Tissue Engineering : basic principles for proper cells' function

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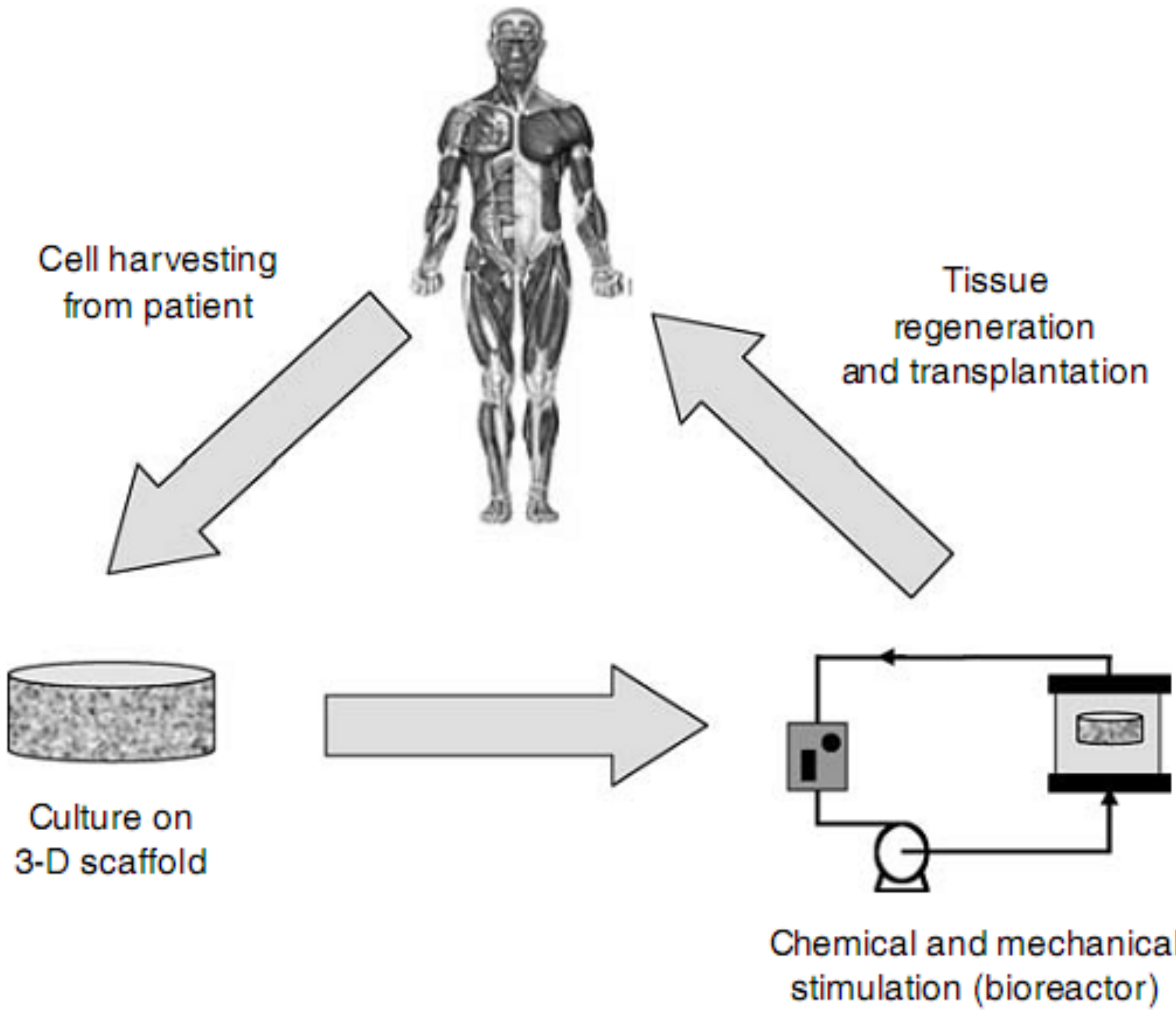
School lecture, Madrid, June 19, 2015

Basic steps in the process of tissue engineering



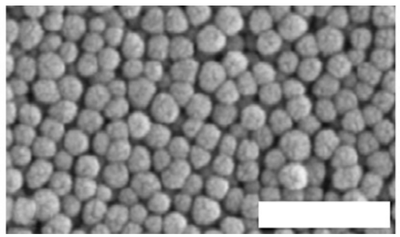
Cell's microenvironment

- Soluble/insoluble chemical factors
- Surface topography
- Mechanical forces

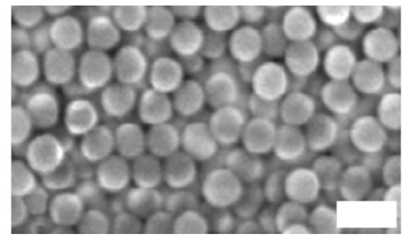
Effects of biomaterial – scaffold properties on f-actin

Morphological characteristics of a biomaterial surface play an important role in determining cellular behaviour

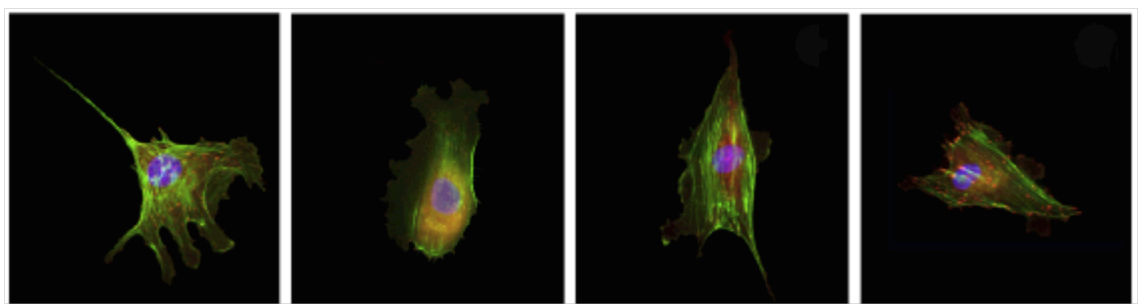
Glass substrates coated with monodispersed silica nanoparticles (NP) of 50, 100 and 300 nm in diameter are surfaces of identical chemistry, but varied roughness



100 nm silica NP-modified Gl



300 nm silica NP-modified Gl



glass 50 nm NP 100 nm NP 300 nm NP

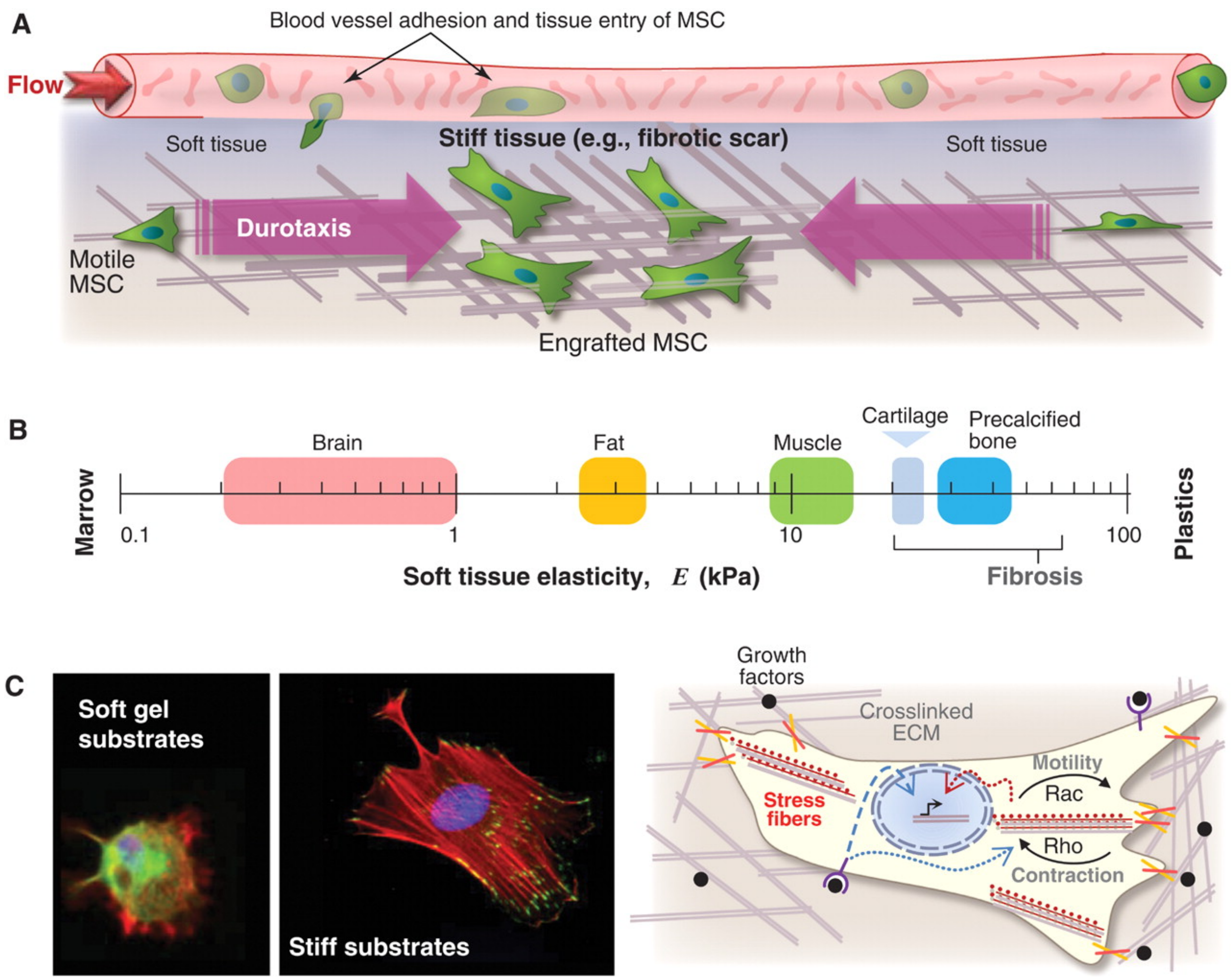
Images of BAEC cells cultured on NP-modified surfaces. Cytoskeletal F-actin fibers (green), FAC (red), and nucleus (blue)

