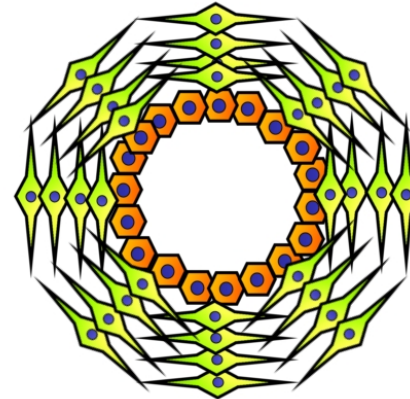
Mancanza di materiale nucleare residuo in colorazioni con DAPI o H&E

[Crapo PM, Gilbert TW, Badylak SF, *Biomaterials*, 2011]

Decellularized biological samples for testing



- Collagene
- Elastina
- Proteoglicani
- Fattori di crescita



- Segnali
- Adesione
- Differenziamento
- Supporto

Decellularized biological samples for testing

Parametri limite

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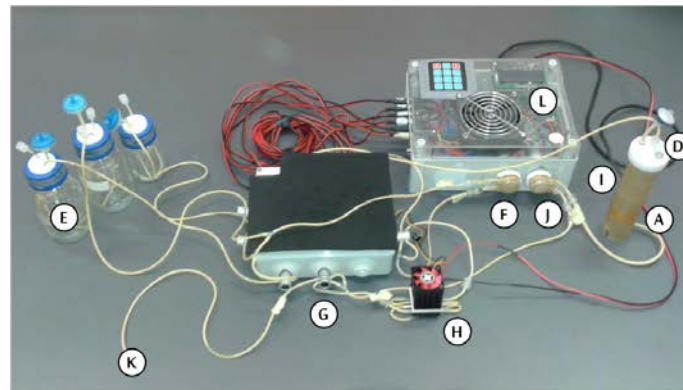
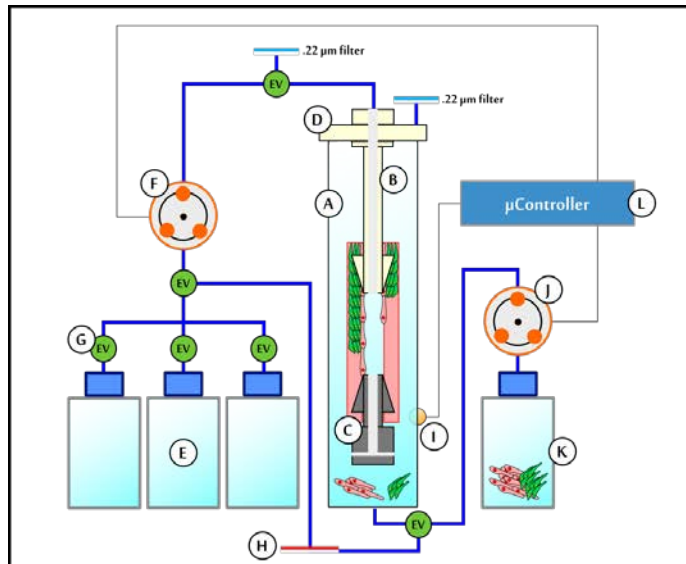
Characteristics

Biological ECM → proper protein composition
and architecture

Proper anatomy, geometry, size.

