Lezione 4 – 14/12/2015

Bioreactors for Tissue Engineering

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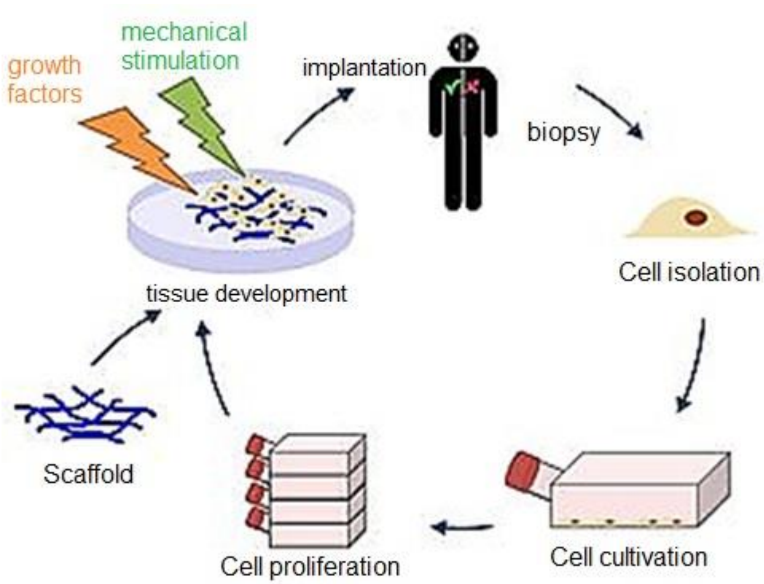
Tissue Engineering

Tissue Engineering is an interdisciplinary field, involving difference sciences such as engineering, biochemistry, biology, medicine and physics, that applies the principles of engineering and life sciences toward the development of biological substitutes that **restore**, **maintain**, or **improve** tissue function or a whole organ

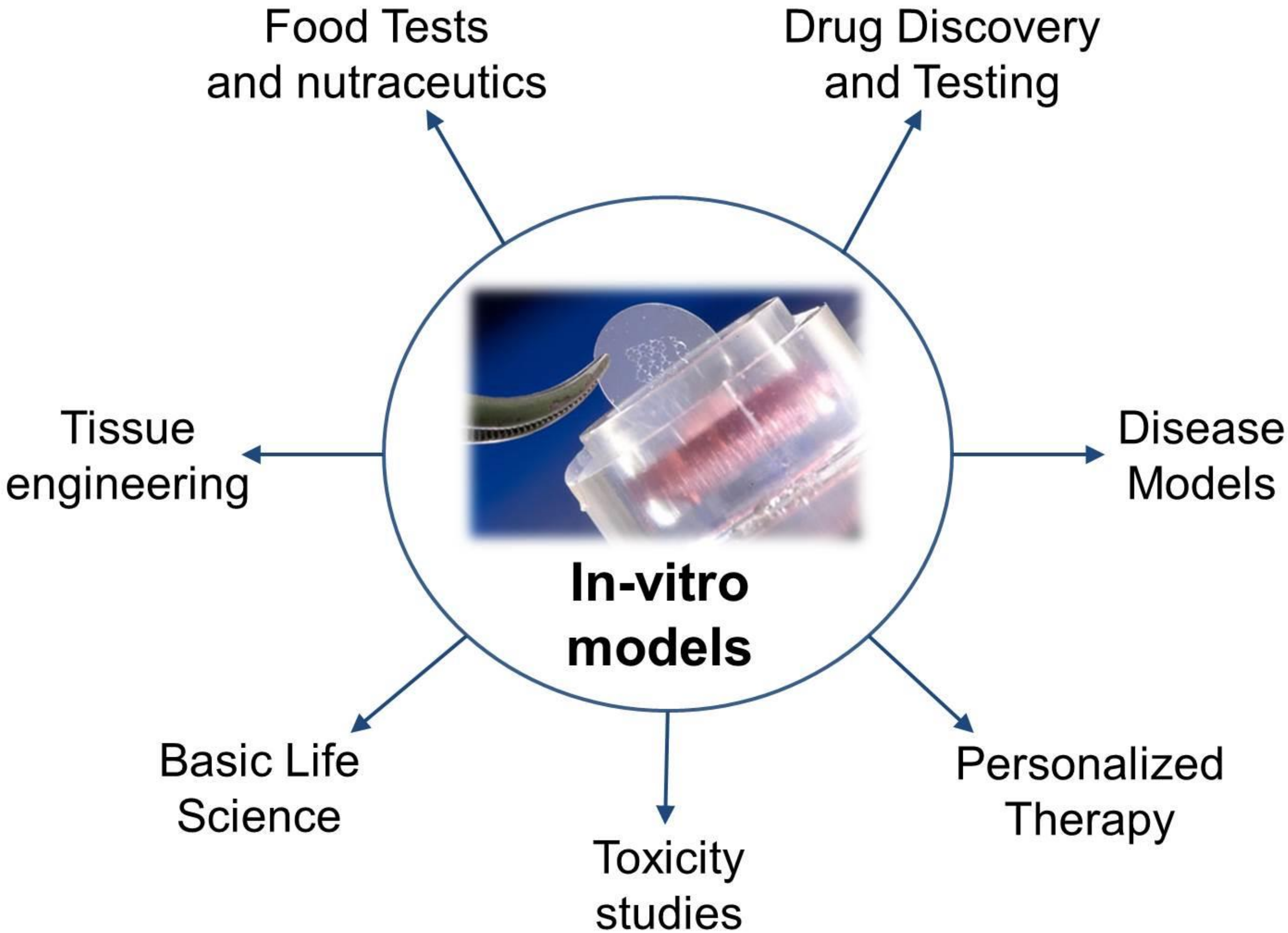


Engineered tissues are developed for:

in-vitro model

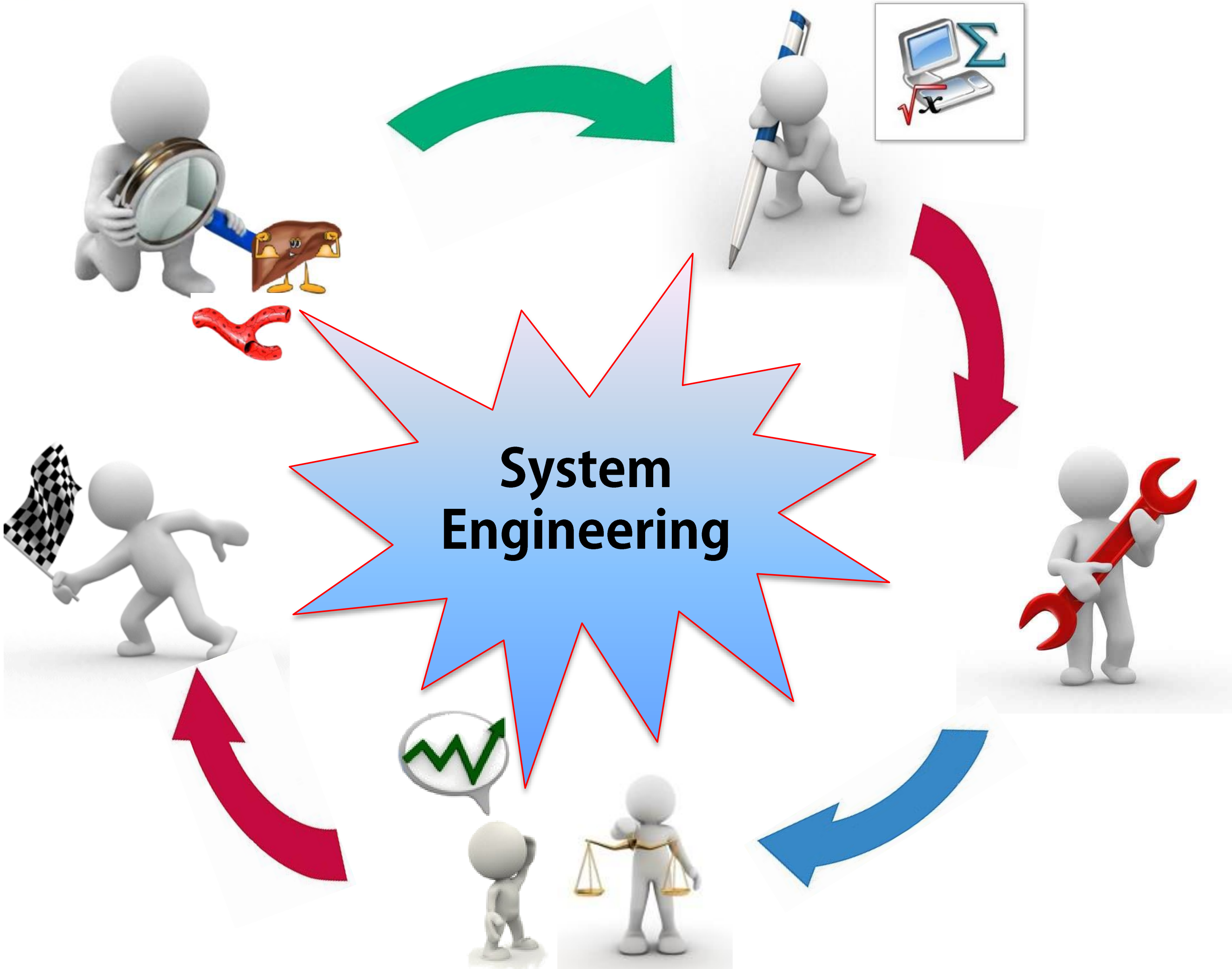
in vitro tissue substitutes (regenerative medicine)

In-vitro models



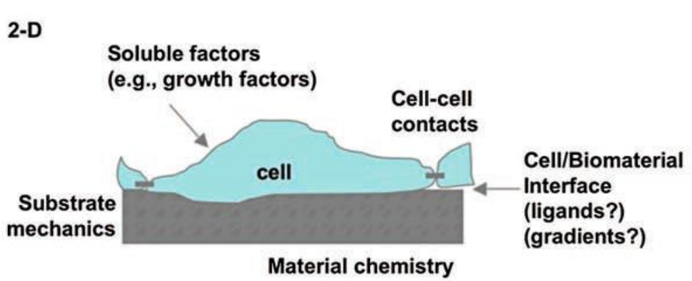
A good in-vitro model should come as close as possible to the in-vivo environment

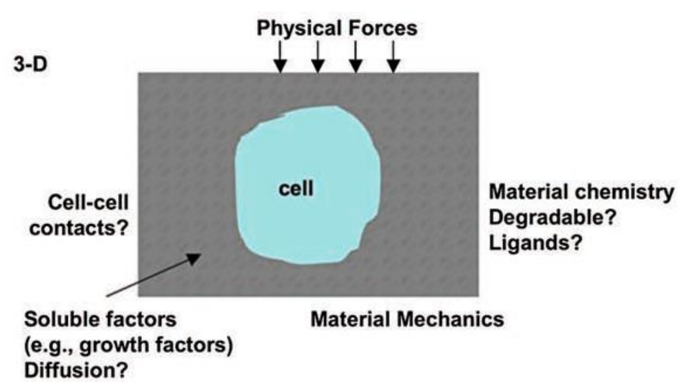
Engineering of Biological Environments



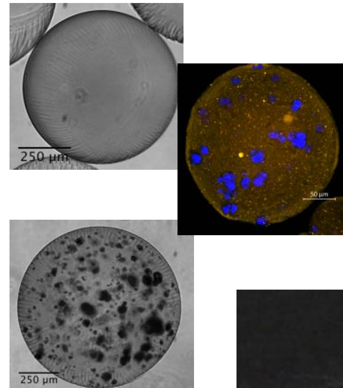
Why 3D cell culture?

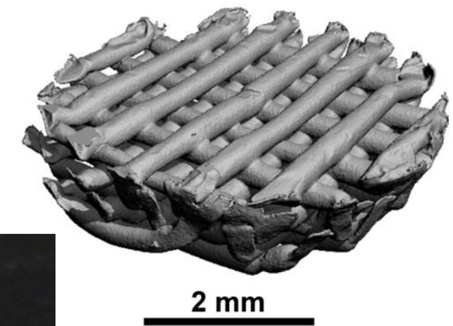
	2D	3D
Shape	**Flat** with typical **thickness of 3 µm**	**Ellipsoids** with dimensions of **10-30 µm**
Environment	~ **50 %** of **cell surface exposed** to **fluid** ~ **50 % exposed to** the **flat culture surface** Very **small % exposed** to **other cells**	~ **100 %** of **cell surface exposed** to other **cells** or **matrix**
Behaviour	**Differences in:** Differentiation, Drug Metabolism, Gene and Protein expression, General Cell Function, In Vivo Relevance, Morphology, Proliferation, Response to Stimuli and Viability	

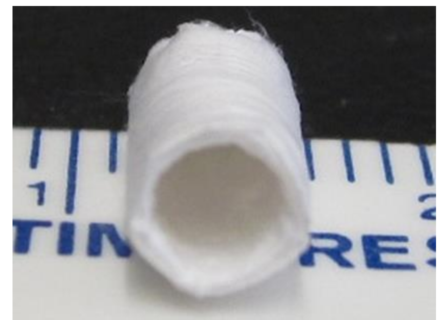




3D scaffolds: cell seeding and culture







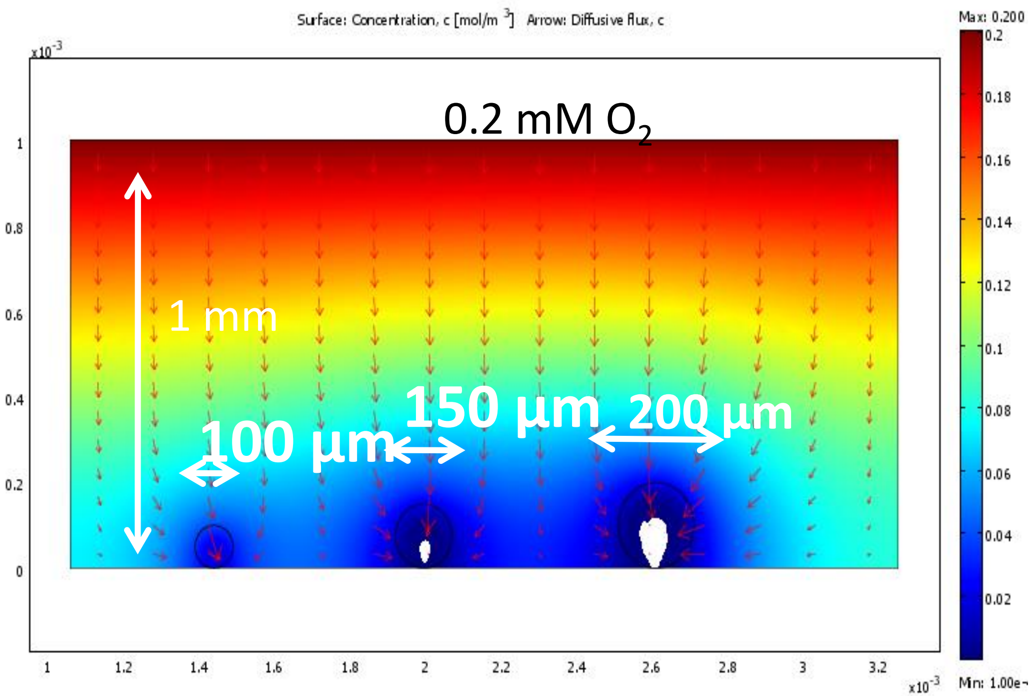
L’utilizzo in-vitro di strutture 3D comporta diversi problemi::

- È molto difficile ottenere una semina uniforme in condizioni statiche
- L’apporto di nutrienti, in particolare l’ossigeno, è fortemente limitato, così come la rimozione dei prodotti metabolici dannosi per le cellule

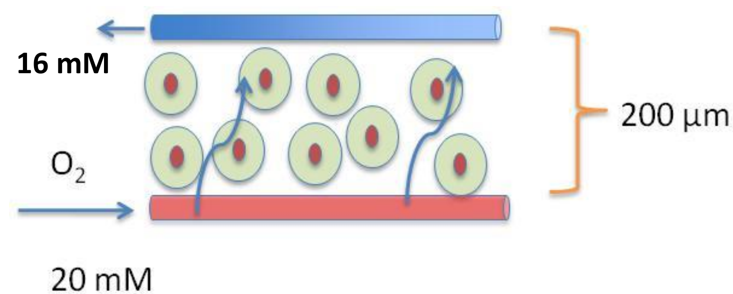
È per tali motivi che l’Ingegneria Tessutale ha abbandonato i classici metodi di coltura cellulare, sviluppando nuove metodologie ed apparecchiature quali il **bioreattore**, che è un vero e proprio “simulatore di organismo vivente”, ovvero un dispositivo sterile, termostatato e con adeguate concentrazioni di metaboliti e gas

The oxygen problem

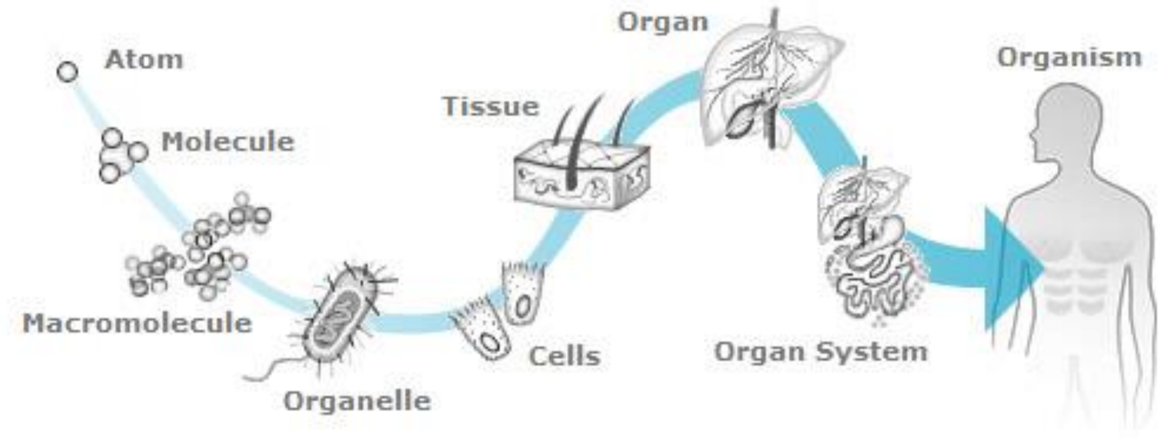
The first bioreactors were designed to solve the oxygen problem



$$J = -D\frac{dc}{dx}$$



In-vitro models



A good in-vitro model should come as close as possible to the in-vivo situation[1] better represent human response

- Cells live in a dynamic environment
- Physical stimuli are critical for cell behaviour[2]
- Cross-talking between different cells and tissue

Use of bioreactors

1 Rouwkema J., Gibbs S., Lutolf M.P., Martin I., Vunjak-Novakovic G., Malda J. In vitro platforms for tissue engineering: implications for basic research and clinical translation., J Tissue Eng Regen Med 5, e164, 2011

2 Bilodeau K, Mantovani D. Bioreactors for tissue engineering: focus on mechanical constraints. A comparative review. Tissue Eng 12, 2367, 2006