Increasing NP size, which results in an increase in surface nanoroughness, increases F-actin alignment with respect to the cell's major axis

F-actin fibers begin to span the entire length of the cell, and FACs appear to localize at the cell periphery where the fibers terminate.

A. M. Lipski et al., (2008). The effect of silica nanoparticle-modified surfaces on cell morphology, cytoskeletal organization and function. *Biomaterials, 29,* 3836–3846.

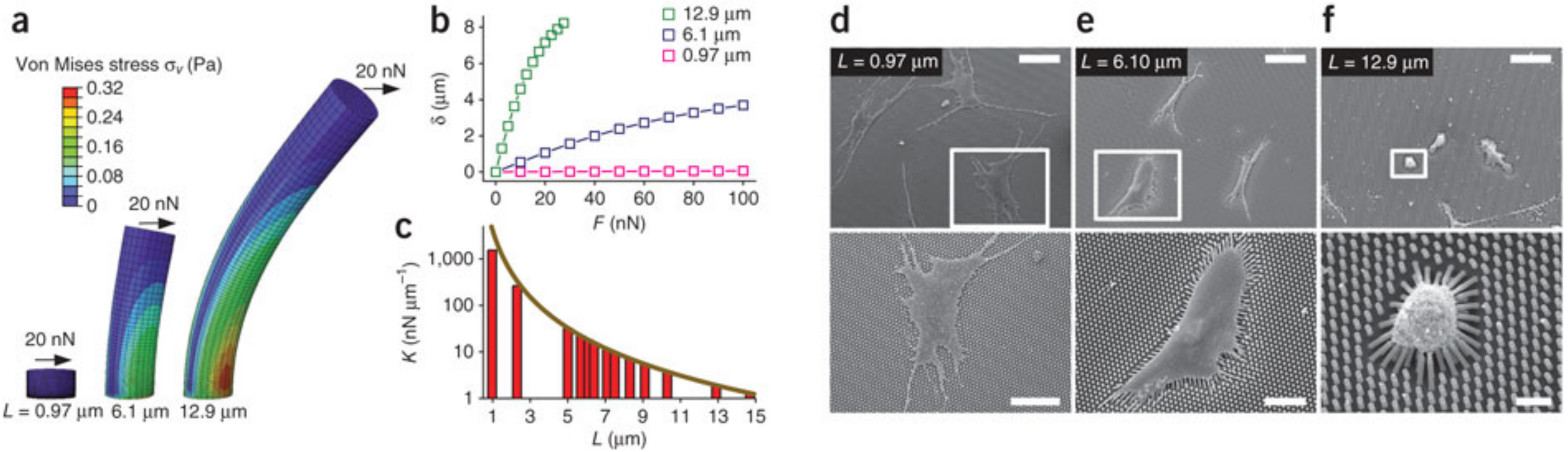
Fig. 2 Forces and ECM in stem cell trafficking.

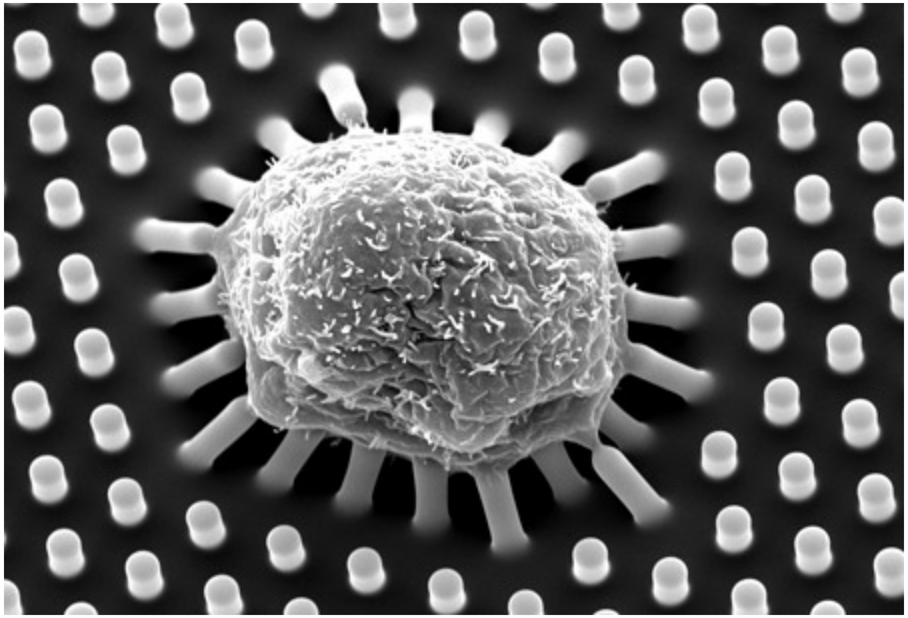


D E Discher et al. Science 2009;324:1673-1677

Science AAAS

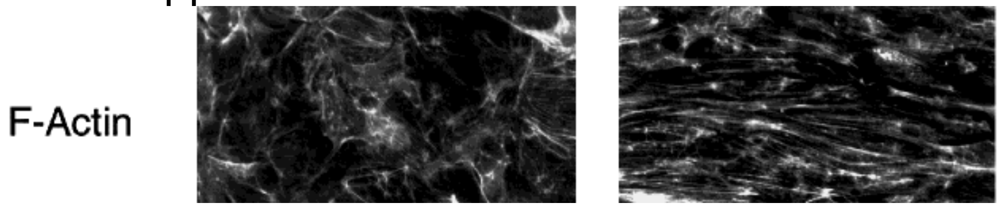
Published by AAAS


a
Von Mises stress σv (Pa)
0.32
0.24
0.16
0.08
0
20 nN
20 nN
20 nN
L = 0.97 µm 6.1 µm 12.9 µm
b
12.9 µm
6.1 µm
0.97 µm
δ (µm)
F (nN)
c
K (nN µm−1)
L (µm)
d
e
f
L = 0.97 µm
L = 6.10 µm
L = 12.9 µm



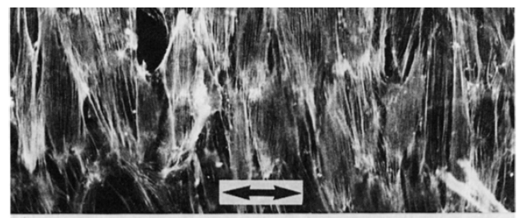
Effects of mechanical stimulation on f-actin

Fluid shear stress stimulation induces endothelial cells to elongate and align in the direction of applied flow



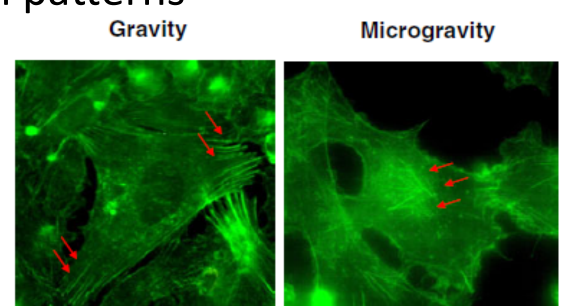
A. M. Malek et al., (1996). Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. *Journal of Cell Science 109*, 713-726.

Cyclic stretching (diameter oscillations, strain along the axis) cause actin filaments to orient in parallel alignment perpendicular to the stretch direction



P. C. Dartsch, (1989). Response of cultured endothelial cells to mechanical stimulation. *Basic Res Cardio, 184,* 268-281.