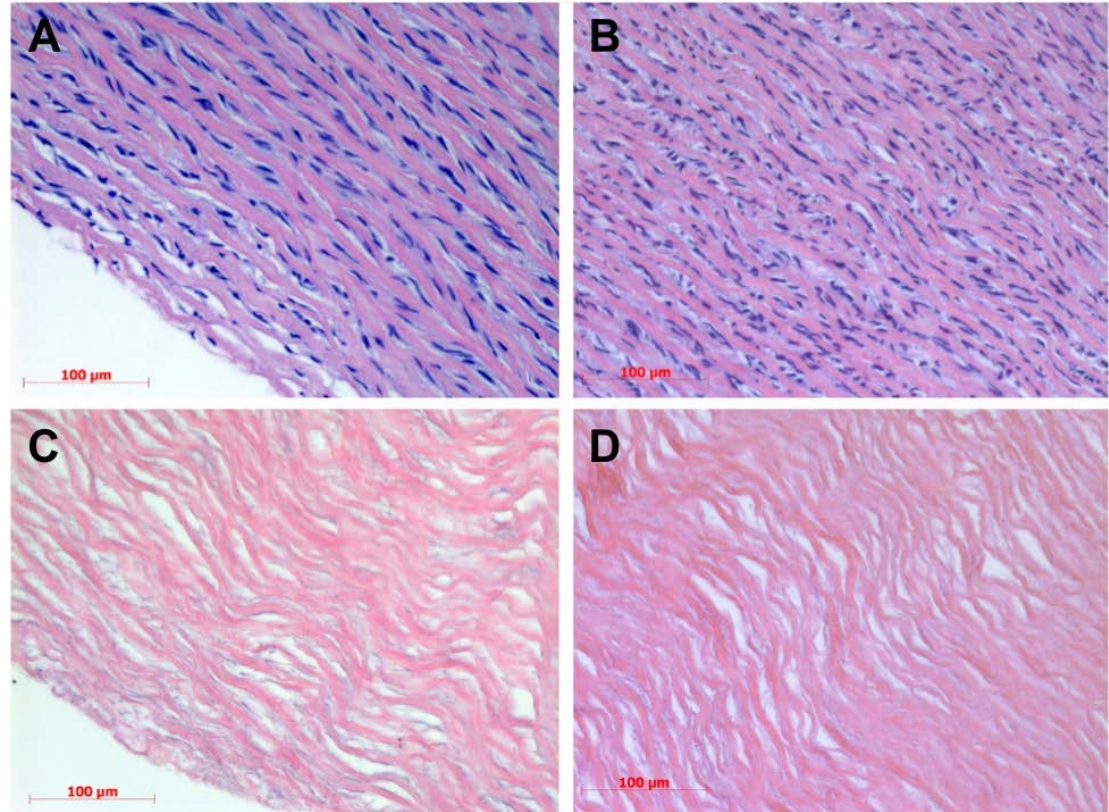
Very low immunological potential

With autologous cells → Patient specific

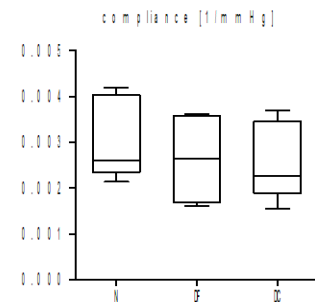
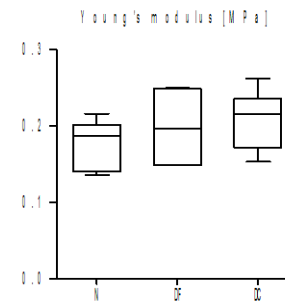
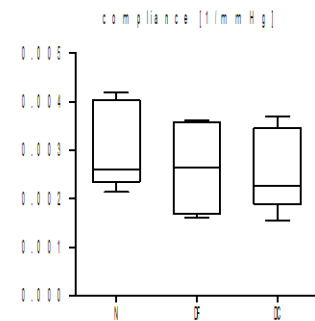
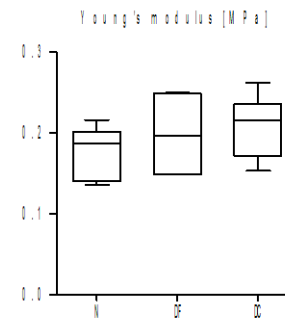
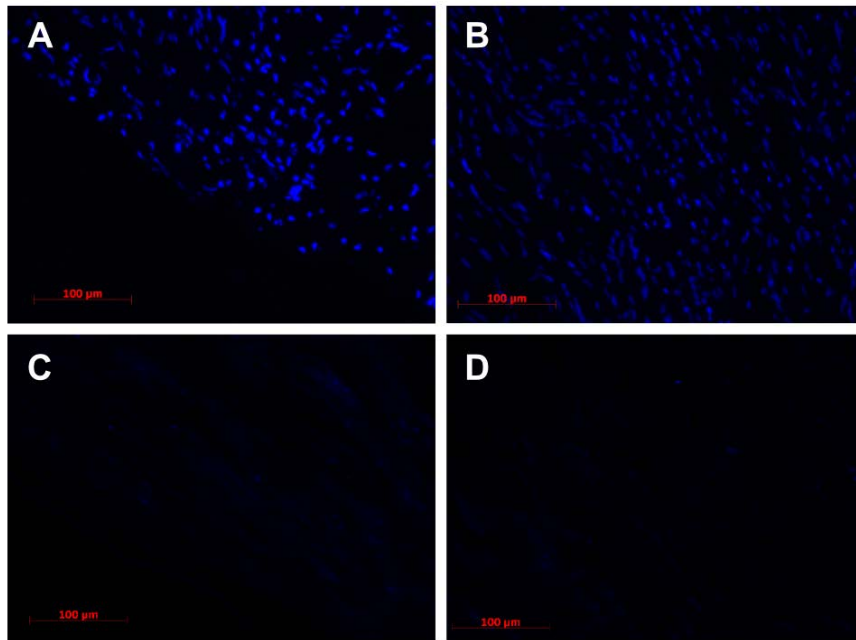