Definition of bioreactors

Important paper, 2004

Review TRENDS in Biotechnology Vol.22 No.2 February 2004
ELSEVIER
Full text provided by www.sciencedirect.com
SCIENCE DIRECT

The role of bioreactors in tissue engineering

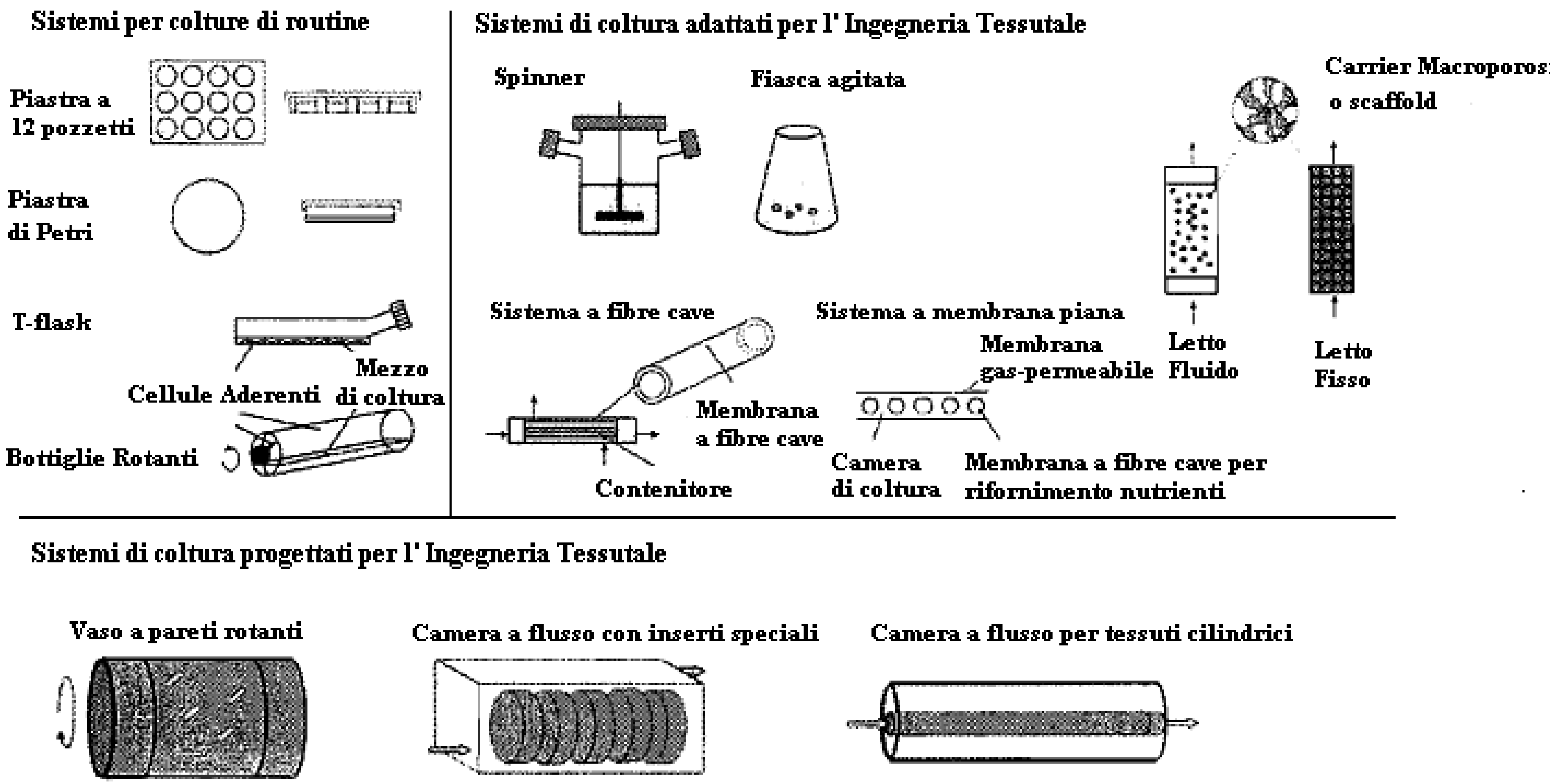
Ivan Martin, David Wendt and Michael Heberer

Bioreactors are generally defined as devices in which biological and/or biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions (e.g. pH, temperature, pressure, nutrient supply and waste removal).

Tissue engineering bioreactors are defined as in vitro culture systems designed to perform at last one of the following functions:

1. establish spatially uniform cell distributions on 3D scaffolds;
2. maintain desired concentration of gases and nutrients in the culture medium;
3. provide efficient mass transfer to the growing tissue;
4. expose developing tissue to physical stimuli.

Evolution of bioreactors



Classification of bioreactors

Bioreactors can also be classified in:

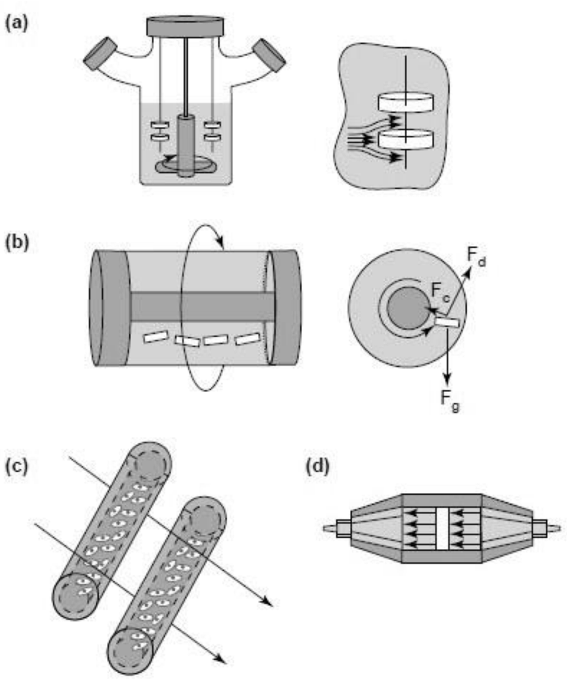
- **Shaken bioreactors** (rotating, lift, spinner flask, orbital shaker, etc)
- **Bioreactor for applying physical stimuli** (shear, pressure, stretch, compression, etc)
- **Bioreactors for connected cell cultures** (Shuler’s one, Ingber’s one, etc)

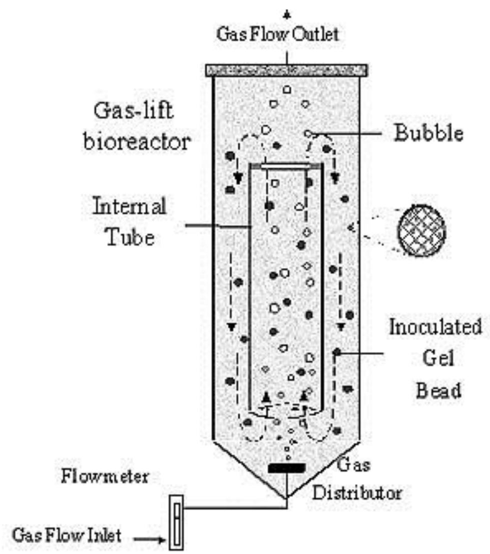
Physical stimuli that the bioreactor is able to perform depend on the functional requirements of the tissue to be engineered

Specific mechanical forces, which are known to be important modulators of cell physiology, might increase the biosynthetic activity of cells in bioarticial matrices and, thus, possibly improve or accelerate tissue regeneration in vitro

Shaken bioreactors

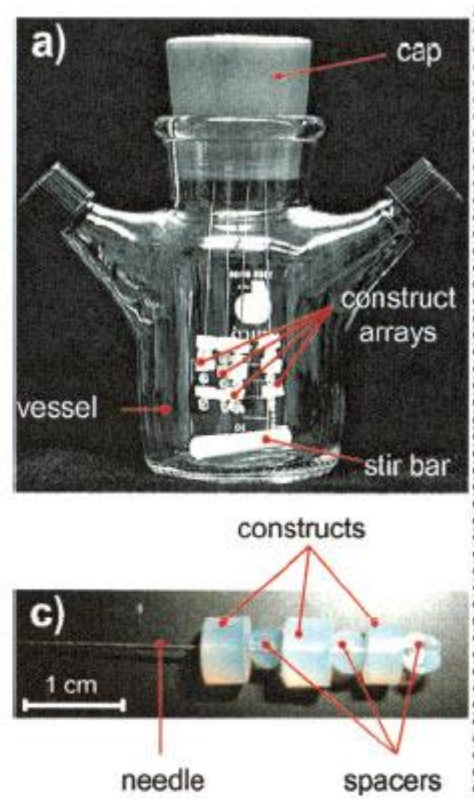
- La cultura è agitata meccanicamente o mediante inserimento di gas.
- L'agitazione ha come scopo quello di tenere in sospensione le cellule per permettere l'adesione a strutture come scaffold , e migliorare il trasporto dei soluti.
- <u>L'agitazione può essere studiata o dimensionata per imporre stimoli di intensità nota</u>





Shaken bioreactors -> Spinner Flask

- Usate per mantenere in sospensione le cellule evitando che si depositino sul fondo del contenitore
- Le cellule non amano stare in sospensione, pertanto tendono a colonizzare gli scaffold che vengono tenuti sospesi grazie ad appositi supporti
- Molto usate per la colonizzazione di scaffold porosi tipo: ceramici o fiber spinned.
- Vengono usate anche dopo la colonizzazione per stimolare le culture (Shear Stress)



Volume di terreno utilizzato: 20-500 mL

Shaken bioreactors -> Spinner Flask

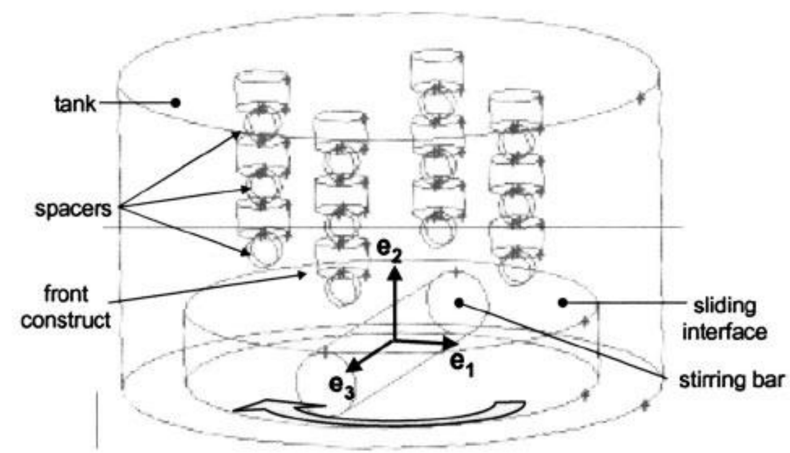
In the experiments, the four equiangularly spaced construct arrays were positioned at 25 mm from the center of the cap of the model bioreactor. The vertical distance between the lower surface of the bottom construct and the stir-bar was fixed at 10 mm.

	Prototype bioreactor
Bioreactor material	Glass
Construct material	Polyglycolic acid (PGA)
Spacer material	Silicone tubing
Working fluid	Cell culture medium
Fluid kinematic viscosity	0.971 cSt
Fluid density	1.03 g/cm^3
Fluid volume	120 cm^3
Bioreactor diameter	6.50 cm
Free-surface height	3.8 cm
Stir-bar length	4.52 cm
Stir-bar diameter	0.787 cm
Construct diameter	0.693 cm
Construct thickness	0.377 cm
Spacer diameter	0.396 cm
Spacer thickness	0.168 cm
Stirring rate	50 rpm

$$\boxed{Re_p = \frac{N_p L_p^2}{\nu_p} = 1758.}$$

Laminar or turbulent flow?

Tested with chondrocytes
(cells from articular cartilage)

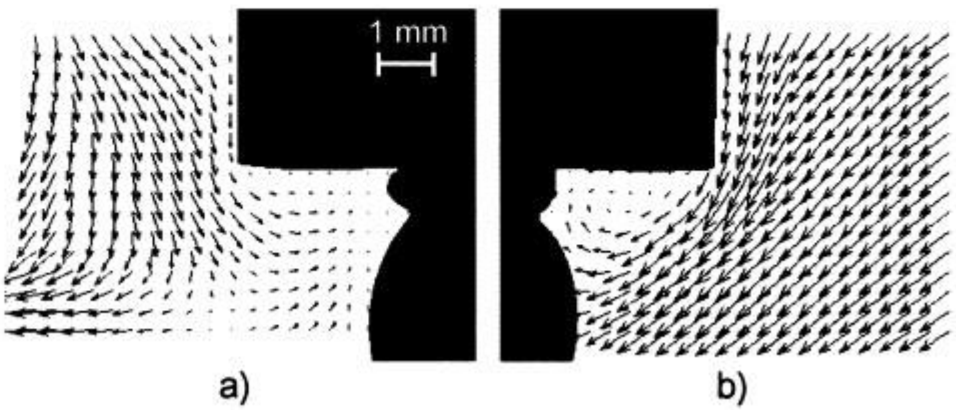


Fluid Mechanics of a Spinner-Flask Bioreactor

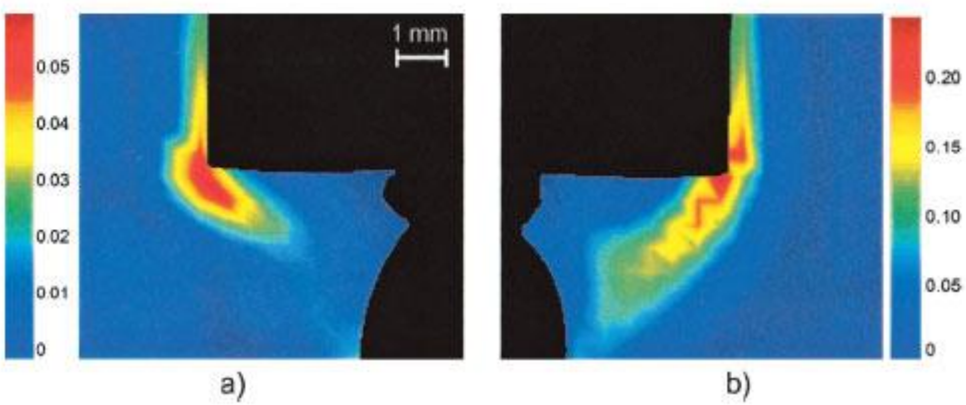
Philippe Sucosky, Diego F. Osorio, Jason B. Brown, G. Paul Neitzel

Shaken bioreactors -> Spinner Flask

Velocity field around the construct



Shear Stress field around the construct



Chondrocytes increase matrix synthesis (Glycosaminoglycan)

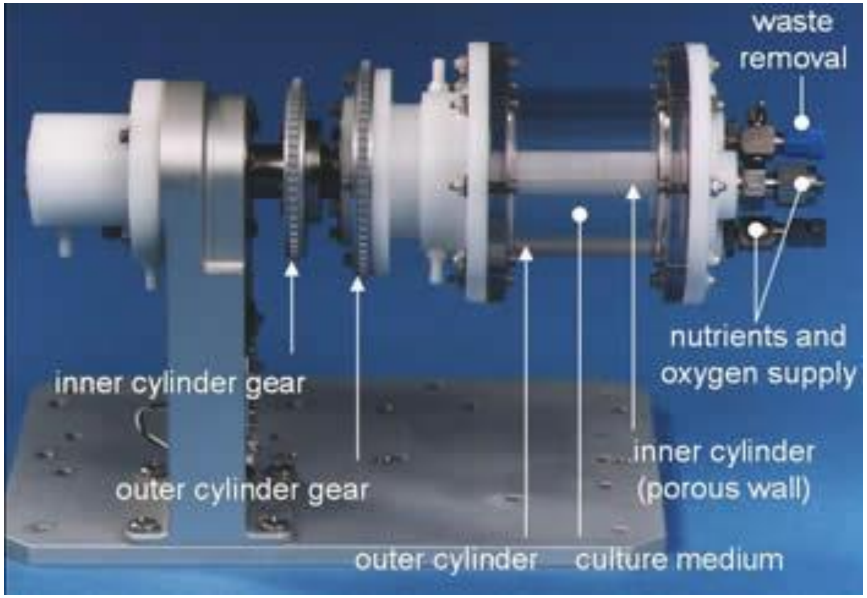
Turbolent flow reduces the GAG accumulation into the construct, in particular at the edge of it.

Dopo 24h di cultura a 10^{-2} Pa di shear stress, una cultura di condrociti (mono-layer) mostra gravi “danni”

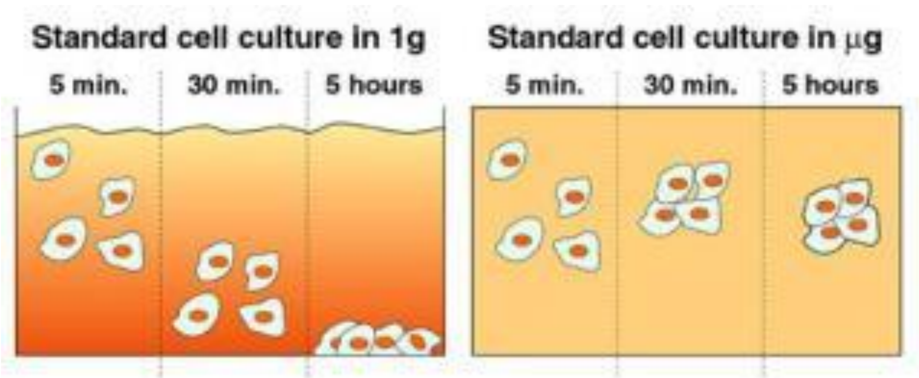
Rotating-Wall Vessels device (NASA)

This rotating vessel device was developed by NASA within the framework of the ideas and concepts originally pointed out by Briegleb, who recognized the need to study the influence of weightlessness on living cells.

Access to real weightlessness was always very limited……



The RWV uses the principle of **clinorotation** - that is, the cancellation of the force of gravity by rotation around one or two axes—to create a microgravity environment in which cells can be grown.



Because cells are suspended weightlessly in fluid, the attractive forces between those cells have a greater proclivity to act on each other, allowing cells to become associated with one another and grow in three dimensions.