Gravity influences the migration profile of endothelial cells and is correlated with actin polymerization patterns

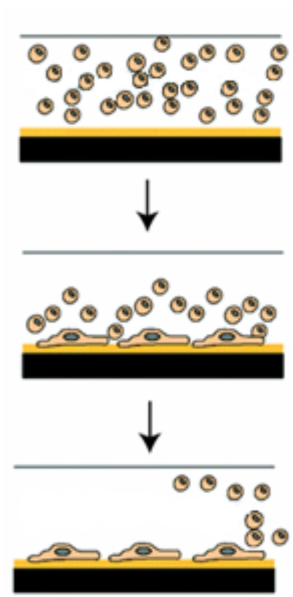


J. H. Siamwala, (2010). Simulated microgravity perturbs actin polymerization to promote nitric oxide-associated migration in human immortalized Eahy926 cells. *Protoplasma, 24,* :3–12.

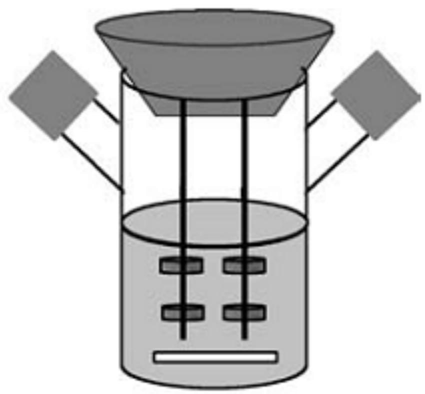
Cell seeding in bioreactors

The aim of the seeding process is to ensure quality, reproducibility, efficiency and uniformity

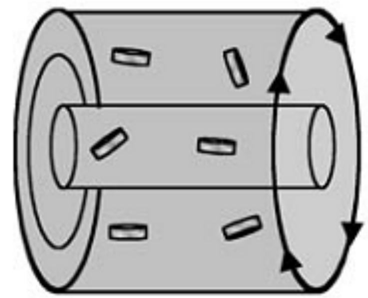
Available protocols



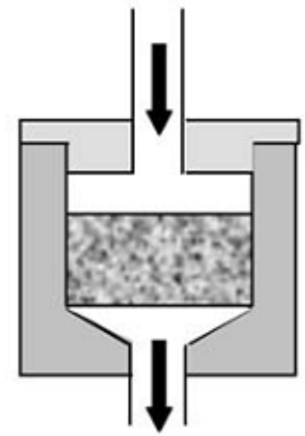
Static seeding



Spinner flask



Rotating wall



Perfusion system

From experimental, trial and error protocols → To computational models :

- complex pore architecture
- fluid dynamics
- kinetics of cell adhesion
- biomaterial properties

Physical conditioning of developing tissues

Fluid-driven mechanical stimulation

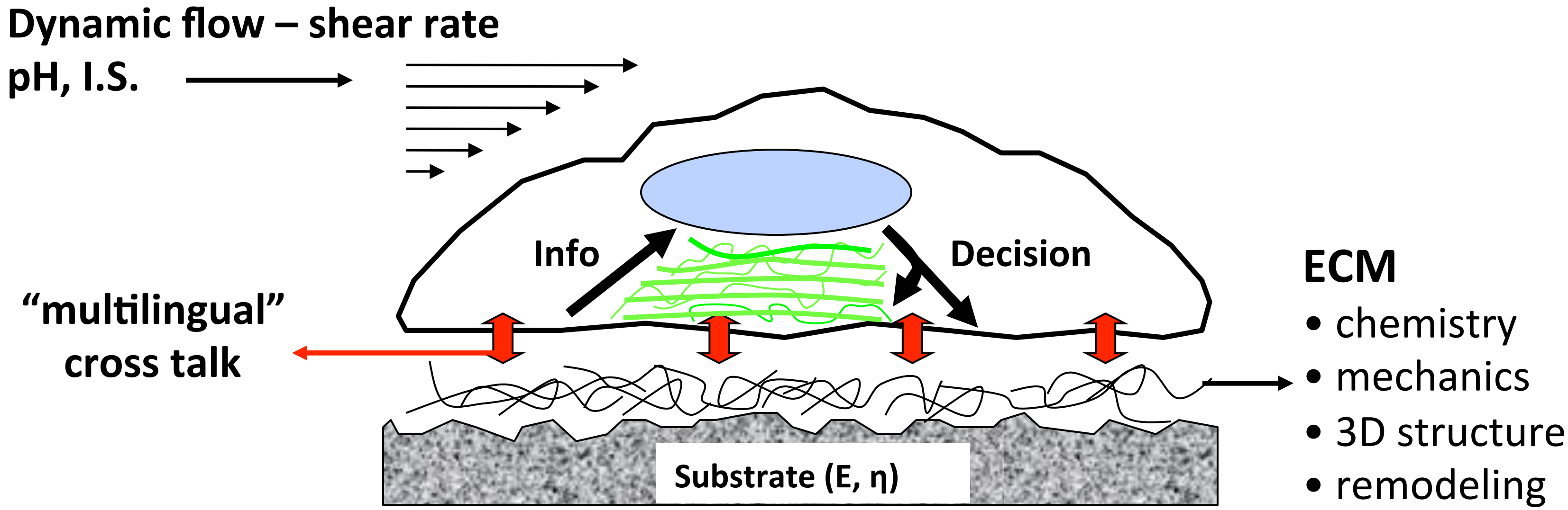
- shear stress (cartilage, bone, endothelial cells)
- differential pressure (blood vessels, heart valves)
- combination of the above (blood vessels, heart valves)

Mechanical conditioning

- tension (tendons, ligaments, skeletal muscle tissue, cardiac tissue)
- compression (cartilage)
- bending (bone)
- gravity orientation and magnitude (cartilage, endothelial cells)

Electrical stimulation

- application of electrical field (skeletal muscle, cardiac constructs, sensory neurons)