Decellularized biological samples for testing

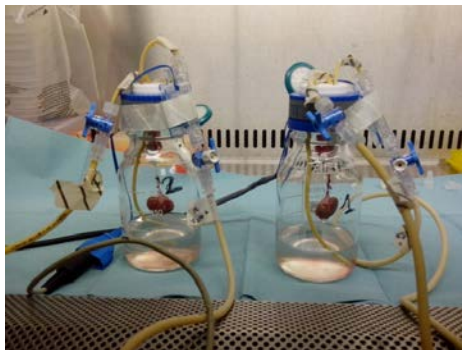
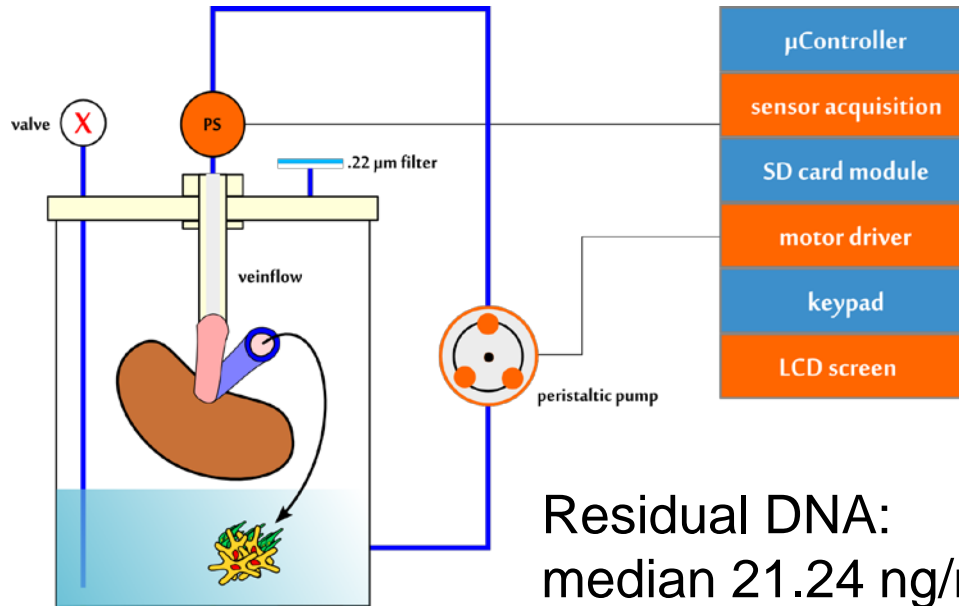
- dH₂O
- SDS
- DNase



Decellularized biological samples for testing



Decellularized biological samples for testing

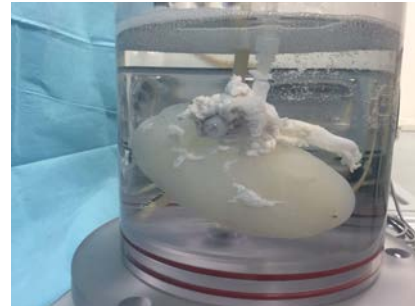
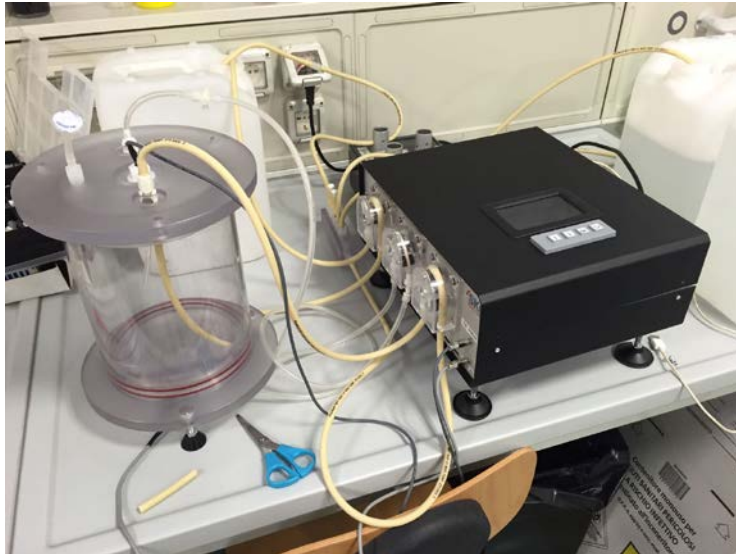


Residual DNA:
median 21.24 ng/mg dry weight

No residual cells

Preserved tissue structure

Decellularized biological samples for testing



Soluzione	Durata [h]	Portata [ml/min]	Volume [L]
Eparina	2	10	3
SDS 1%	72	10	54
dH ₂ O	2	10	3
Triton X-100 1%	12	10	9
PBS	48	10	36

Conclusion

3R TEST SAMPLE

Hybrid → (artificial material and cells)

Biological → (decellularized tissue or organs and cells)

Patient specific → biological and autologous cells for testing