Rotating-Wall Vessels device (NASA)

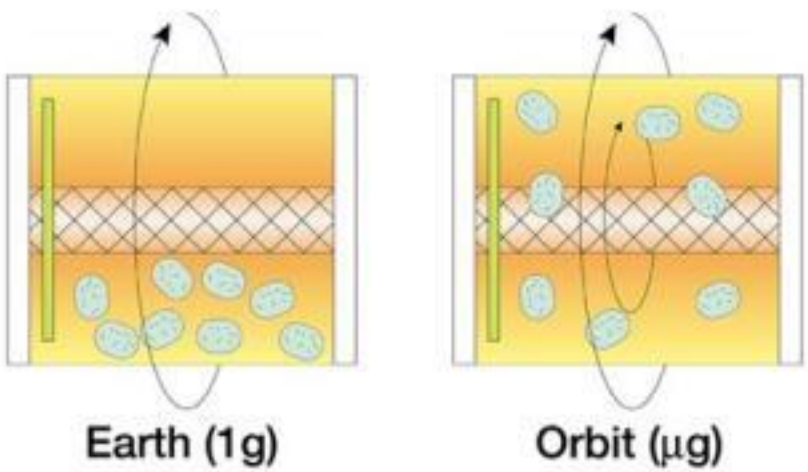
The rotating-wall perfused-vessel (RWPV) bioreactor developed by NASA was tested in two different conditions, in order to evaluate the gravity's effect on biological tissue:

- on Earth (gravity force)
- in space (microgravity)

Even if the RWPV bioreactor creates similar fluid-dynamic conditions, the shape if the cell seeded constructs was different (spherical in space, disk-shape on Earth)

On Earth:
- the inner cylinder, disk, and outer cylinder are all rotated at the same rate (15-35 rpm, depending on the construct size)
- cells move in circular orbits or in stationary location

In Space:
- Rotation is required only for mass transport
- differential rotation mode, the inner cylinder and disk rotate together at a higher rate than the outer wall



Rotating-Wall Vessels device (NASA)

The rotating-wall perfused-vessel (RWPV) bioreactor, used for both **microgravity** and **Earth-based** cell science experiments, is characterized in terms of the fluid dynamic and fluid shear stress environment.

The RWPV was designed specifically to allow the long time culture of shear-sensitive mammalian cells in a microgravity environment

- Replenish fresh media
- Monitoring and control of pO_2, pH and temperature

Design Requirements:
- Shear levels of around 10^{-3} Pa
- Laminar flow
- Cells must be suspended in the media

This bioreactor was characterised by mathematical and CFD models, assuming:
1. Incompressible fluid
2. Uniform density and viscosity

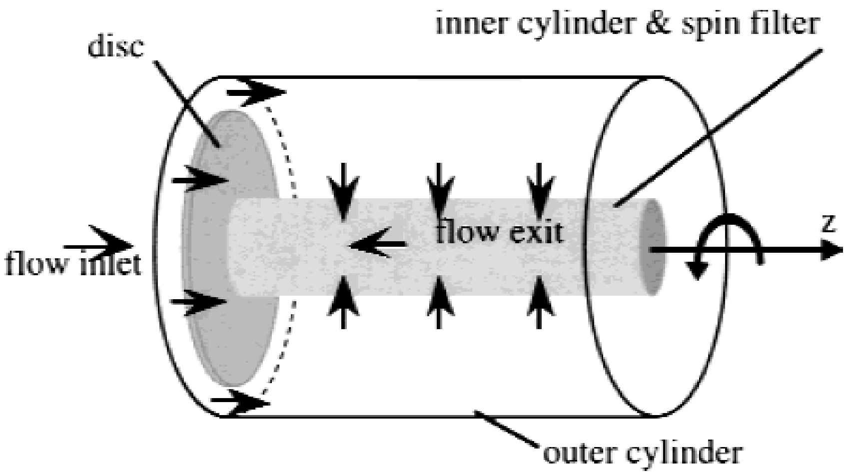


Figure 1. Rotating-wall perfused-vessel geometry.

Rotating-Wall Vessels device (NASA)

D_{ext}= 5 cm

D_{in}= 1.5 cm

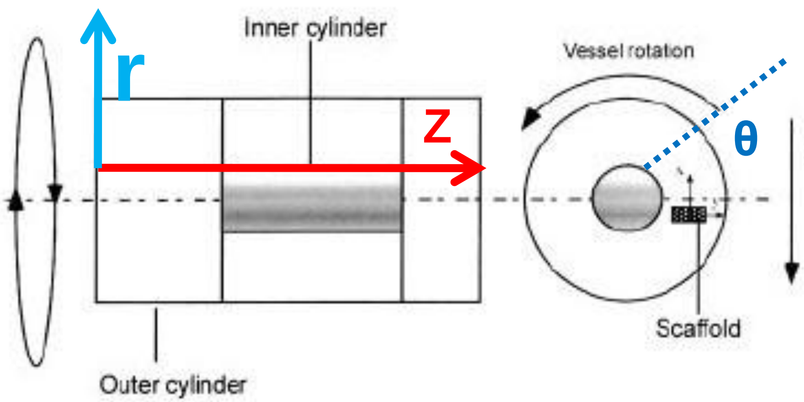
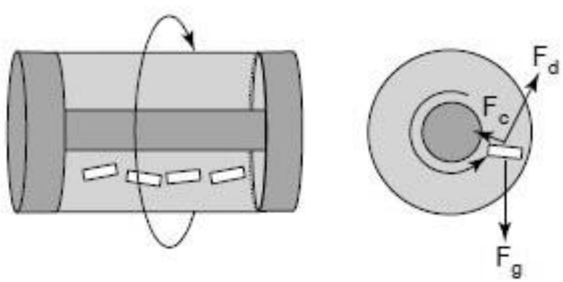
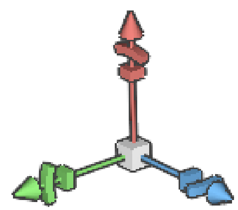


Figure 1. Sketch of the rotating bioreactor (on-ground conditions).

The three forces are balanced. The construct is in free-fall through the culture media





Three Shear Stress components

$$S_{r\theta} = \mu\left[r\frac{\partial}{\partial r}\left(\frac{v}{r}\right)\right]$$

$$S_{\theta z} = \mu\left[\frac{\partial v}{\partial z}\right]$$

$$S_{rz} = \mu\left[\frac{\partial w}{\partial r} + \frac{\partial u}{\partial z}\right]$$

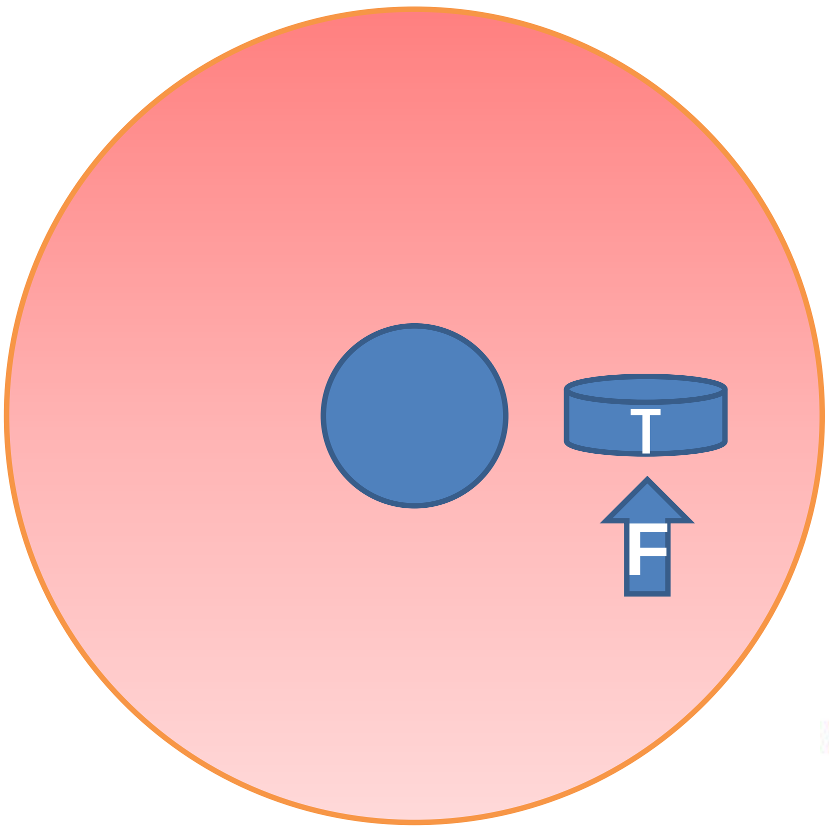
$$S_m = \frac{1}{3}(S_{r\theta}^2 + S_{\theta z}^2 + S_{rz}^2)^{1/2}$$

The Fluid Dynamic and Shear Environment in the NASA/JSC Rotating-Wall Perfused-Vessel Bioreactor

Cynthia M. Begley, Stanley J. Kleis

Rotating-Wall Vessels device (NASA)

$S_m = \mathbf{T} = F/A$



V_{rotaz}=30 rpm
V_{media}= U = 6 cm/s

PGA disk scaffold:
0.5 cm diameter
0.2 cm thick
formed as a 97% porous mesh of 13µm diameter fibers
Culture medium = DMEM

$C_D = f$ = Fattore di attrito (detto anche di fanning) del costrutto in caduta libera
E = L/d aspect ratio
$\Delta\rho$ gradiente di densità tra il costrutto e il fluido

$$C_D \mathrm{Re}^2 = \frac{2g\Delta\rho E d^3}{\rho \nu^2}$$

7.4 10^4

$$C_D = \frac{64}{\pi \mathrm{Re}}\left(1 + 0.138\,\mathrm{Re}^{0.792}\right)$$

$$\mathrm{Re} = \frac{Ud}{\nu} = 290 \rightarrow U = 4.64\ [\mathrm{cm/s}]$$

The net weight of the tissue is balanced by the viscous resistance and the flow around the construct is axisymmetric

Organic Tissues in Rotating Bioreactors: Fluid-Mechanical Aspects, Dynamic Growth Models, and Morphological Evolution

Marcello Lappa

Rotating-Wall Vessels device (NASA)

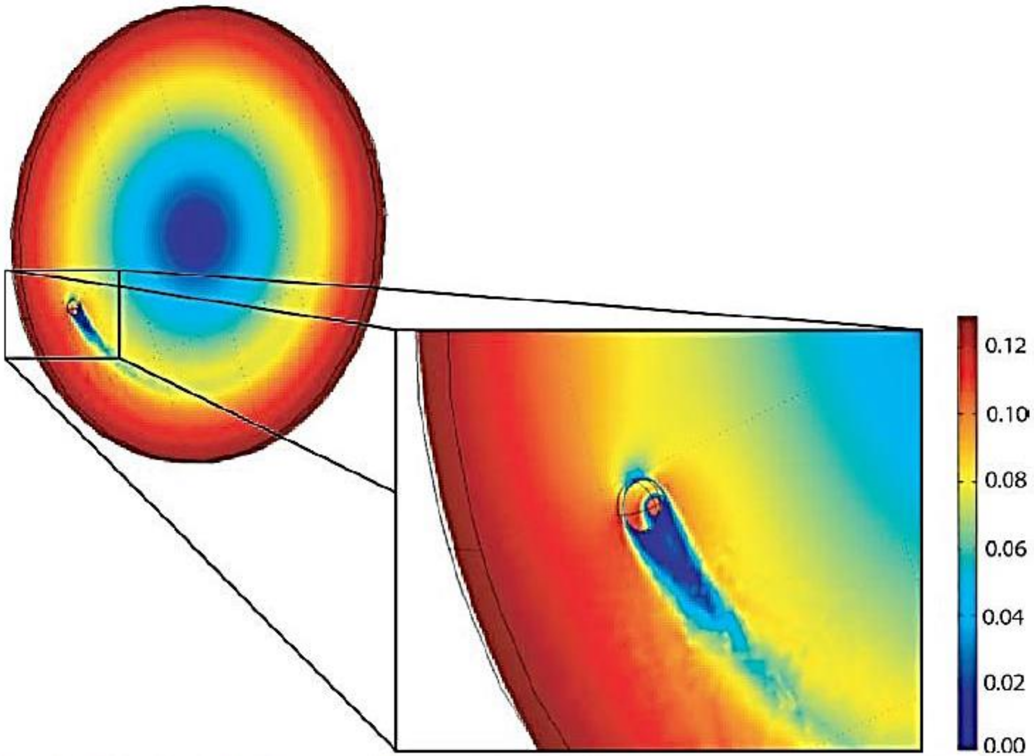
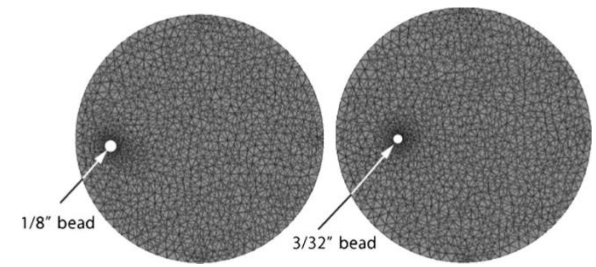


FIG. 3. Fluid velocity distribution in the bioreactor. The velocity (measured in m/s) increases with radius from the center of the bioreactor except in the region near the spherical bead (see inset). The disruption in the velocity field is responsible for the elevated shear stresses in the fluid.



- Buona distribuzione delle cellule nello scaffold
- Buon apporto di ossigeno e nutrienti
- Basso shear stress

Coltura di cellule ossee, cartilaginee, cardiache, epatiche

?????

Bioreactors for physical stimuli

Current Drug Discovery Technologies, 2006, *3*, 211-224

Engineered Tissue as a Model to Study Cell and Tissue Function from a Biophysical Perspective

Manuela Teresa Raimondi*

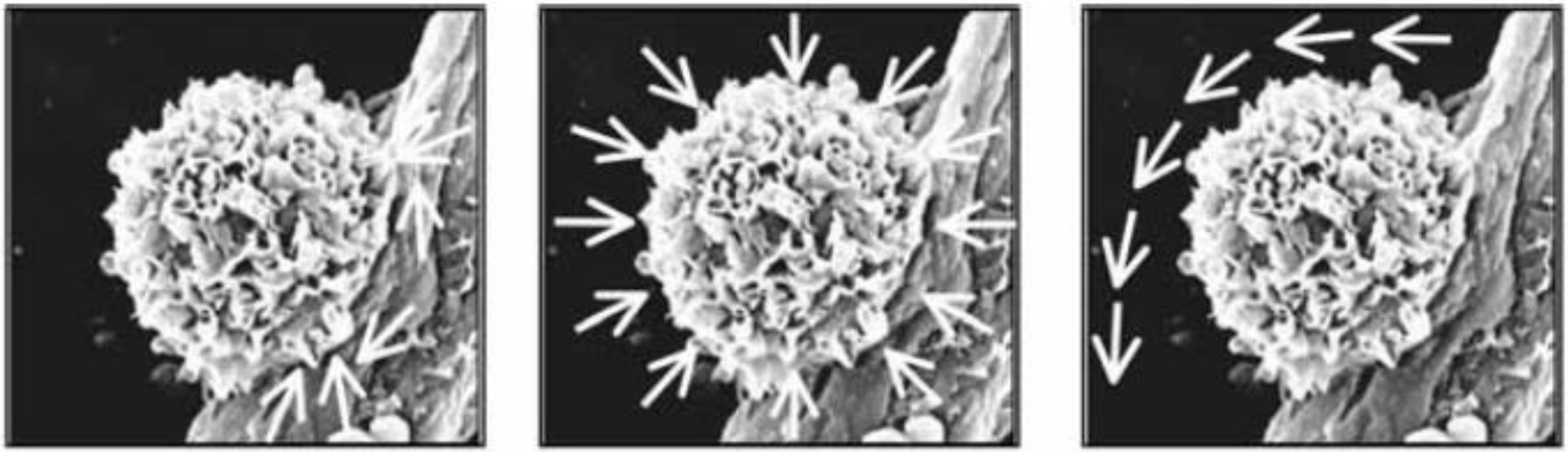
global approach

quantification of physiologic loading conditions in living tissue → development of systems able to impose physiologic loading on tissue explants to verify maintenance in vitro of the biosynthetic activity of cells → development of culture systems able to impose physiologic loading on engineered tissue

local approach

quantification of physiologic loading conditions in living tissue → extrapolation of the local mechanical environment responsible for the stimulation of the cell biosynthetic activity → development of culture systems able to mimic the local mechanical environment on cells within engineered tissue

The Local approach: mechanotrasduction



matrix stresses (tension, compression, shear) | hydrostatic pressure | hydrodynamic shear

I meccanismi precisi di come uno sforzo meccanico carico viene trasferito e traslato in un segnale chimico e biochimico che accende i pathway di reazioni e che vanno a modulare l'espressione genica non è stato ancora chiaramente compreso: ci si riferisce a questo processo con il nome di **meccanotrasduzione**

Theoretical models

Experimental models

Mechanobiology

Bioreactors for applying physical stimuli

E’ possibile applicare alle culture stimoli specifici

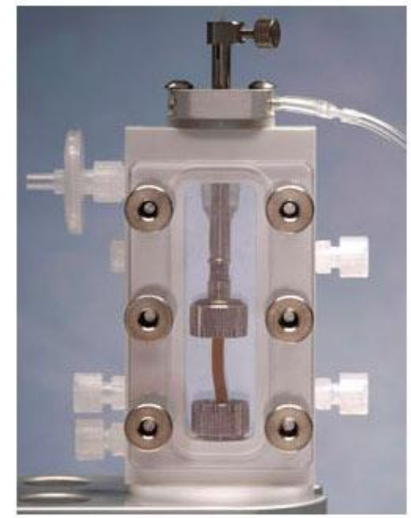
La stimolazione è **nota**, **modellata** e finemente **controllabile**

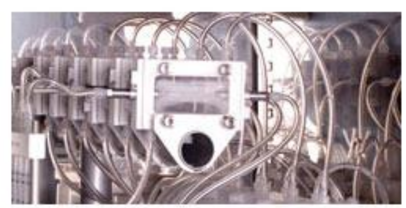
Si possono applicare diverse tipologie di stimolazione:

- Shear stress
- Pressione idrostatica
- Compressione
- Trazione
- Torsione
- Pressione differenziale