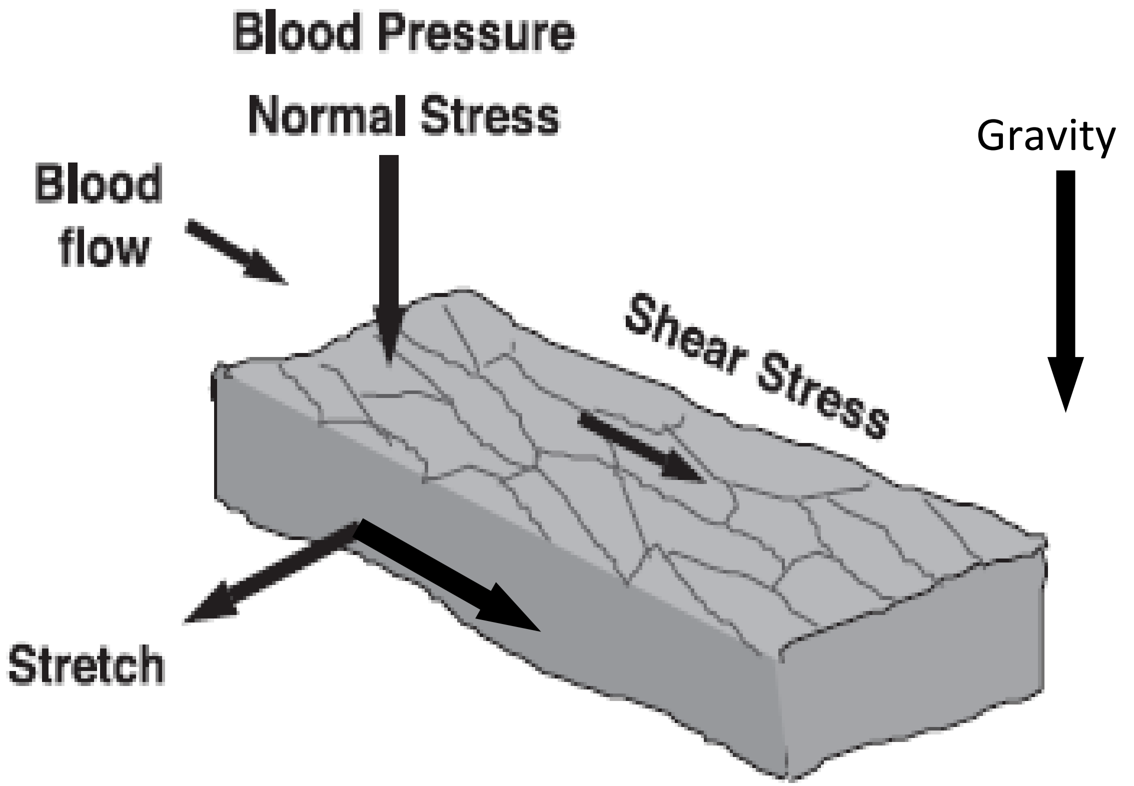


Physicochemical effects of fluid – Cell – ECM microenvironment

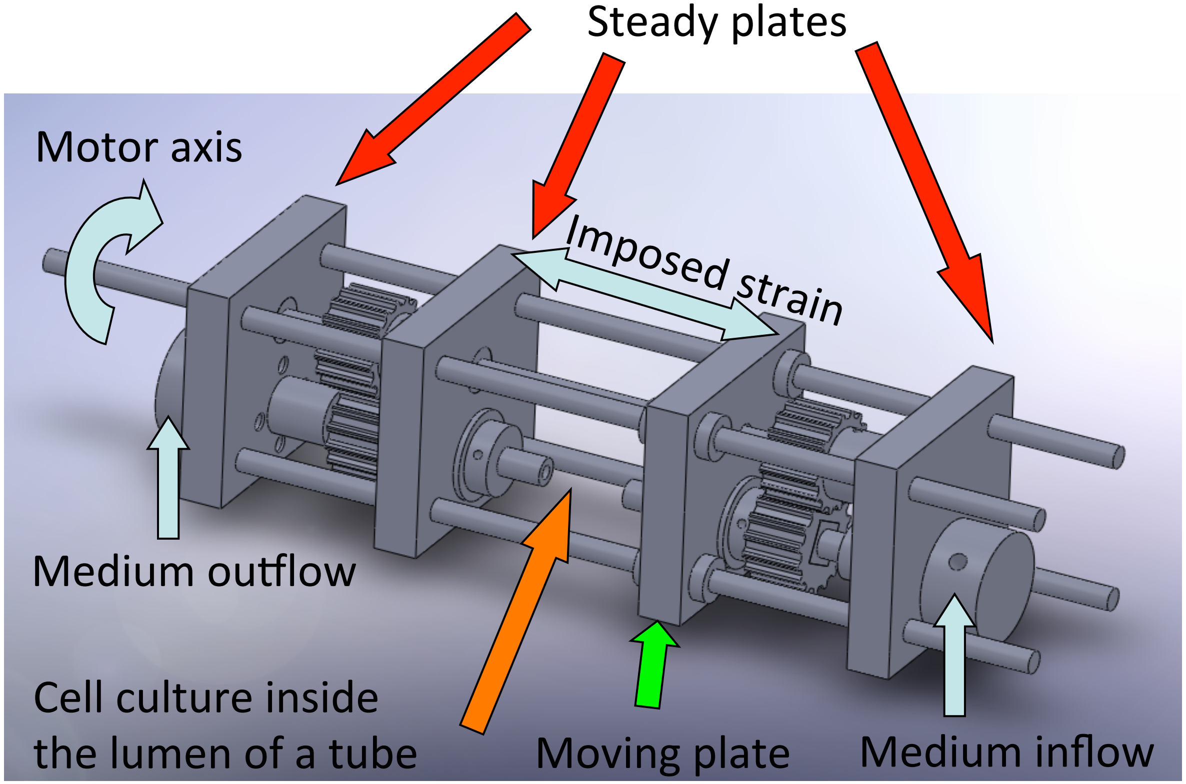
Purpose : Culturing of cells under simulated *in-vivo* mechanical environment

In the case of Endothelial Cells (ECs) :

- Flow – Shear stress
- Tensile stretch
- Gravity
- Pressure



Design and construction of a multipotent testing device

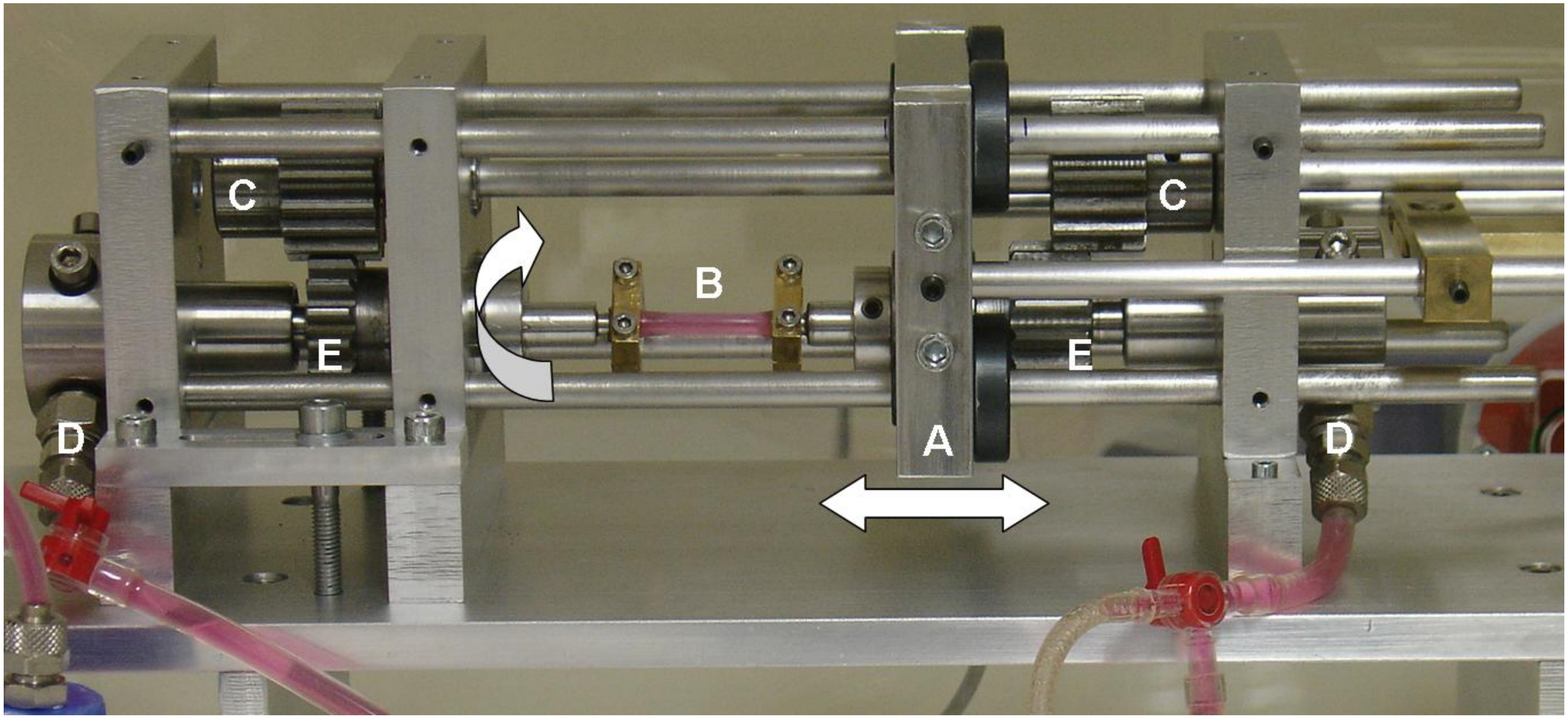


Drawing of the rotating wall bioreactor created by 3-D CAD software (Solidworks)

Functional modes of the bioractor :