…

LigaGen L30-4C Chamber

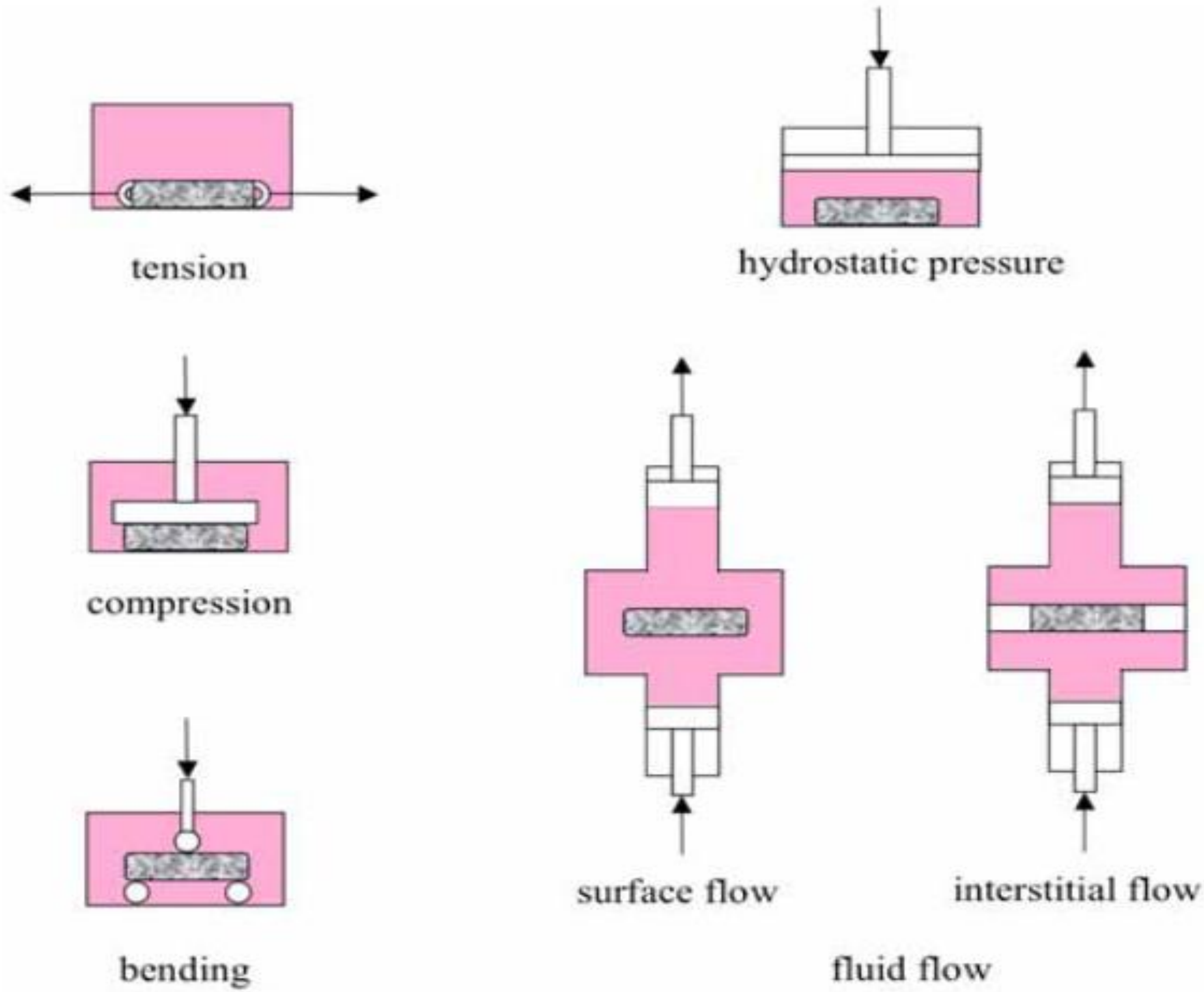




LumeGen V60 Chamber: 6VC System

LumeGen V60 Chamber

Different kind of stimulation



Definition of bioreactors

Bioreactors are essential in tissue engineering because:

- they provide an in vitro environment mimicking in vivo conditions for the growth of **tissue substitutes**
- they enable **systematic in-vitro studies** of the responses of living tissues to various mechanical and biochemical cues

Essential parts:

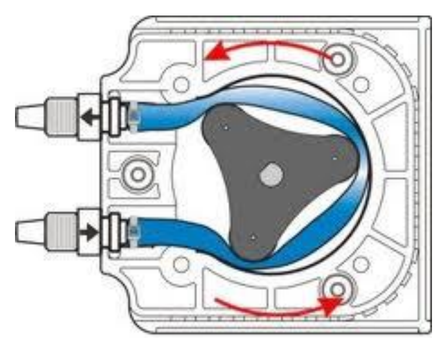
- pH sensor
- pO_2 sensor
- Temperature sensor

sensors

- Heating system
- Peristaltic pump
- Actuators

Active components

La **pompa peristaltica** è un apparecchio che applica il principio della peristalsi, in base al quale la prevalenza al fluido trattato viene impressa da una strozzatura che scorre lungo il tubo.



Sensors and actuators

Sensore (elettrico):

è un trasduttore che si trova in diretta interazione con il sistema misurato, e la trasforma in una grandezza misurabile (corrente, tensione, etc)

I sensori possono essere classificati in base al loro principio di funzionamento oppure al tipo di segnale in uscita, ma più comunemente vengono classificati in base al tipo di grandezza fisica che misurano

Attuatore:

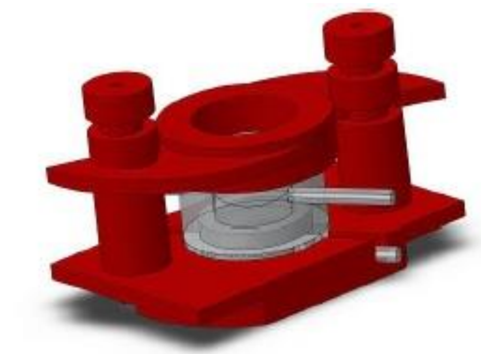
Dispositivo che converte energia da una forma ad un'altra, in modo che questa agisca nell'ambiente fisico al posto dell'uomo.

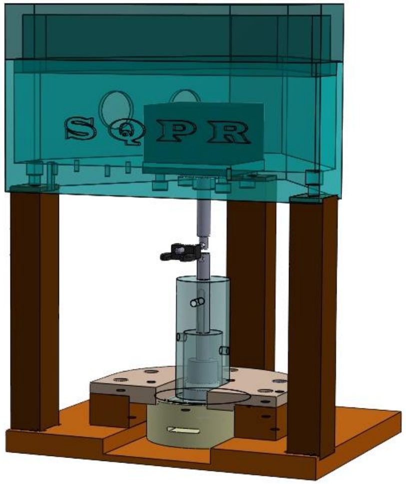
In generale, gli attuatori sono capaci di trasformare un segnale in input (tipicamente elettrico) in movimento.

Esempi: idraulici, pneumatici, elettrici

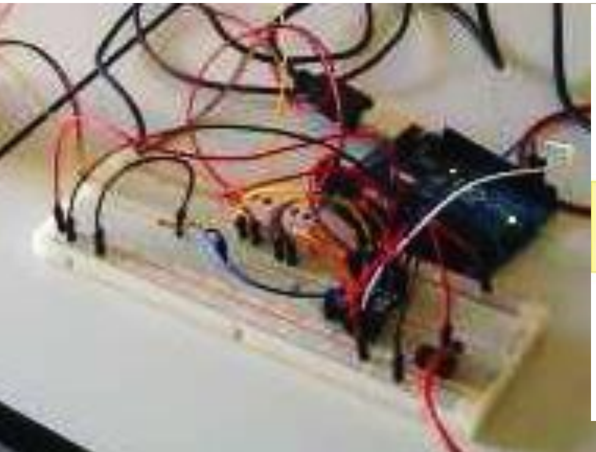
Design and realization of the system

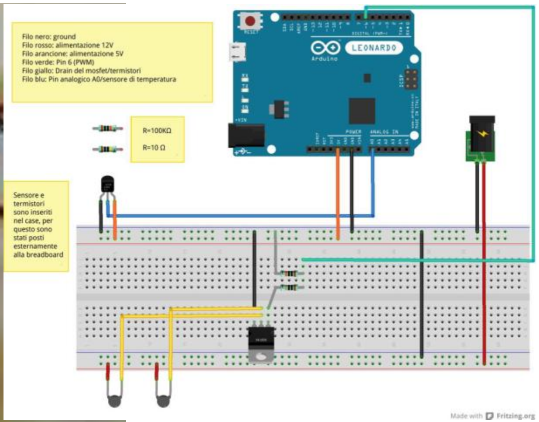
- Design meccanico con sistemi software per la progettazione assistita da computer (Computer-Aided Design, CAD)

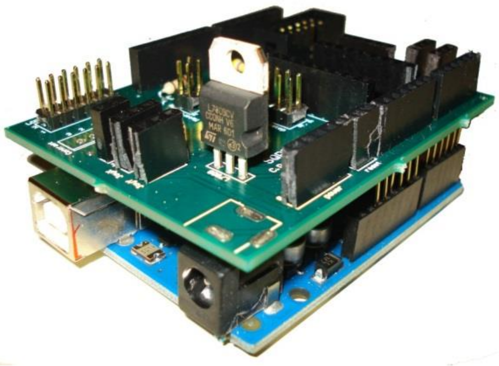




- Design elettronico

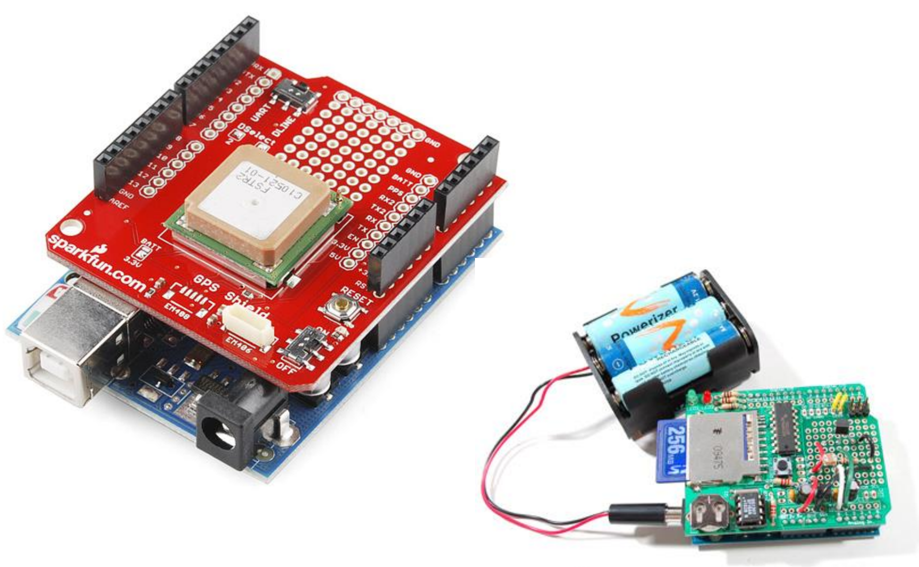


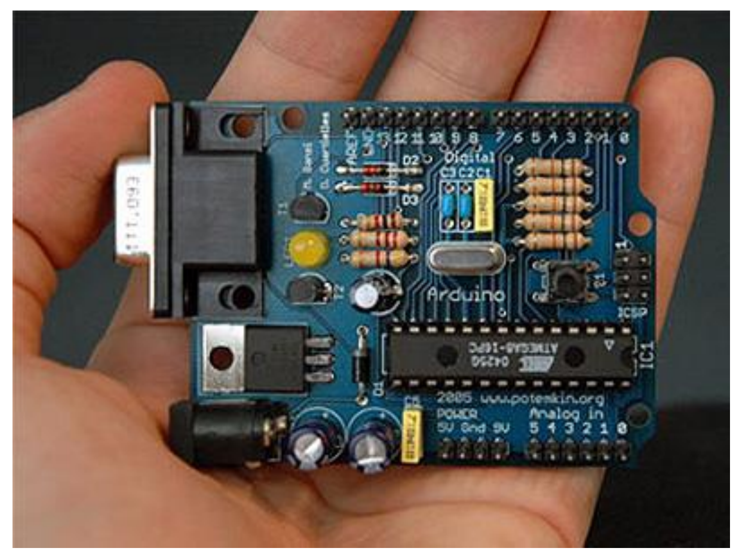




What it is Arduino?

- “Arduino is an open-source physical computing platform based on a simple i/o board and a development environment that implements the Processing / Wiring language. Arduino can be used to develop stand-alone interactive objects or can be connected to software on your computer.“
- A physical Input / Output board (I/O) with a programmable Integrated Circuit (IC).



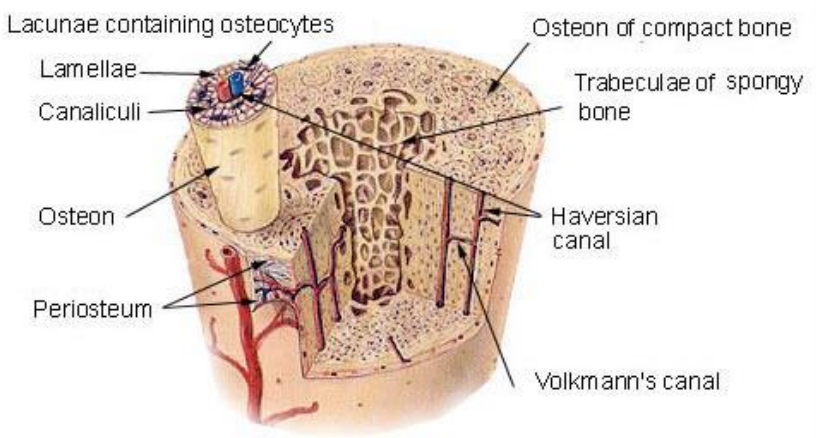


Some examples:

- Bone
- Cartilage
- Ligament
- Heart
- Intestine
- Eye
- Blood vessel
- Lung

Tissue engineering of bone

Bone remodeling is controlled by mechanical as well as metabolic factors. It is postulated that bone contains **sensor cells** that monitor mechanical strain, comparing it to a physiologically desirable range of value, and activating several biological processes when the sensed values are out of range.



The main three types of cells constituting the bone are:

- Osteoblasts
- Osteocytes
- Osteoclasts

It is evident that mechanical loading at physiological strain magnitudes results in an increase of the metabolic activity of **osteocytes** and provides evidence for their involvement in bone mechanotransduction.

<u>Unfortunately, it is not clear how osteocytes actually sense mechanical loading and transduce it into cellular signal.</u>

Osteoblasts have been shown to respond to mechanical stimuli by increased secretion of several proteins, regulated the cell activity and also increased the intercellular communication.

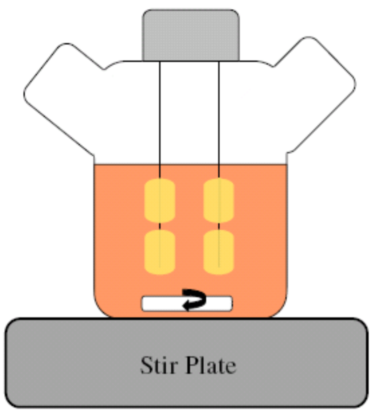
Tissue engineering of bone

Forces applied on bone during movement result in changes of:

- hydrostatic pressure
- direct cell strain
- fluid flow induced shear stress
- electric fields (as a result of fluid flow)

For this reason, a large number of in vitro systems have been developed to simulate in vivo loading environments, using different techniques included:

- Hydrostatic Pressure
- Stretching
- Bending
- Fluid shear stresses
- Direct Compression



Fluid flow -> fluid shear stress
Velocity field
nutrient diffusion

<u>Oxygen level and nutrients supplies are also very important factors</u>

Tissue engineering of bone

The **Rotary Cell Culture System (RCCS)** is a unique cell culture technology for culturing both suspension and anchorage-dependent cells. It is the first bioreactor system designed to simultaneously integrate the ability to co-culture cells, and the features of low shear force (and consequently low turbulence), and high mass transfer of nutrients. Together these properties encourage spheroid formation and proliferation of cells within the three-dimension spheroids.
http://www.synthecon.com/rotary-cell-culture-systems.html

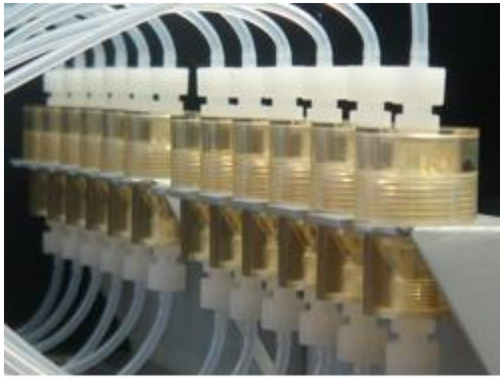
The **Zetos System** is a novel 3D Bioreactor (Zetos™) capable of maintaining human bone biopsies in a viable and responsive state for up to 45 days.
Human bone samples can be mechanically stimulated, maintaining physiological levels of mechanical loads
http://www.smtc.ed.ac.uk/zetossystem.htm

Tissue engineering of bone

TGT's **OsteoGen bioreactors** impart perfusion through cell seeded cylindrical scaffolds. Applications include investigating cell function, modulating the growth and development of engineered tissues, or acting as a test bed for drug and regenerative medicine technologies. Researchers are currently utilizing these systems in a wide range of research areas including:

- Bone stem cell phenotype research
- Mineral deposition of marrowstromal cells

Optional features such as; **transducers**, **non-contact micrometers**, **pressure sensors**, etc., and/or modules to customize the instrument to specific needs can be added to accomodate the research application.



010 - 1 OsteoGen Chambers

120 OsteoGen

BENEFITS		
All-autoclavable components	S	S
Excellent sample access	S	S
Medium change without operning chamber via luer fittings	S	S
Increased sterility with fast and clean sample change and access	S	S
Lightweight design for easy handling	S	S
Easily cleaned surface finishes	S	S
Compact design for fit in standard incubators	S	S
100% satisfaction guaranteed warranty	S	S

Tissue engineering of bone

Bose Corporation has developed a multi-specimen **ElectroForce® BioDynamic®** test instrument for intervertebral disc and other orthopaedic applications to mimic the complex loading that tissues experience in vivo. Spinal discs, cartilage and bone tissues, scaffolds and tissue-engineered constructs can be characterized under multiaxial stimulation.

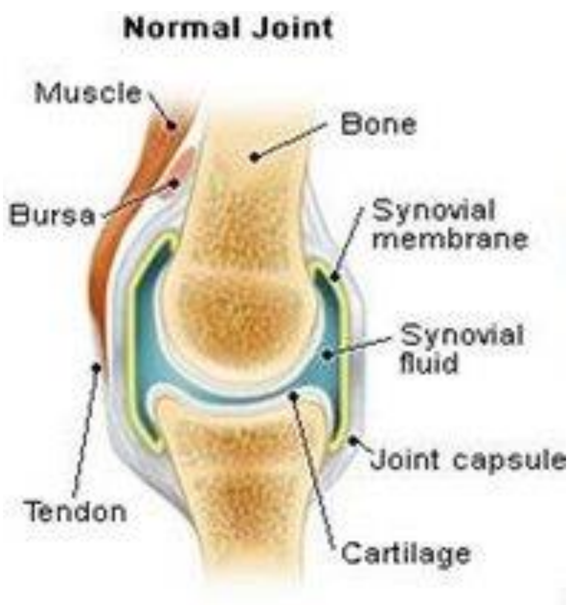
The 5900 BioDynamic test instrument accommodates four disc specimens in a single chamber mounted between porous compression platens. The specimens are subjected to **axial compression**, **pulsatile flow** through porous platens, and radial cyclic **hydrostatic pressure** while maintaining sterility in a cell culture incubator. All system components in contact with the samples and the fluid are sterilizable to allow long term stimulation and characterization in an incubator (See datasheet)

	Axial Compression	Hydrostatic Chamber Pressure	Pulsatile Flow Through Porous Platens
Force	±405 N*	N/A	N/A
Dynamic Volume	N/A	N/A	0.3 mL/pulse
Maximum Pressure	N/A	2250 mmHg (0.3 MPa)	2250 mmHg (0.3 MPa)
Displacement	6 mm	N/A	N/A
Maximum Test Frequency	2 Hz	1 Hz	2 Hz
Transducers	Displacement Four load cells (one per sample)	Pressure	Displacement Eight pressure (two per sample)
Mean Flow Rate	N/A	Mean flow pump: 1-102 mL/min	Four mean flow pumps (one per sample): 1-102 mL/min
Sample Specifications	Number of samples: Four or two samples can be used in the chamber Sample diameter: 5 or 10 mm Sample length: 0-10 mm		
Optional Measurements	Digital video extensometer for strain Laser micrometer for outer diameter pH, dissolved oxygen, carbon dioxide, and lactate/glucose		
Environment	Cell culture incubator-compatible (consult Bose)		

Cartilage Tissue Engineering

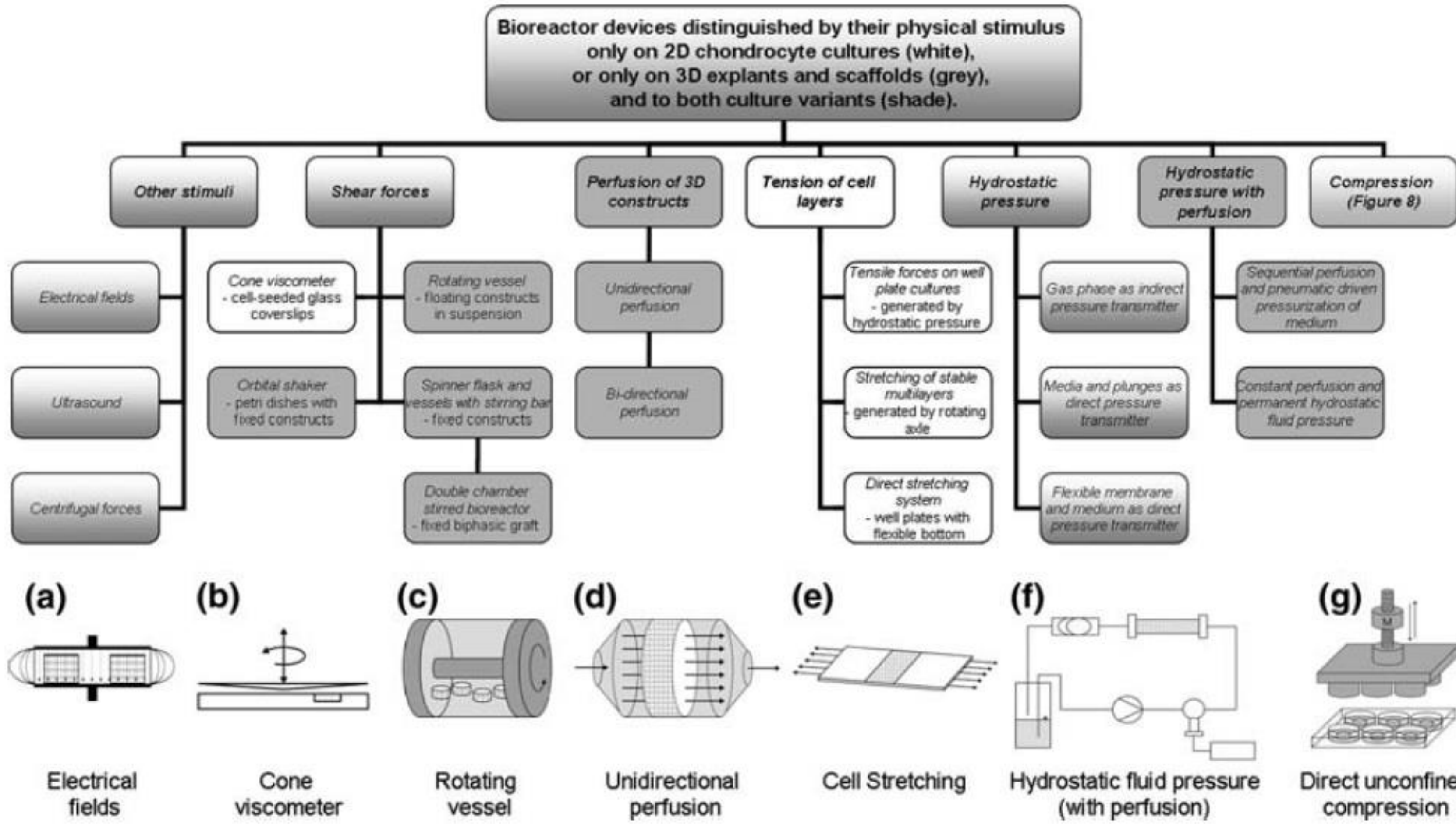
Articular cartilage is affected in vivo by several biomechanical forces and electric gradients as well as changes in the pH.
The dynamic processes that occur in cartilage are necessary to maintain its structure and function and have to be applied in the tissue engineering of cartilage as well.

For the articular cartilage of the major weight bearing joints in the hip and the knee average loadings of about 7-10 MPa and a shear modulus of 2.6 MPa



The **compression** is considered to be the most important form of loading to act on cartilage in vivo. Soon after loading the cartilage with a uniaxial compressive force, an increasing **internal hydrostatic pressure** arises in the tissue. This slow movement together with the inability of aqueous solutions to be compressed is the reason for the increasing hydrostatic pressure
Shear stress is another force that cartilage has to resist when the synovial fluid is pressed alongside the smooth surface of the tissue as a consequence of joint movement

Cartilage Tissue Engineering



Chondrocytes react to these mechanical stimuli modifying their metabolic activity and matrix production (evaluated by glycosaminoglycan content).

Cartilage Tissue Engineering

Illustration of the response of superficial, deep and full depth cells subjected to **15% compression** at a range of frequencies (0.3Hz, 1Hz and 3Hz). GAG synthesis and cell proliferation values were all normalized to unstrained control results

	0.3Hz	1Hz	3Hz
Superficial Cells	↓ GAG ↑ Proliferation	↓ GAG ↑ Proliferation	↓ GAG ↑ Proliferation
Deep Cells	↓ GAG ↔ Proliferation	↑ 50% GAG ↑ Proliferation	↔ GAG ↔ Proliferation
Full Depth Cells	↓ GAG ↑ Proliferation	↑ 40% GAG ↑ Proliferation	↔ GAG ↑ Proliferation

↑ = increase ↓ = decrease ↔ = no change

Illustration of GAG and collagen results obtained by Heath for foal and adult chondrocytes seeded in PGA scaffold and subjected to 5 weeks of intermittent pressure

Intermittent Pressure	*GAG Concentration (mg/g tissue)*		*Collagen Concentration (mg/g tissue)*	
	Foal	*Adult*	*Foal*	*Adult*
Native Cartilage	40 - 120	80 - 120	100 - 150	120 - 180
Control (no pressure)	26.0±24.4	2.0±1.0	6.3±1.6	0.5±0.3
500psi	89.3±31.4	5.7±1.0	6.7±1.9	3.0±0.3
1000psi	133.7±38.5	3.5±1.4	11.9±2.7	7.3±0.5

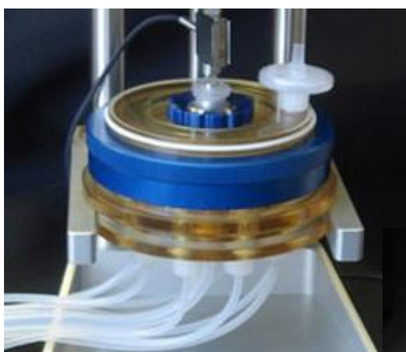
Cartilage Tissue Engineering

CartiGen Bioreactor apply an oscillatory compressive or tensile stimulation to disc shaped samples. A pressurized non-permeable membrane compresses the samples, applying to them a controlled hydrostatic pressure. It integrates a porous platens with the bottom of the chamber to permit perfusion through the sample constructs, to allows the direct flow through the construct while applying oscillatory compressive stimulation.

The **Flexercell®Compression Plus System** is a computer driven instruments that simulate biological compression conditions applying a controlled, static or cyclic compression to the samples: the constructs are compressed between a piston and a stationary plate. This system is composed by three dierent parts: a controller, a compression chamber, and a monitor; it is able to program multiple frequency, amplitude, and wave changes in one regimen. The main limit of this system is its low flexibility: it works just with its BioPress®series culture plates

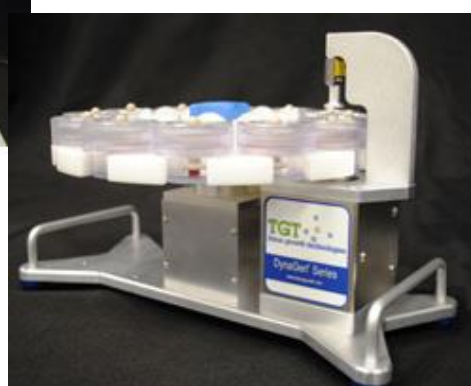
Cartilage Tissue Engineering

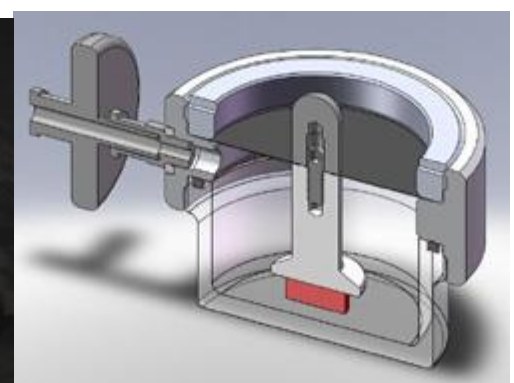
C10 - 12 CartiGen



C9 - X CartiGen Chamber

STIMULATION				
Axial stimulator and reaction frame	S	S	S	
Compressed air and manifold	-	-	-	
CONTROLS				
Controller software, computer, and monitor	S	S	S	
Power supplies and cabling	S	S	S	
Mean flow control	A	-	S	
Dynamic flow control	A	-	S	
TISSUE MONITORING				
Non-contact flow sensors	A	A	A	
Axial load cell	S	S	S	
Pressure transducer	-	-	-	





Cartilage Tissue Engineering

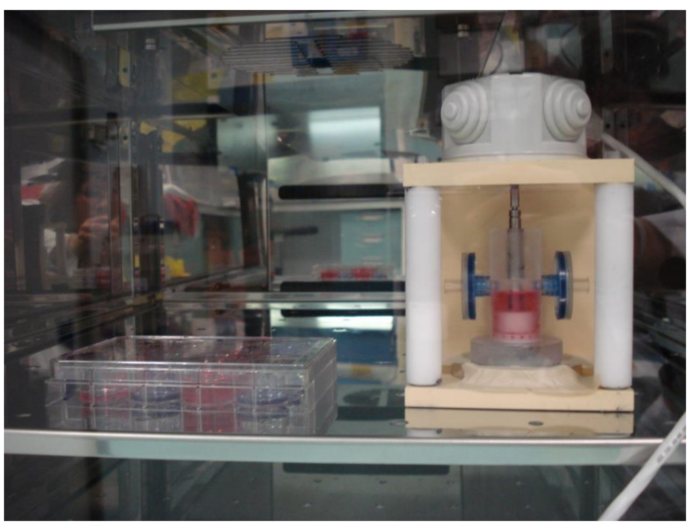
The **Multi Specimen BioDynamic®Test Instruments** can be used for a variety of tissues and biomaterials, using its multimover capabilities for tension/compression loading and dynamic (pulsate) flow stimulation. This system is available in two different designs: multi-frame design, for parametric studies, with three configuration and independent programmability, and multi-chamber system, for statistical analysis, with 4 chambers, in which the same force and pressure are applied.

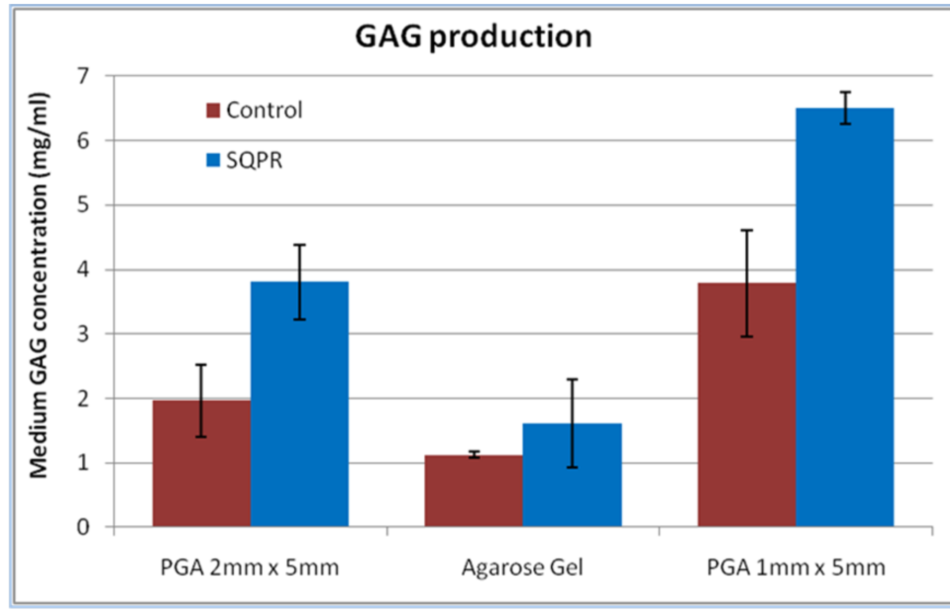
The **TransMIT Pressure system** is projected for cultivation and pressure stimulation of cell-matrix-composites. A special aspect of this bioreactor is that the device is composed in a way that generally available tissue culture plates (with up to 96 cavities) can be used as part of the new bioreactors. The stimulation of the cells or composites can be done with defined or undefined pressure. The durability and frequency of the pressure stimulation are freely programmable by user.

Cartilage Tissue Engineering (SQPR)

The SQPR (Squeeze PRessure) bioreactor was designed to apply a **contactless hydrodynamic pressure** at different frequency, using different cell constructs

Control Unit SQPR





+ 93 % **+ 70 %** **+ 43 %**

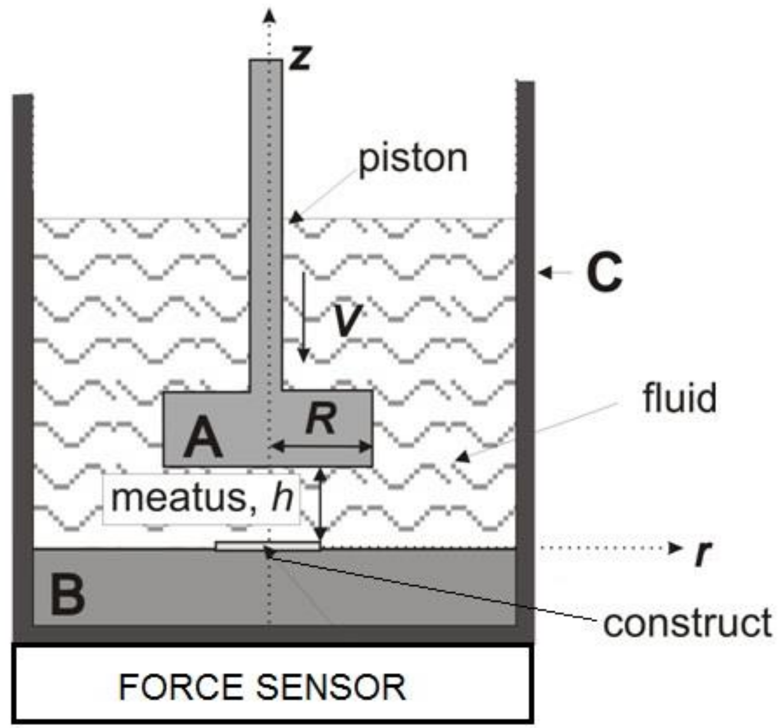
De Maria C, Giusti S, Mazzei, D Crawford A, Ahluwalia A, *Squeeze Pressure Bioreactor a Hydrodynamic Bioreactor for Noncontact Stimulation of Cartilage Constructs*, Tiss Eng, DOI: 10.1089/ten.tec.2011.0002.

Cartilage Tissue Engineering (SQPR)

The SQPR (Squeeze PRessure) bioreactor was designed to apply a **contactless hydrodynamic pressure** at different frequency, using different cell constructs

- il pistone si muove solo in **direzione verticale**
- il movimento del pistone è controllato da un **motore passo-passo**
- sotto la base del bioreattore c'è un sensore di forza

  -> il pistone può toccare il supporto
- posizione iniziale del pistone NON nota



$$p(r) - p_a = \frac{3\mu V}{h^3} R^2 \left(1 - \frac{r^2}{R^2}\right)$$

TE of Ligament and Tendons

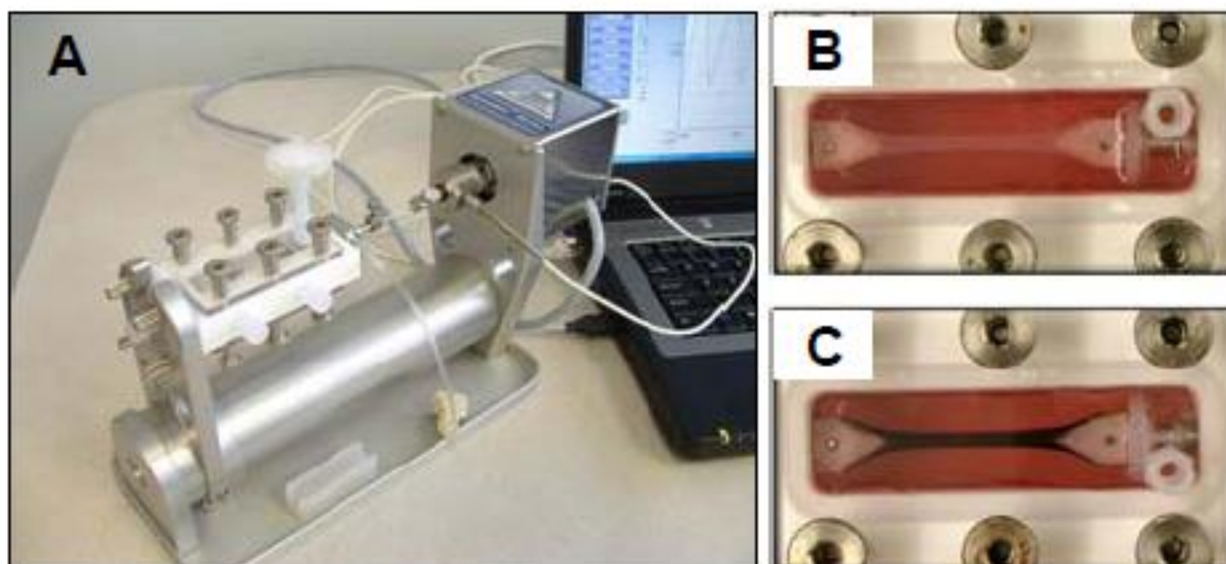
Ligaments and tendons are dense connective tissues dominated by fibroblasts.

It is known that rapid turnover of collagens in the matrix of ligaments and tendons is essential for continuous attachment of muscles to the bone, and that fibroblasts are aligned with collagen fibrils in vivo.

A recent study demonstrates how the ECM in tendons and ligaments reacts to mechanical stress (**axial stretch/compression**) by a molecular adaptation. In healing ligaments, mechanical loading has been shown to affect the organization of collagen fibers and alignment of fibroblasts.

A) Image of stimulator and bioreactor chamber assembly.

B) and C) show details of bioreactor chamber with control and SWNT-loaded constructs, respectively.