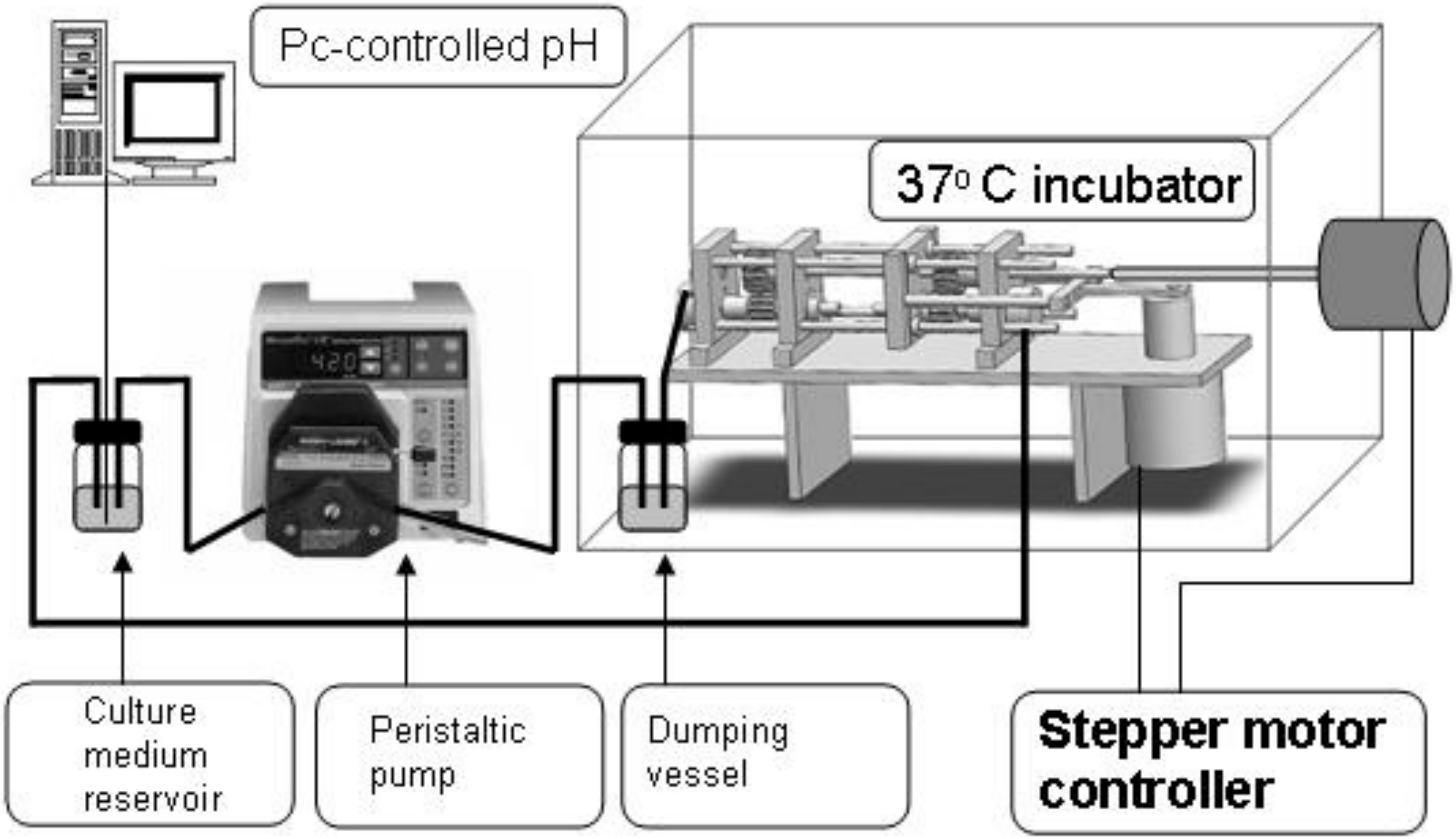
- shear stress, as a result of the medium flow,
- normal stress attributable to internal pressure,
- substrate strain due to vessel's wall compliance and
- gravitational forces (gravity orientation due to rotation)

Design and construction of a multipotent testing device

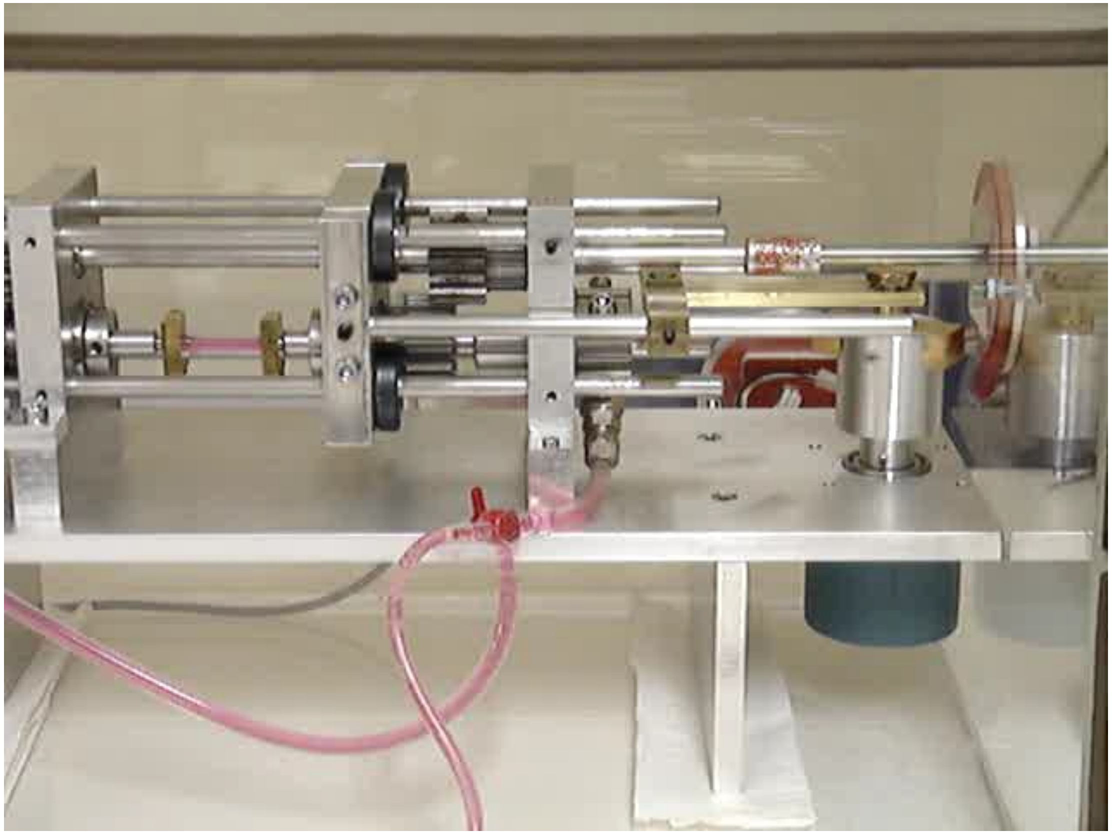


A) Oscillating plate delivering the uniaxial substrate strain or compression. B) Loaded biomaterial specimen seeded with cells. C) Rotation mechanism on both ends of the tubular specimen to avoid torsion. D) Medium inflow and outflow delivering the shear stress and hydrostatic pressure to the cells. E) Free ends of stainless steel ducts entering a hollow drum. The conjunction of the two parts is sealed with O-rings.

Experimental set up of the testing device



Experiment in progress…



All function modes of the device active:

- rotation
- perfusion
- substrate strain

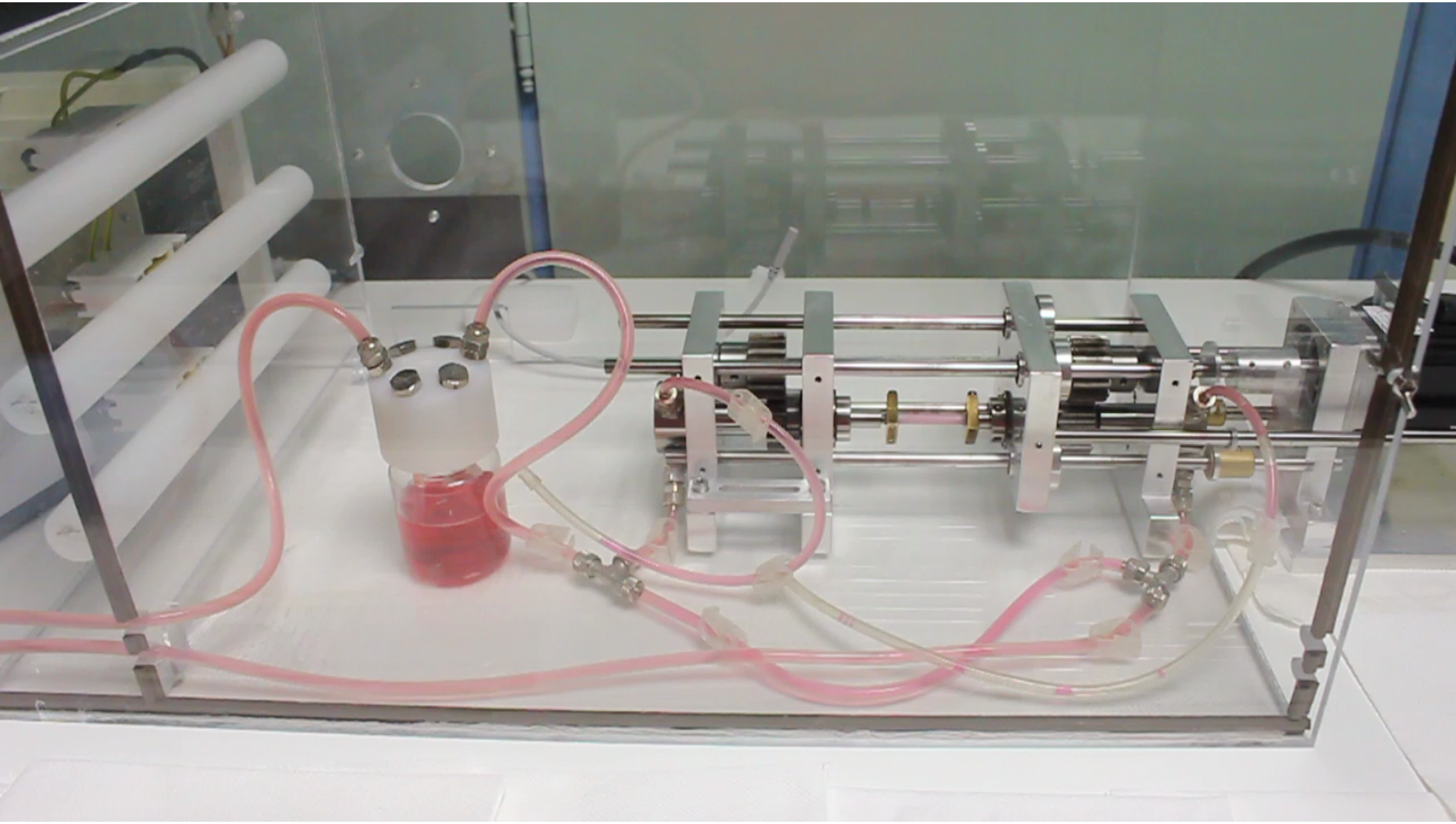
- Ethylene Vinyl Acetate (EVA) tubular specimens are used as cell culture substrate.
  Inner diameter 4 mm, wall thickness 1 mm and total length 4 cm.

- EVA tubes are processed with:

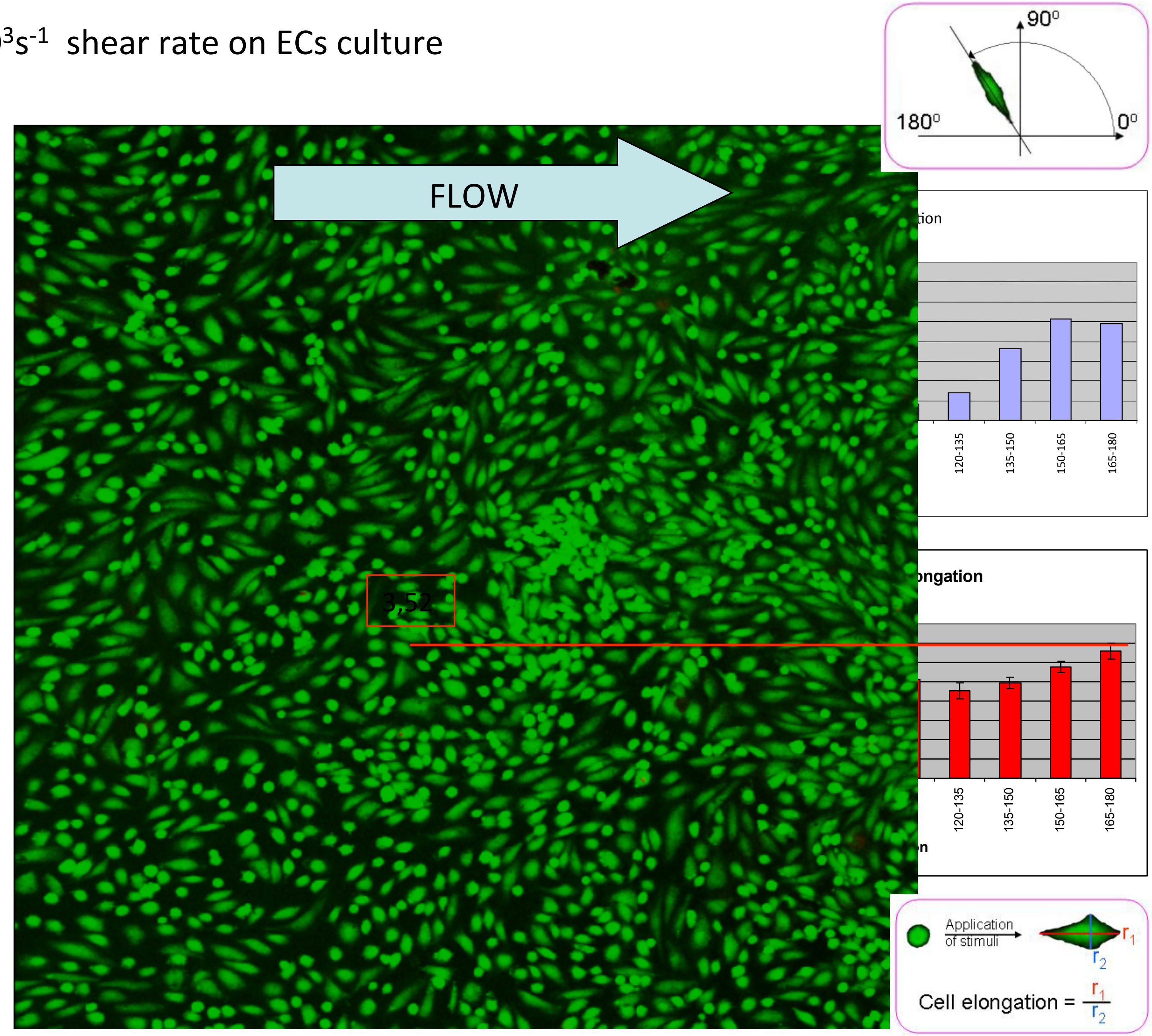
1. 37% HCl 3hrs
2. 70% ethanol
3. 3 times with 1X PBS
4. 4% gelatin solution 2hrs at 37^0C
5. 12,5% glutaraldehyde 1min (for the crosslinking of gelatin)

- 50.000 cells/cm^2 are seeded at the lumen of the tube as it rotates. Four hours after seeding, a mechanical stimulus is superimposed.

2nd generation of our “bioreactor”