Cardiovascular Tissue Engineering

"Studies on the in vitro culture of cardiac tissue are **more complex**, **less advanced**, and **less common** than those concerning cartilage, bones, and ligaments. <u>The effects of the parameters used during cell culture and their mechanisms are still largely unknown</u>.

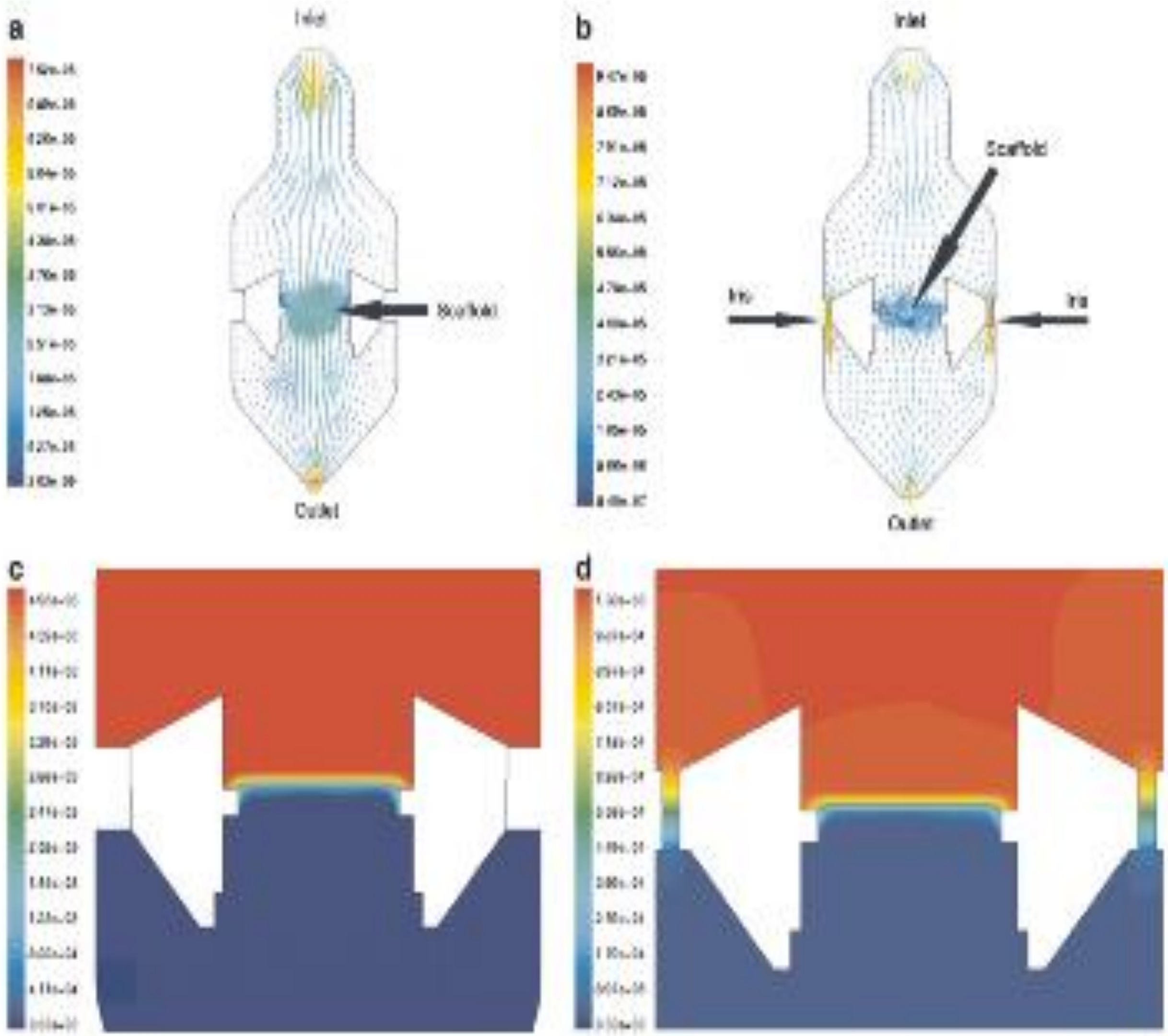
<u>Bioreactors</u> can therefore be used to better understand various phenomena involved in the mechanisms of the cardiac tissue regeneration"

Bilodeau and Mantovani, Tissue Eng, 2007

Blood vessels and Heart valves	Should be coated with endothelial cells, so bioreactors apply **controlled fluid shear stress, pulsatile flow, cyclic strain**
Myocardial substitutes	Stimulationn of cardiac muscle (cardiomyocytes) by applying **fluid flow, cyclic tension and compression, elctric fields**

Cardiac myocytes cannot tolerate hypoxia for long time

Blood vessels and heart valves



Laminar flow bioreactor for **primary human mesenchymal stem cells** [1].

Macroporous ceramic scaffolds (d. 10 mm, h 3 mm, pore size 300 µm)

Estimated Reynolds number:
Irises closed: 2000
Irises opened: 600

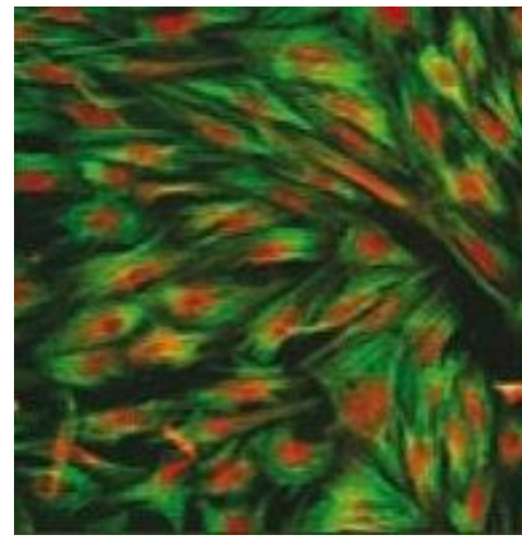
1) Weyand et al. *Bioresearch open Access,* vol 1, num. 3, 2012

Blood vessels and heart valves

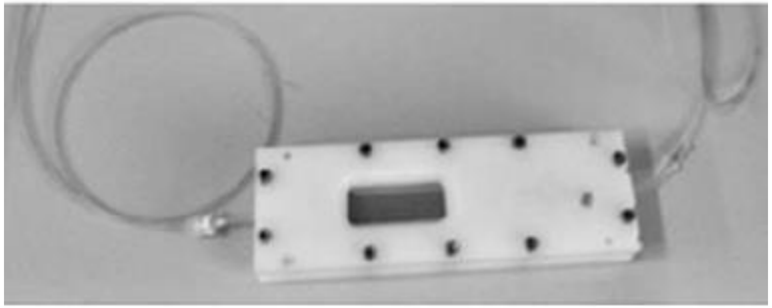
IBIDI® labware:

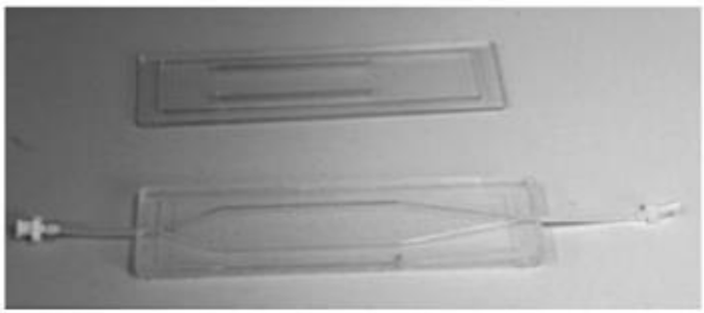
a) High shear stress chamber
b) Suitable conditions for vascular endothelial cells
c) Observable using inverse microscope or confocal microscopy

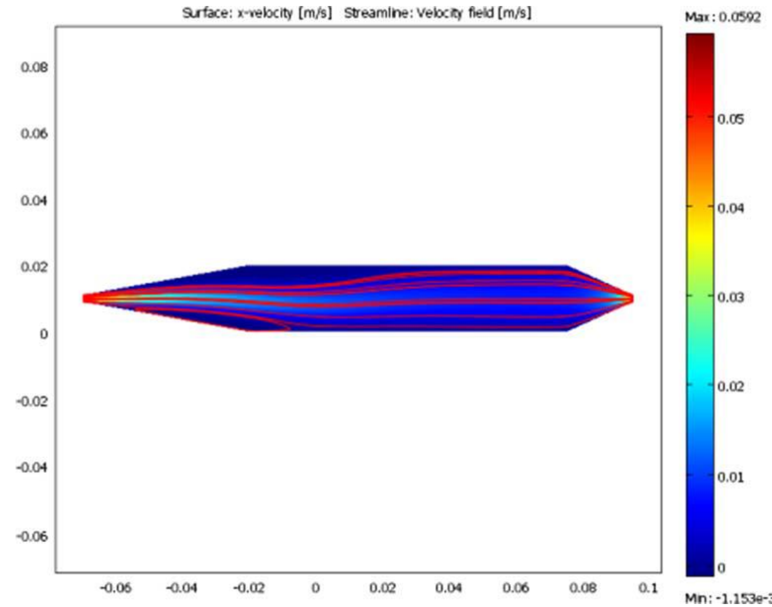
Air bubbles problem

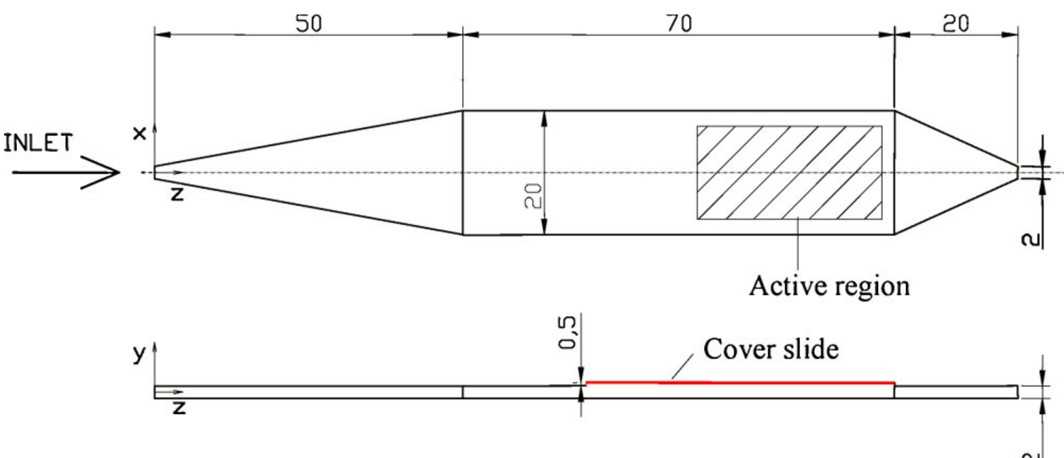


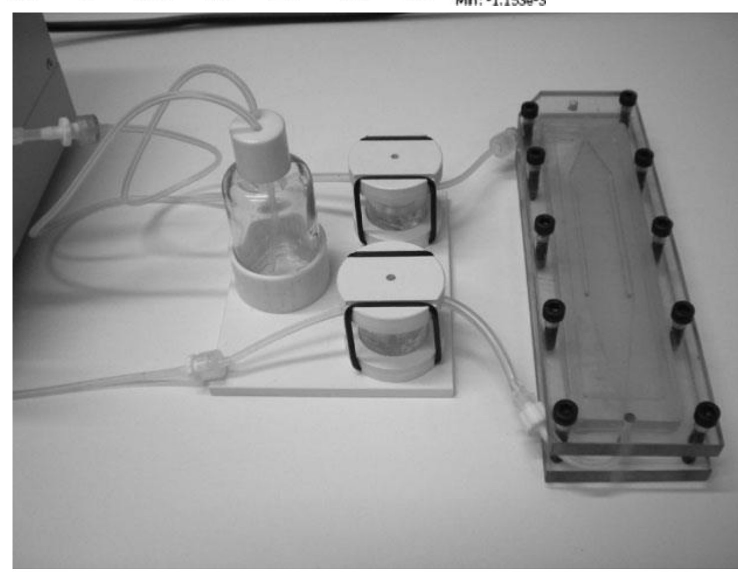
Blood vessels and heart valves





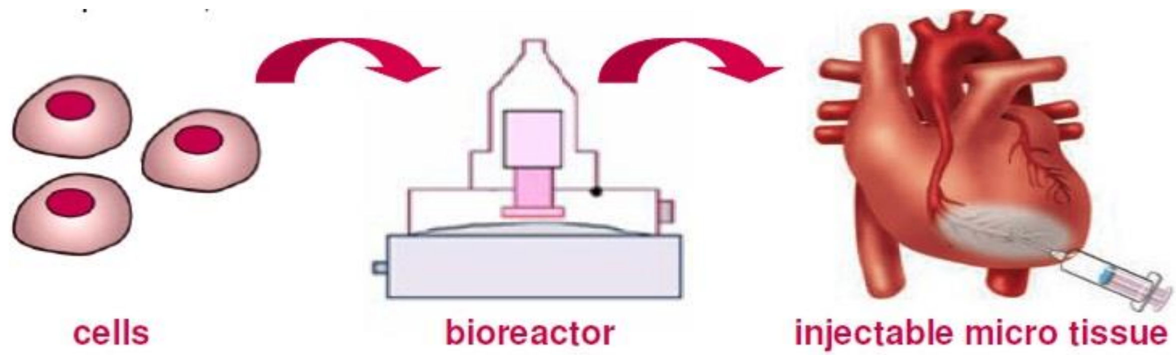


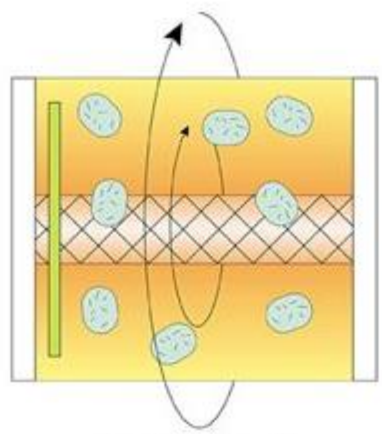


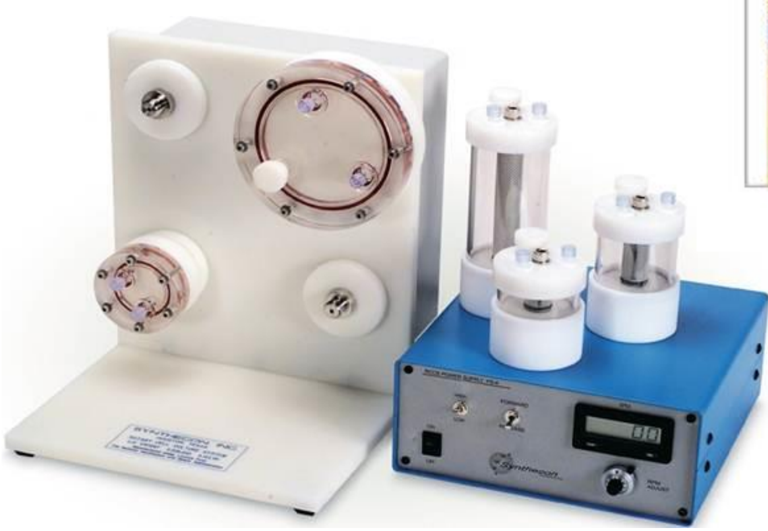


Cardiac Tissue

- Media flow
- Pulsatile flow with perfusion
- Shear stress
- Pressure
- Stretch
- Electrical stimuli
- Direct compression







It has been found that a **low shear stress environment is advantageous for initial cell** and tissue growth in a bioreactor. However, once cultured the **mature tissue can be exposed to an higher range of shear stress**, depending on the in-vivo environment

Barron, 2003

Cardiac Tissue

Biotechnol. Prog. **2008**, *24*, 907–920

- Pressure
- Stretch
- Electrical stimuli
- Direct Compression

Pulsatile Perfusion Bioreactor for Cardiac Tissue Engineering

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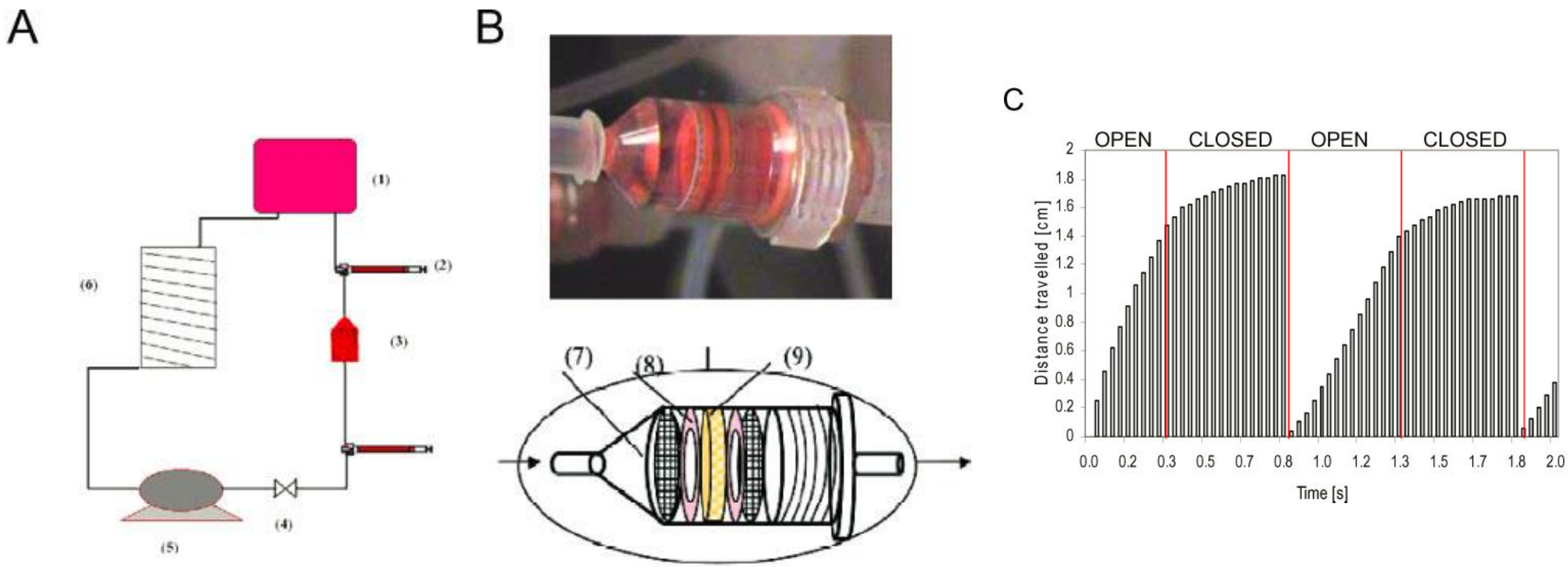
Dept. of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada



Experimental set-up for pulsatile perfusion cultivation.
(A) Schematics of the perfusion loop that includes (1) medium reservoir, (2) debubbling syringes (3) perfusion chamber, (4) solenoid pinch valve, (5) peristaltic pump, and (6) gas exchanger. (B) Cardiac tissue construct (9) is placed in the perfusion chamber between two (8) silicone gaskets and (7) two polypropylene meshes. (C) A representative flow visualization profile indicating the presence of pulsatile medium flow at the frequency of 1 Hz. The distance traveled by the dye front in the tubing (ID 0.063 in., OD 0.125 in., Wall 0.31 in.) was measured with the valve opened and closed at the frequency of 1 Hz (0.5 open/0.5 s closed) and the nominal flow rate set at the pump to 1.5 mL/min.

Cardiac Tissue

- Pressure
- Stretch
- Electrical stimuli
- Direct Compression

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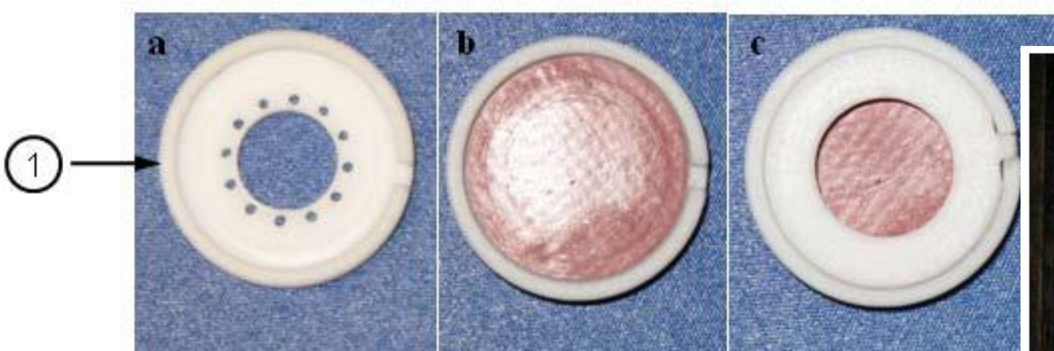
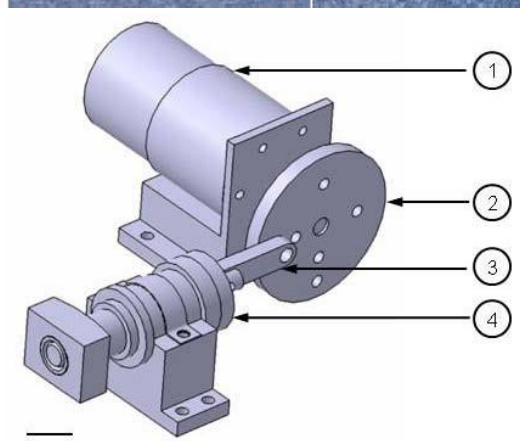
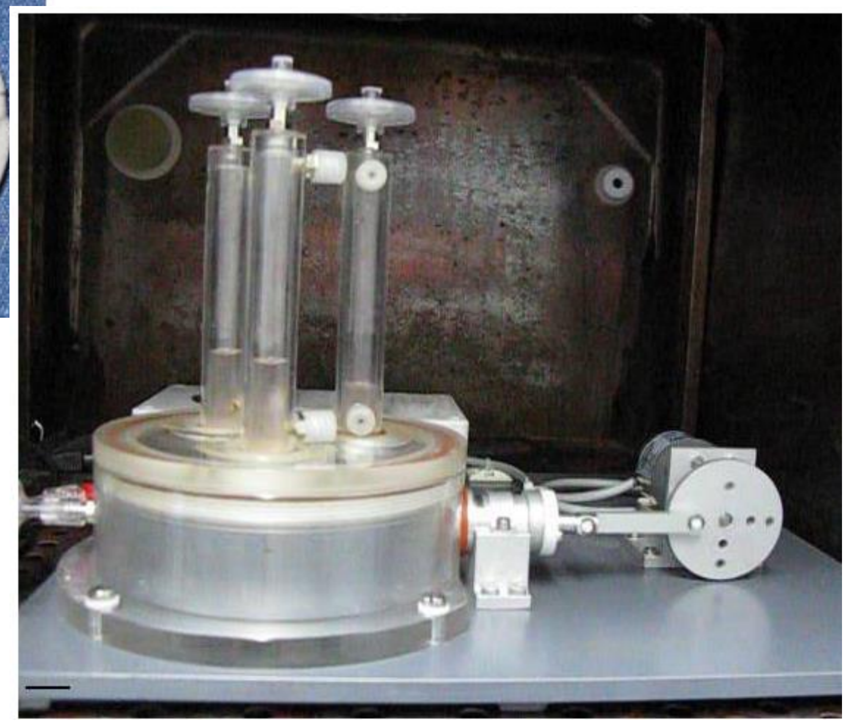
Article

A Novel Pulsatile Bioreactor for Mechanical Stimulation of Tissue Engineered Cardiac Constructs

Trixi Hollweck [1,†], Bassil Akra [1,†], Simon Häussler [1], Peter Überfuhr [1], Christoph Schmitz [1], Stefan Pfeifer [2], Markus Eblenkamp [2], Erich Wintermantel [2] and Günther Eissner [1,*]







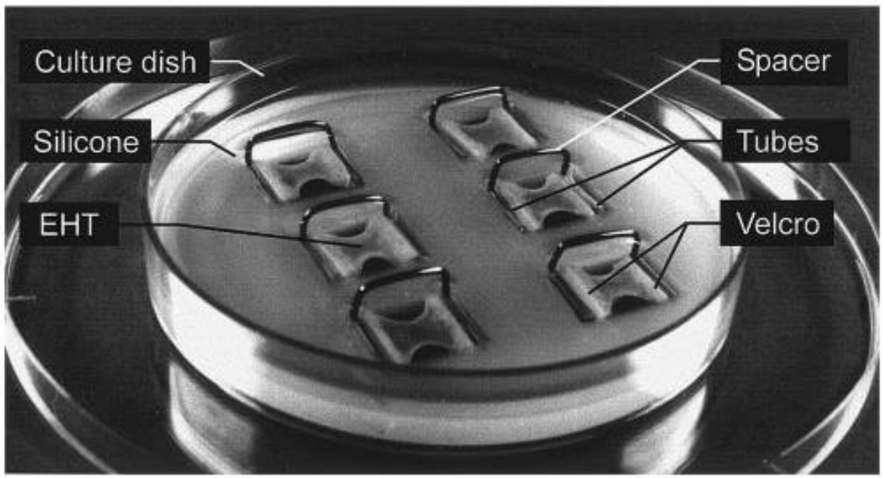
Cardiac Tissue

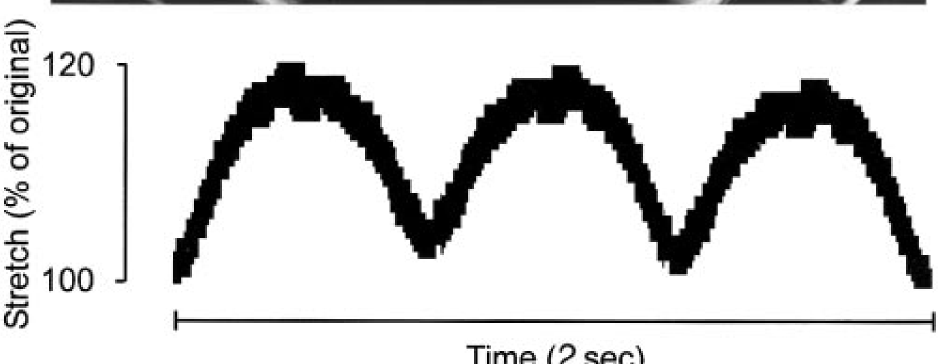
- Pressure
- Stretch
- Electrical stimuli
- Direct Compression

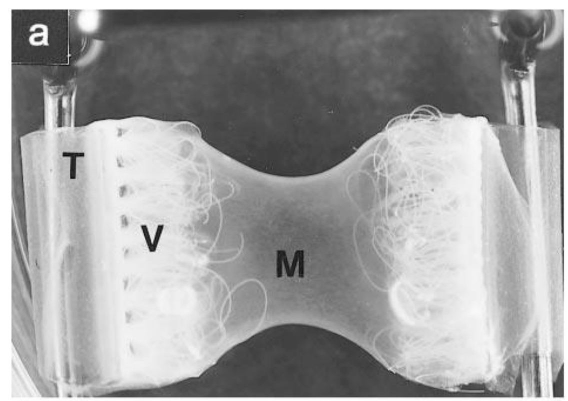
Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement

CHRISTINE FINK, SÜLEMAN ERGÜN,* DIRK KRALISCH, UTE REMMERS, JOACHIM WEI AND THOMAS ESCHENHAGEN[†,1]

Institute of Experimental and Clinical Pharmacology and Toxicology and *Institute of Anatomy, University-Hospital Eppendorf, Hamburg, Germany; and †Institute of Experimental and Clinical Pharmacology and Toxicology, Erlangen, Germany

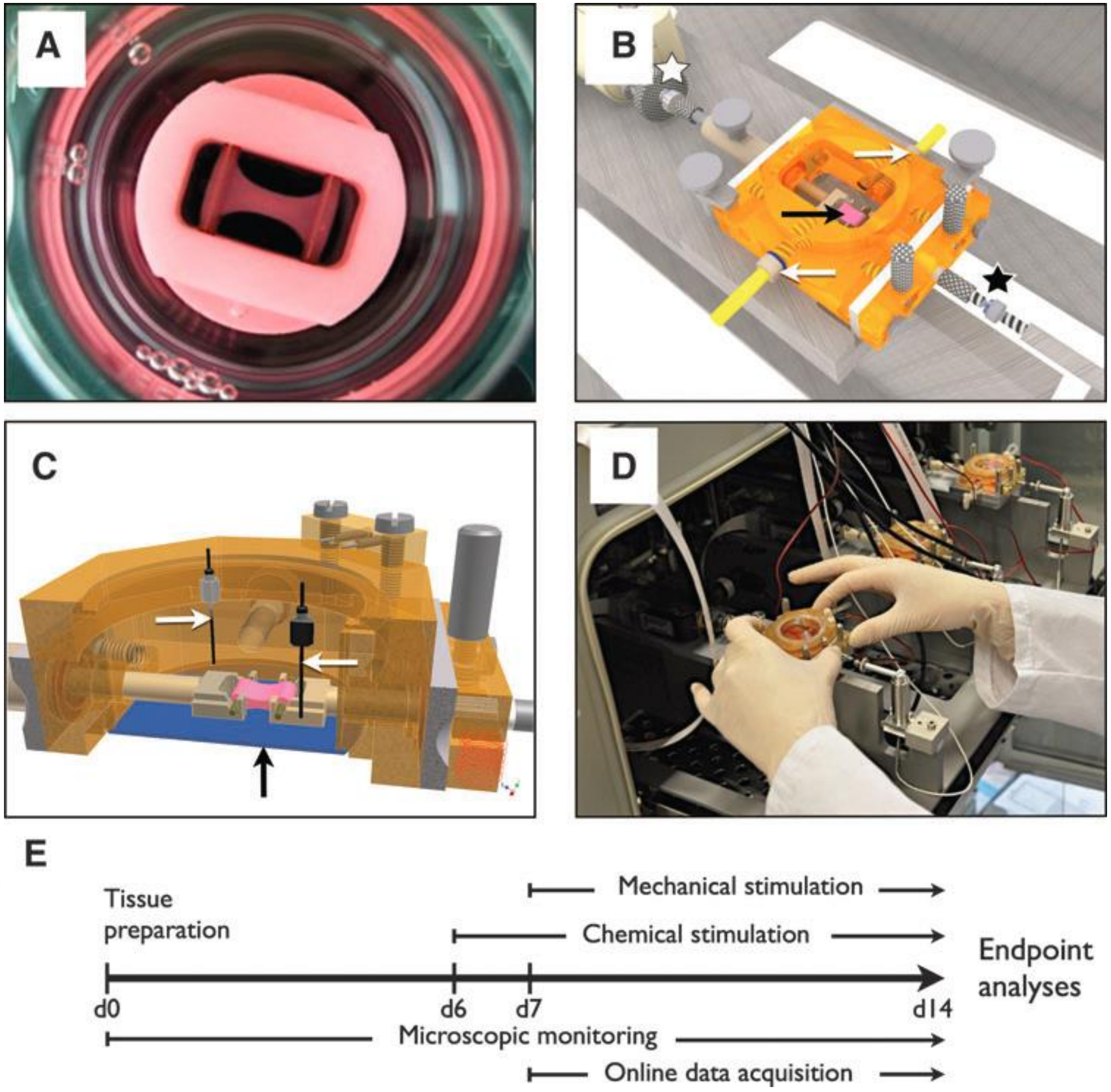






Cardiac Tissue

- Pressure
- Stretch
- Electrical stimuli
- Direct Compression



TISSUE ENGINEERING: Part C
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DOI: 10.1089/ten.tec.2010.0405

A Novel Miniaturized Multimodal Bioreactor for Continuous *In Situ* Assessment of Bioartificial Cardiac Tissue During Stimulation and Maturation

George Kensah,[1,2,*] Ina Gruh, Ph.D.,[1,2,*] Jörg Viering,[3] Henning Schumann, Ph.D.,[3]
Julia Dahlmann,[1,2] Heiko Meyer, Ph.D.,[2,4] David Skvorc,[1,2] Antonia Bär, Ph.D.,[1,†] Payam Akhyari, M.D.,[1,‡]
Alexander Heisterkamp, Ph.D.,[2,4,5] Axel Haverich, M.D.,[1,2] and Ulrich Martin, Ph.D.[1,2]

Cardiac Tissue

Construction of three-dimensional vascularized cardiac tissue with cell sheet engineering

Katsuhisa Sakaguchi [a], Tatsuya Shimizu [b], Teruo Okano [b,*]

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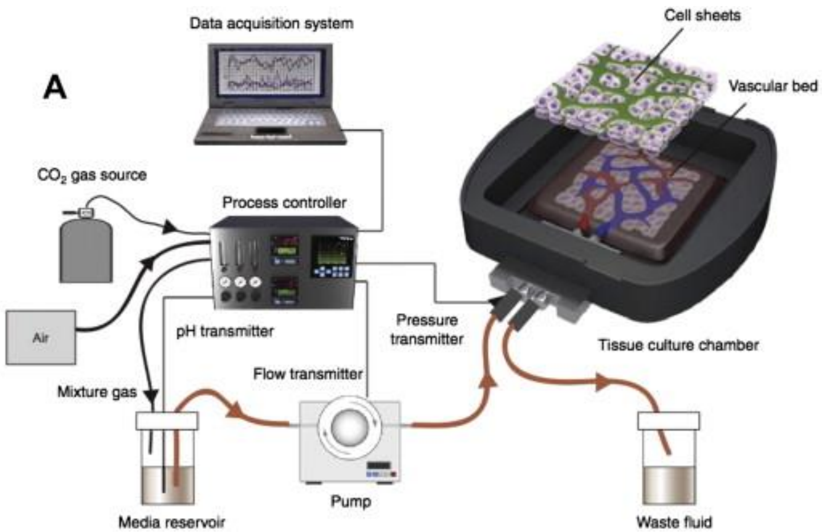
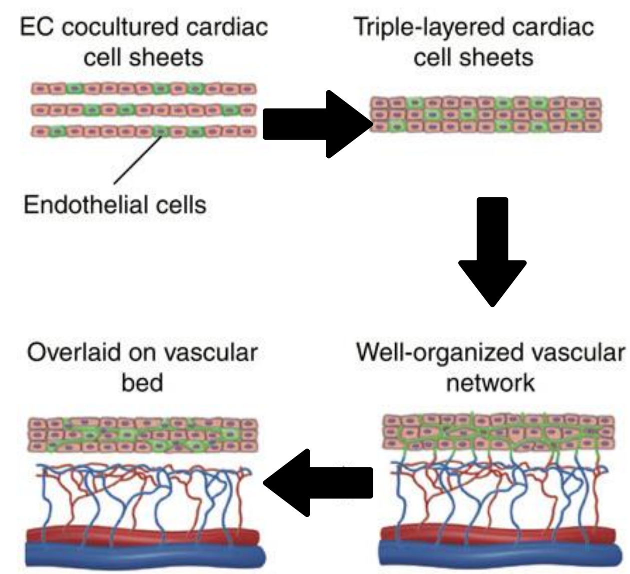
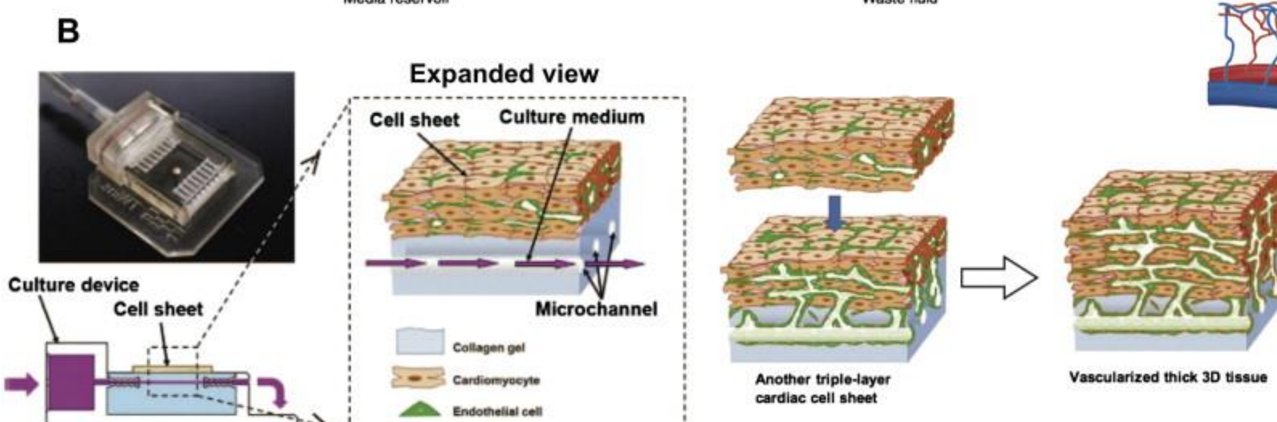
[b] Institute of Advanced Biomedical Engineering and Science, TWIns, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