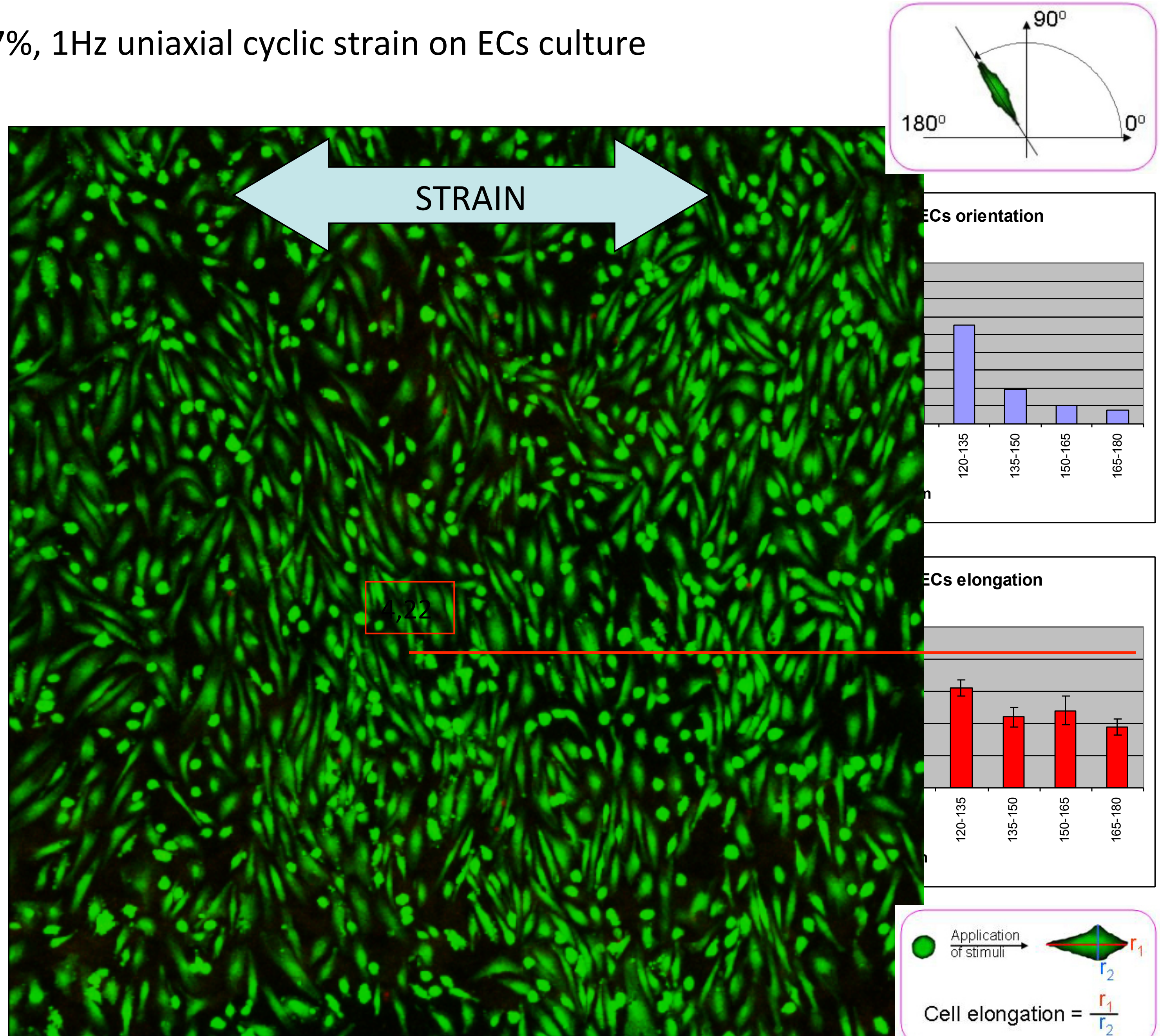


Effect of $10^3 s^{-1}$ shear rate on ECs culture



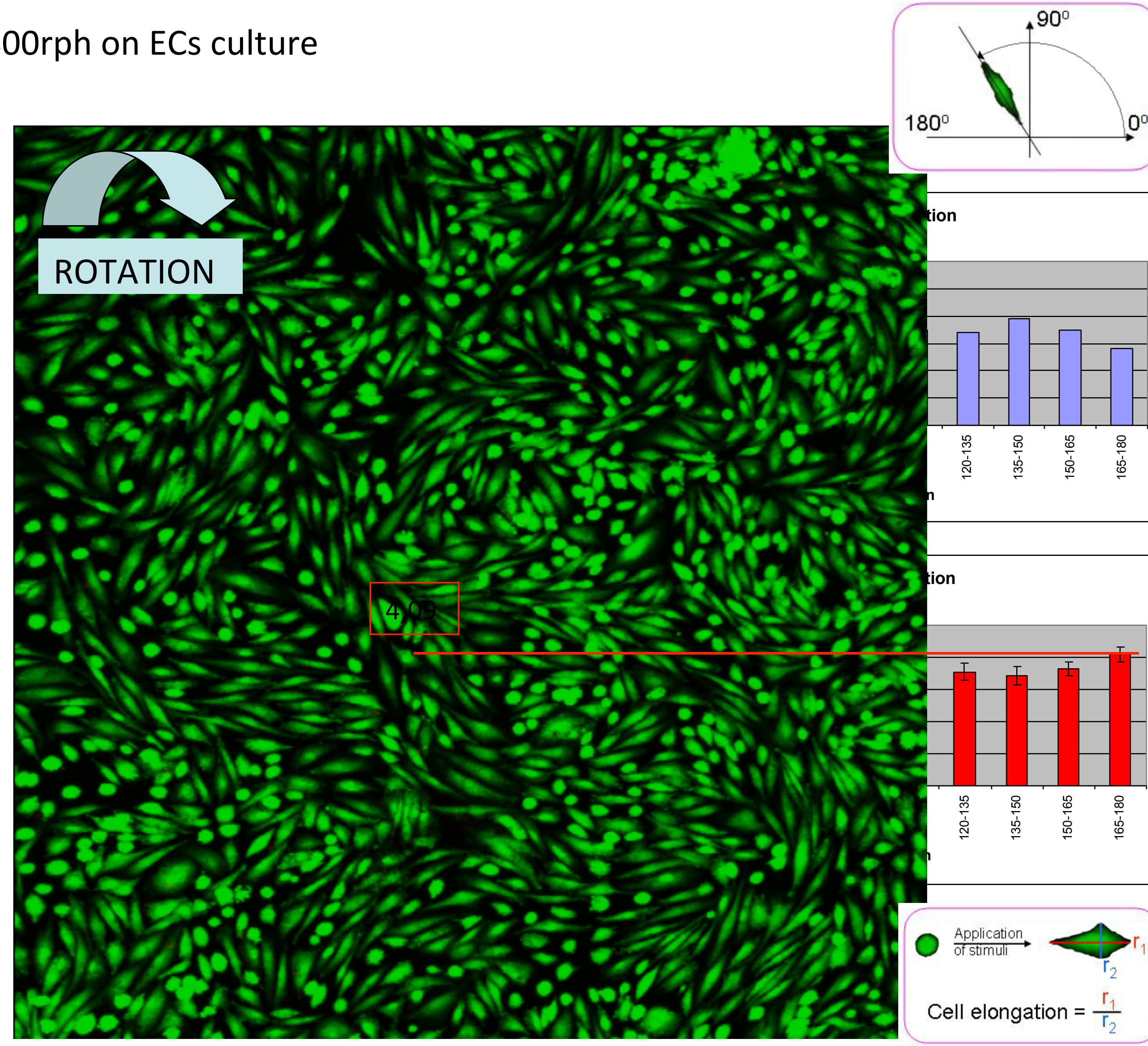
FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

Effect of 6,7%, 1Hz uniaxial cyclic strain on ECs culture



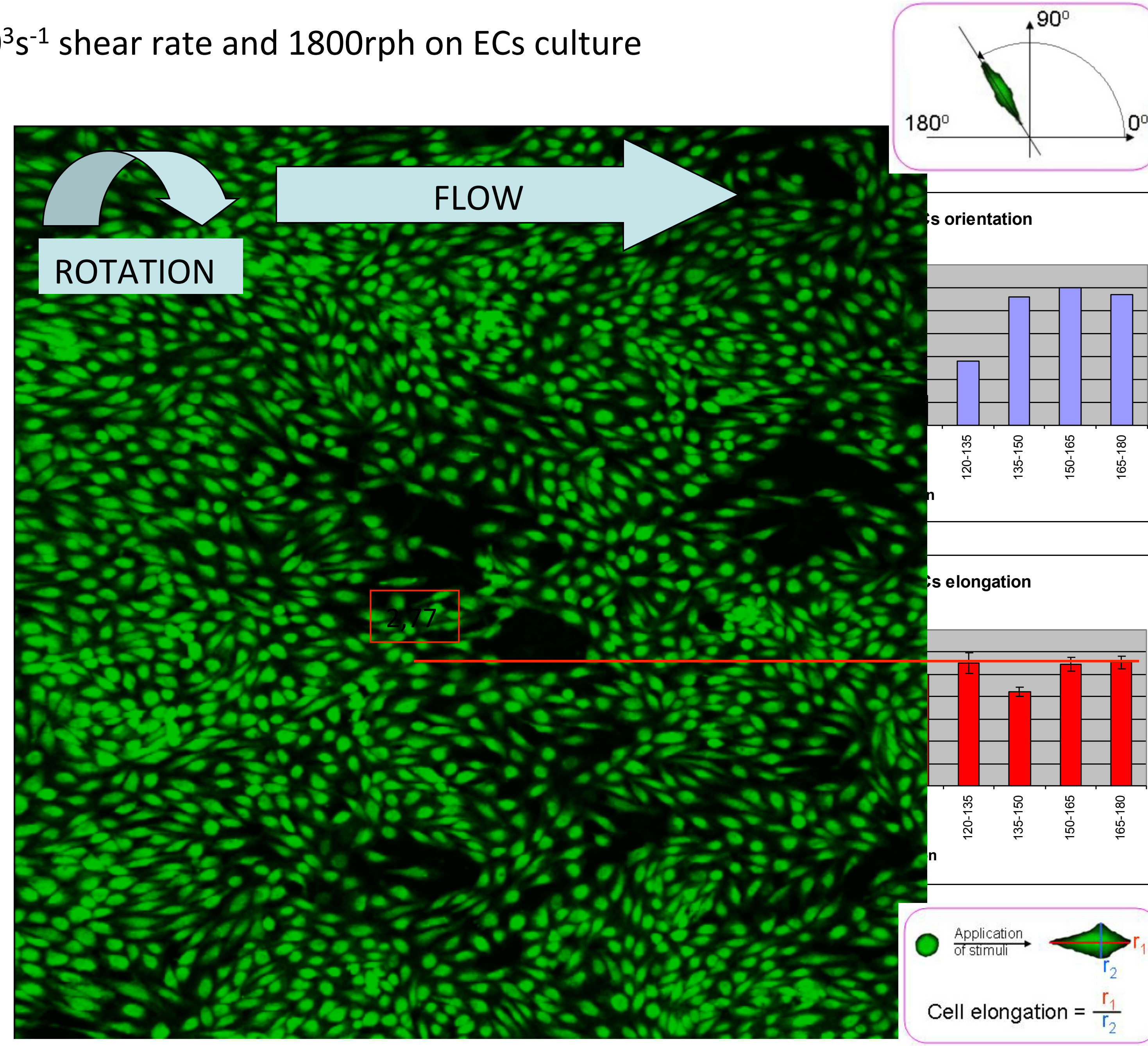
FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