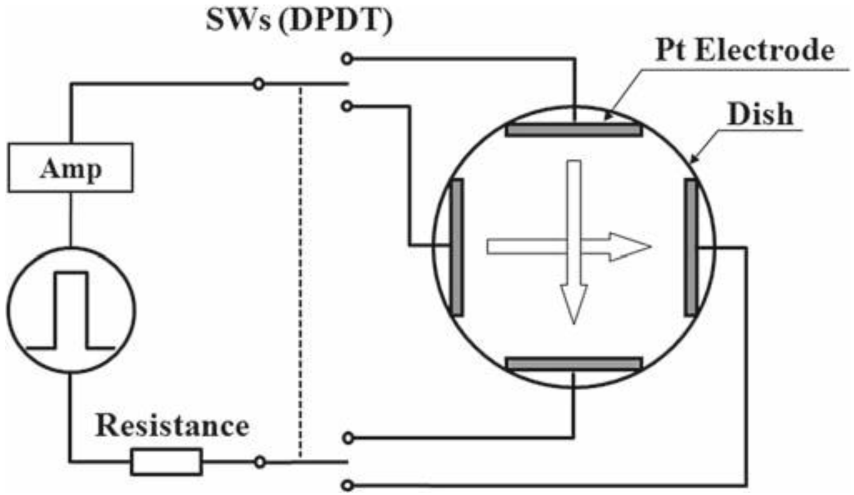




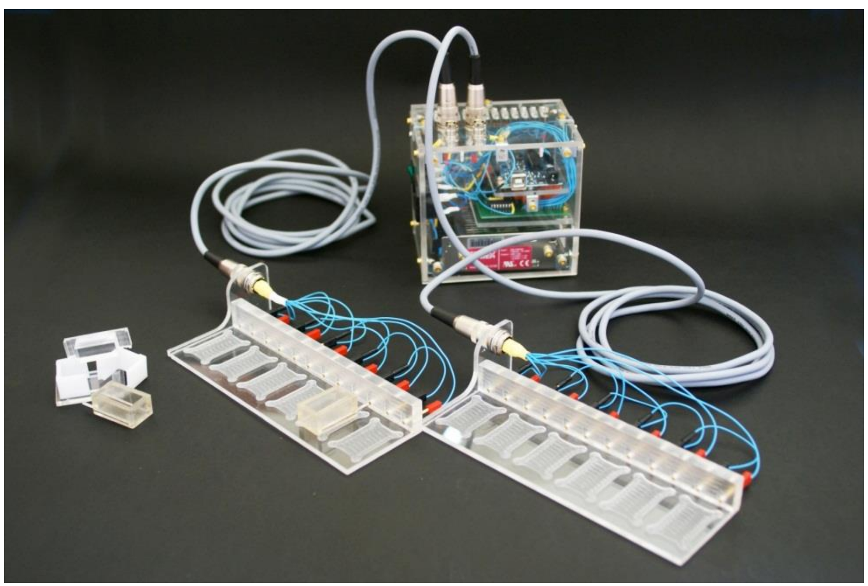


Cardiac Tissue

- Pressure
- Pulsatile flow
- Shear stress
- Stretch
- Electrical stimuli



Cells are seeded on myotubes



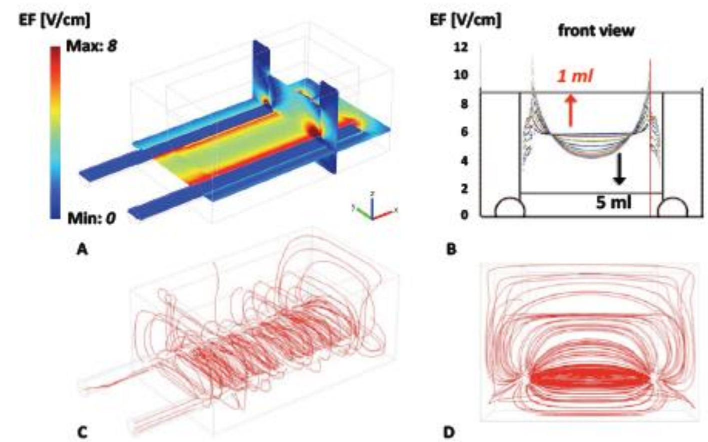
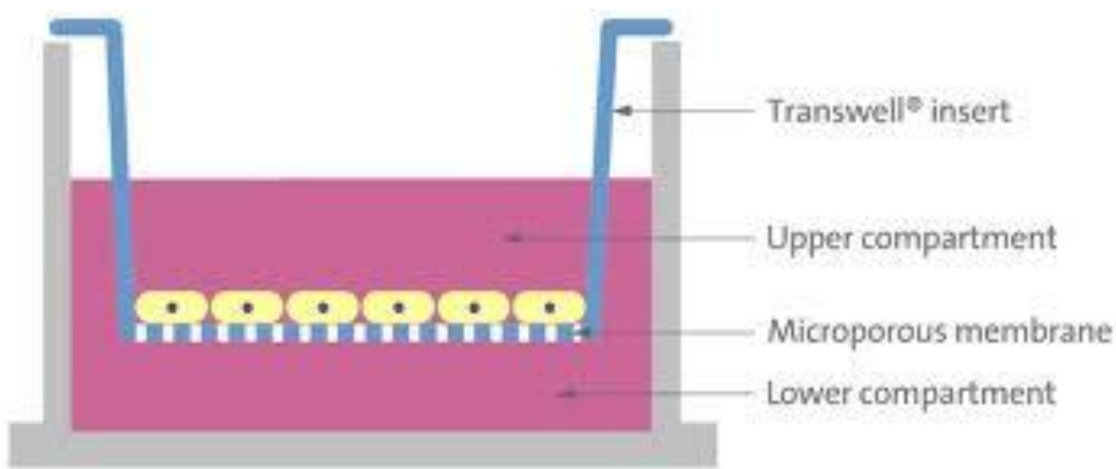


Fig. 3. Distribution of the EF within the culture chamber: contours of the EF magnitude (A) and EF streamlines distribution (C, D) with a 8-V amplitude square voltage input; (B) EF magnitude and distribution evaluated at different medium volumes within the chamber.

In-vitro models of physiological barriers

- Intestine
- Lung
- Brain-Blood Barrier
- Bladder
- Retina



Static “classic” model

(0.4 µm pore size, 2 mL, media change for each permeability assay)

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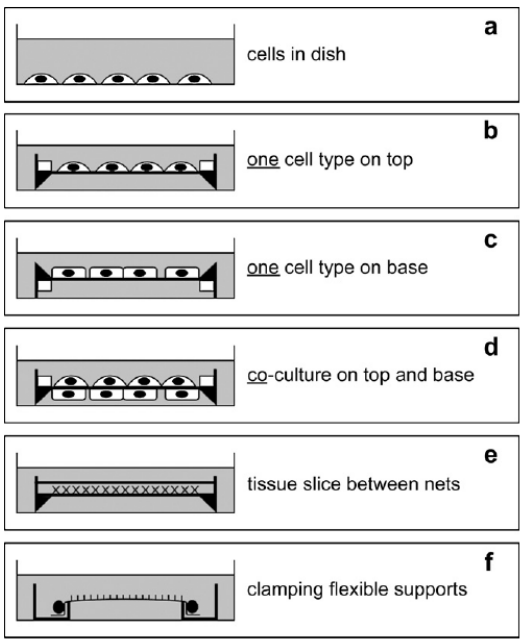

A modular culture system for the generation of multiple specialized tissues☆

Will W. Minuth*, Lucia Denk, Anne Glashauser

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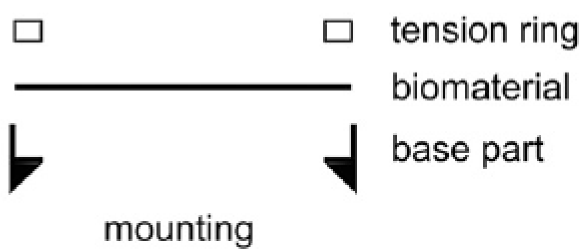

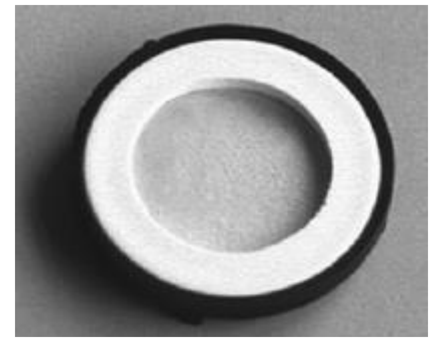

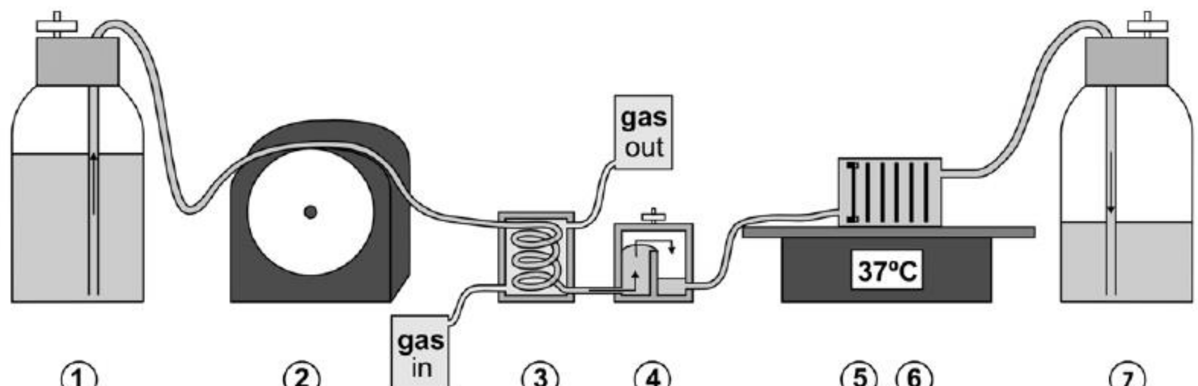

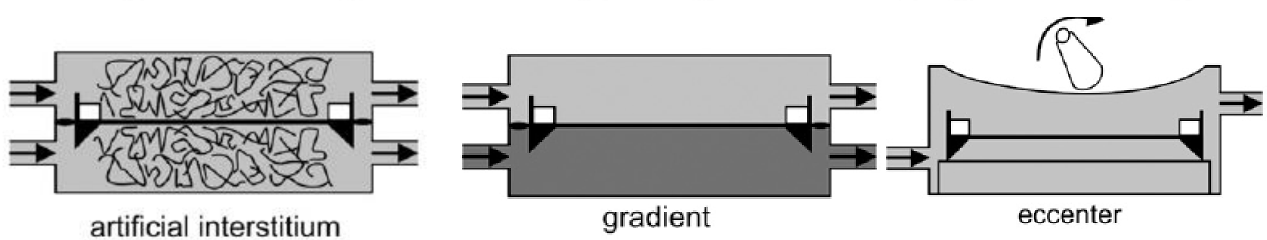

In-vitro models of physiological barriers

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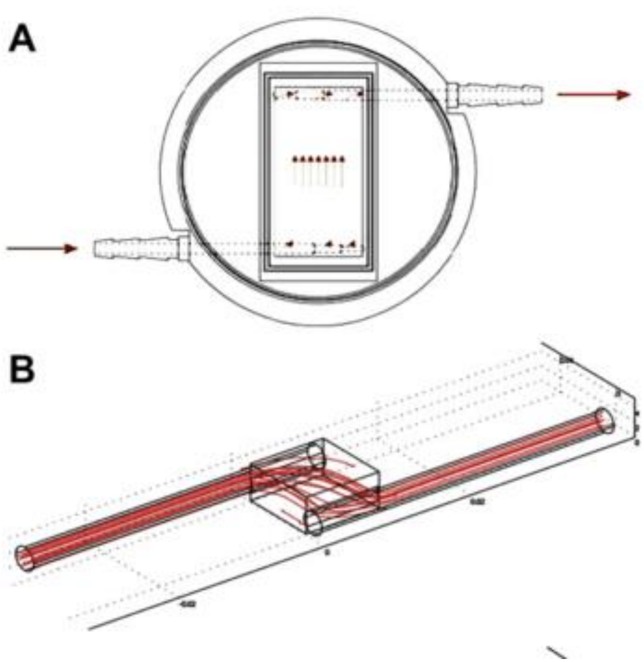
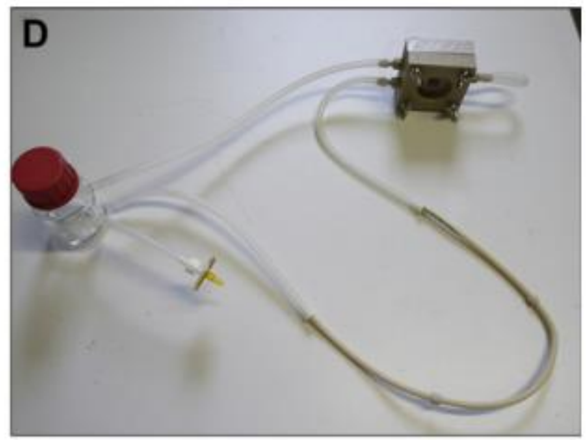
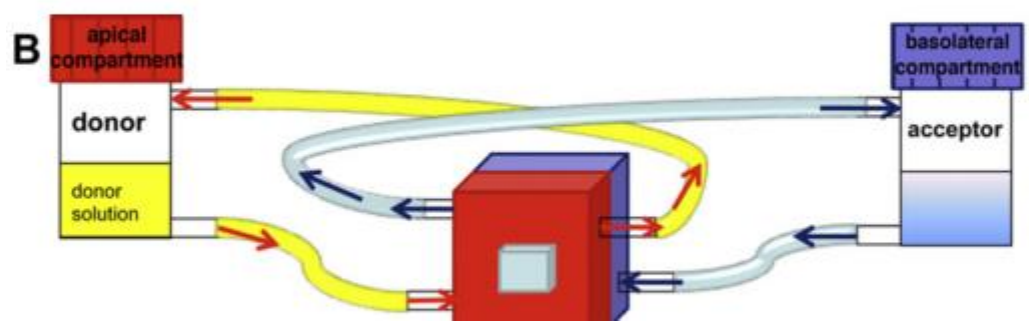
The physiological performance of a three-dimensional model that mimics the microenvironment of the small intestine

Jacqueline Pusch [a], Miriam Votteler [a,b], Stella Göhler [a], Jasmin Engl [a], Martina Hampel [a,c], Heike Walles [a,d], Katja Schenke-Layland [a,b,*]







In-vitro models of physiological barriers

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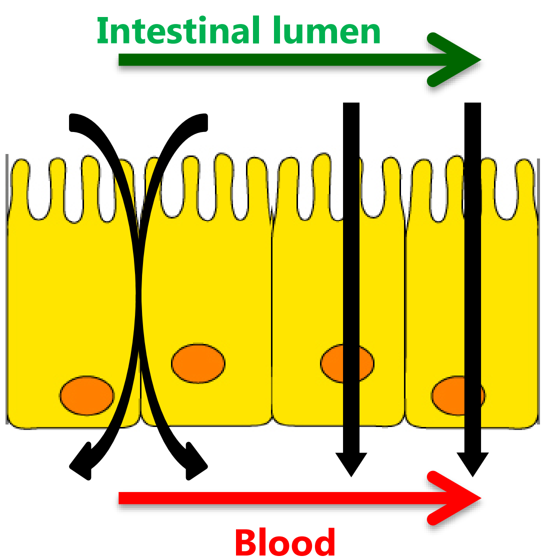
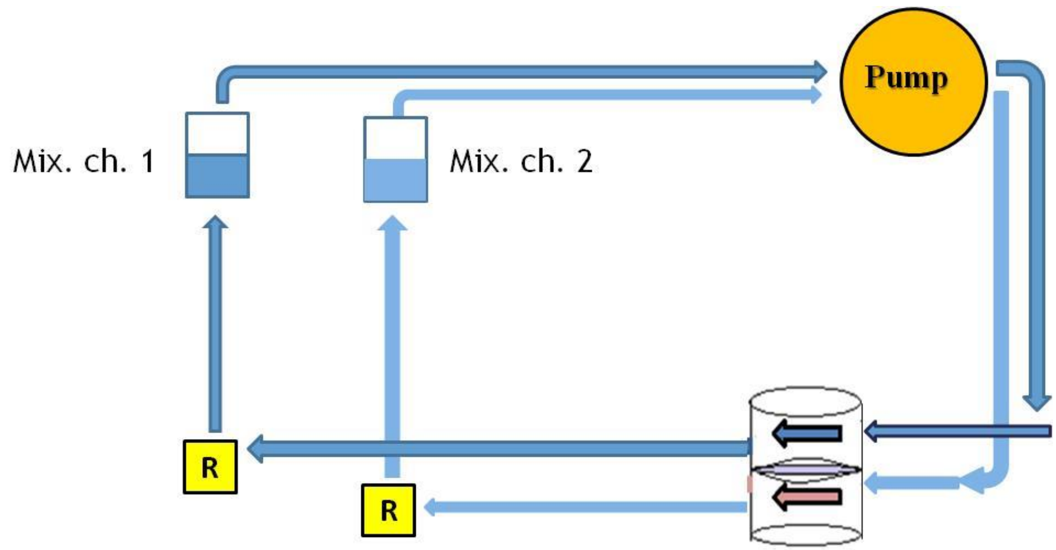
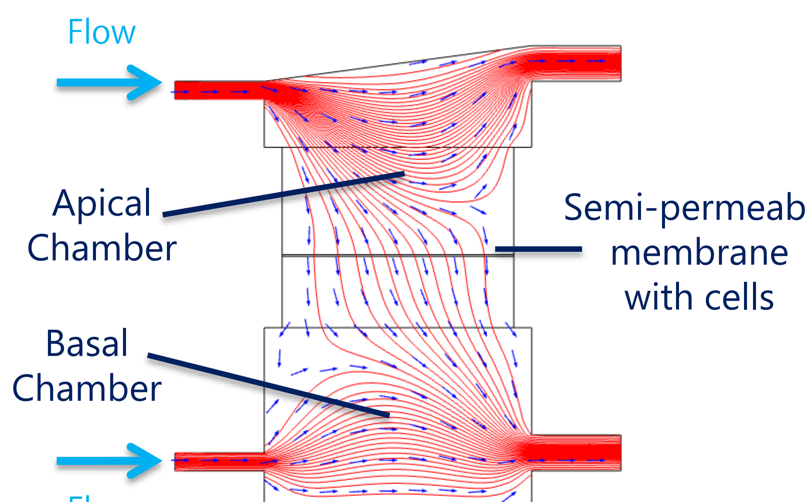
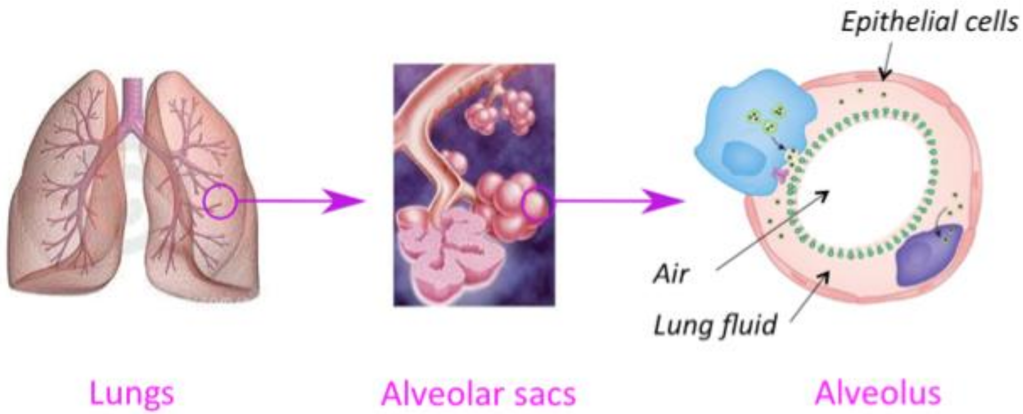






Dynamic Model

In-vitro models of physiological barriers



- Intestine
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In-vitro model of lung:

- Air-liquid interface
- Blood-like circulation in the bottom circuit
- Alveolar movement