Effect of 1800rph on ECs culture



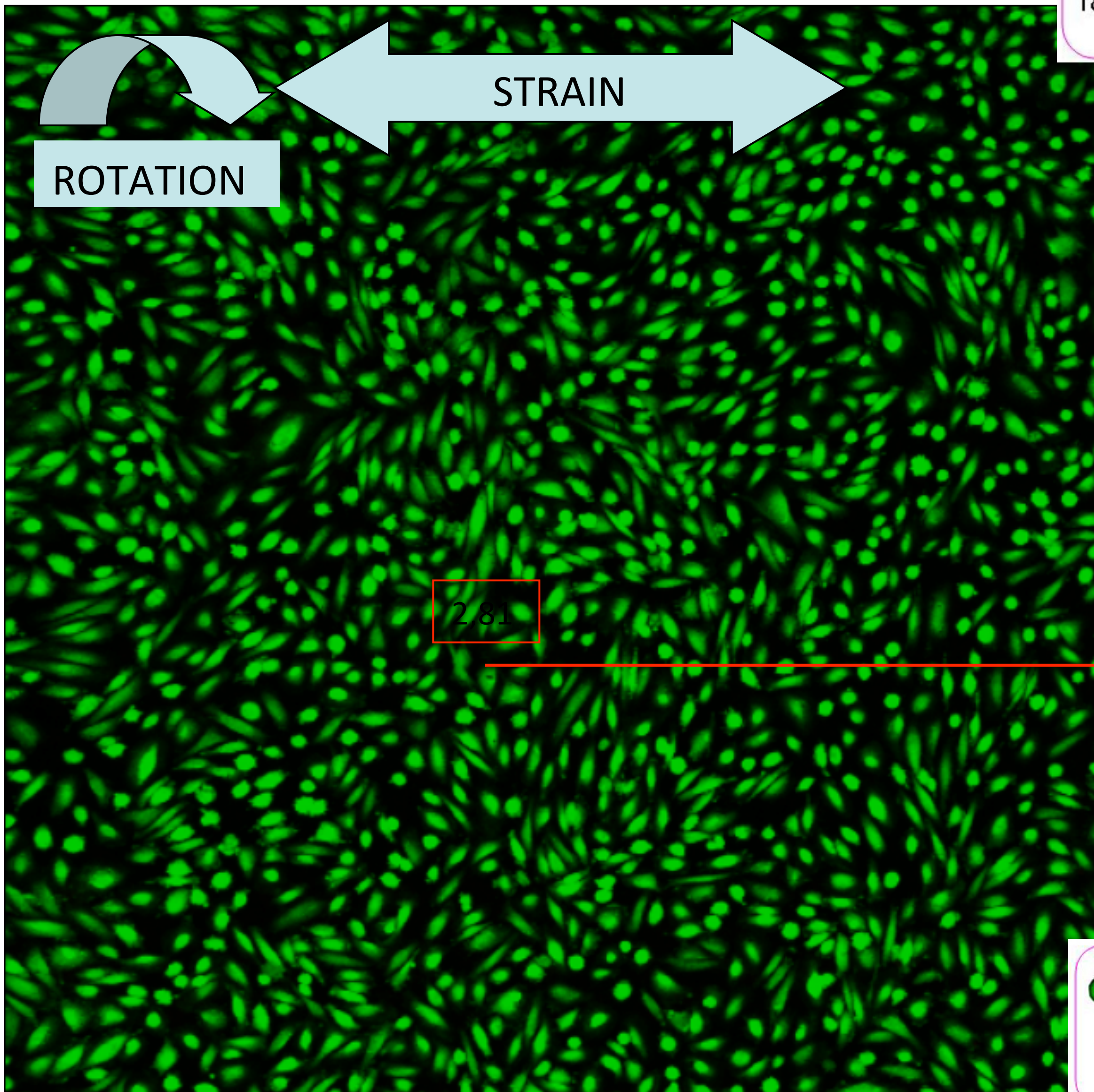
FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

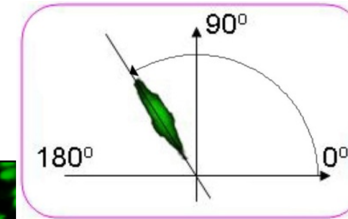
Effect of $10^3 s^{-1}$ shear rate and 1800rph on ECs culture

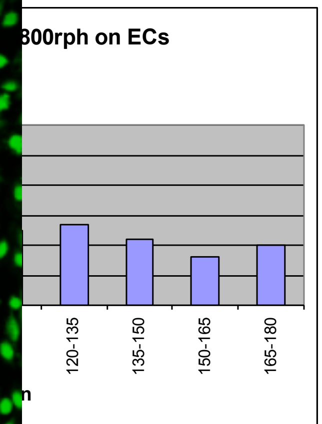


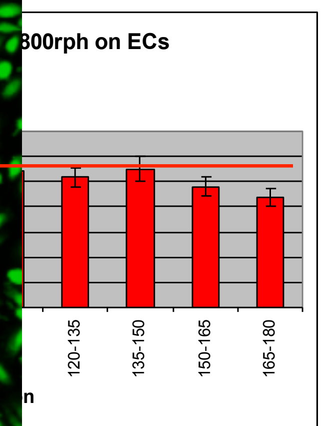
FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

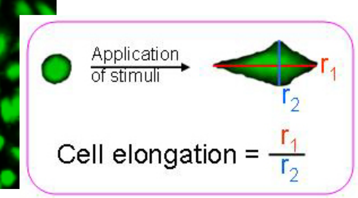
Effect of 6,7%, 1Hz uniaxial cyclic strain and 1800rph on ECs culture





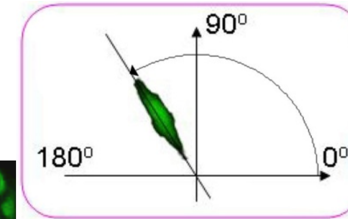


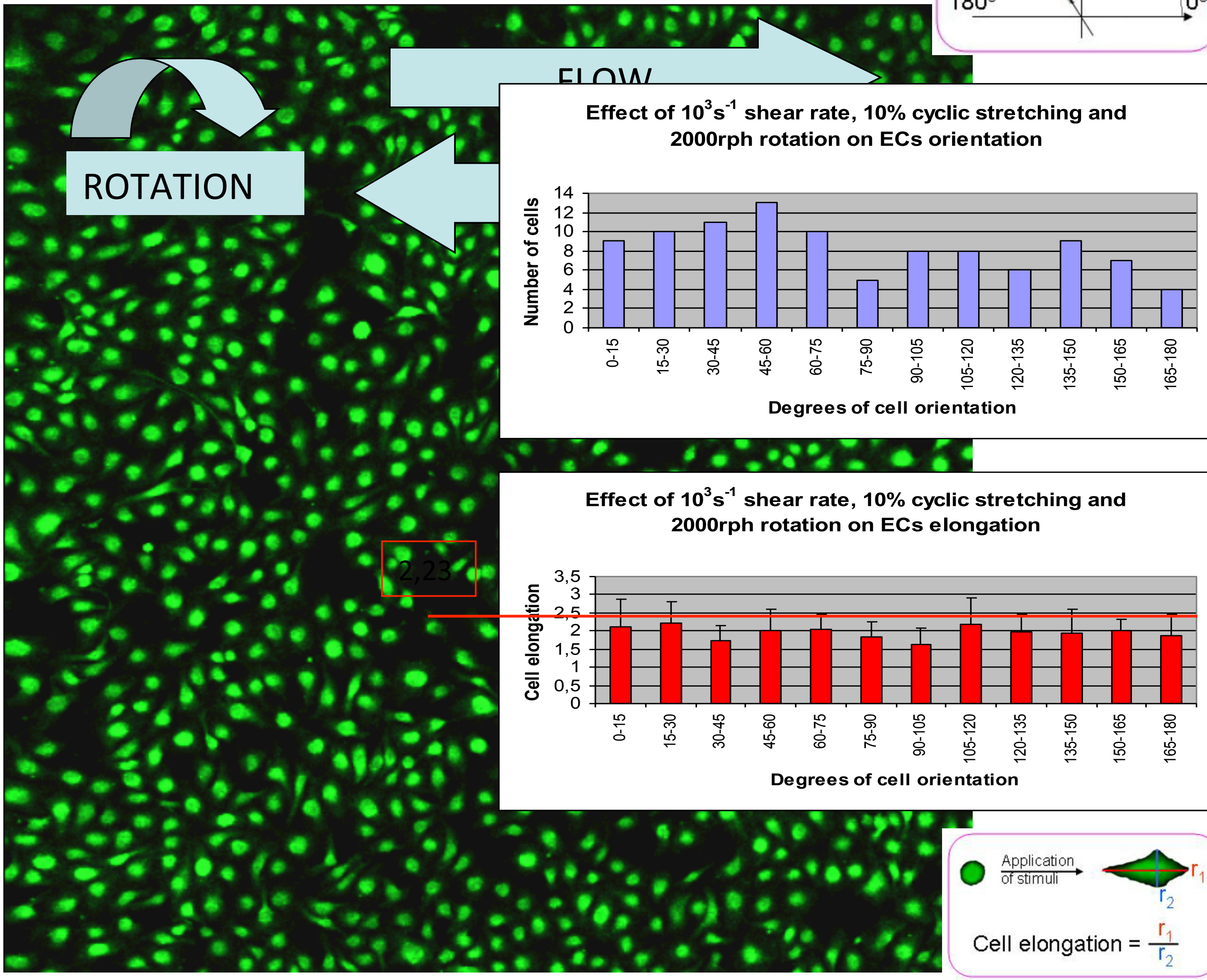




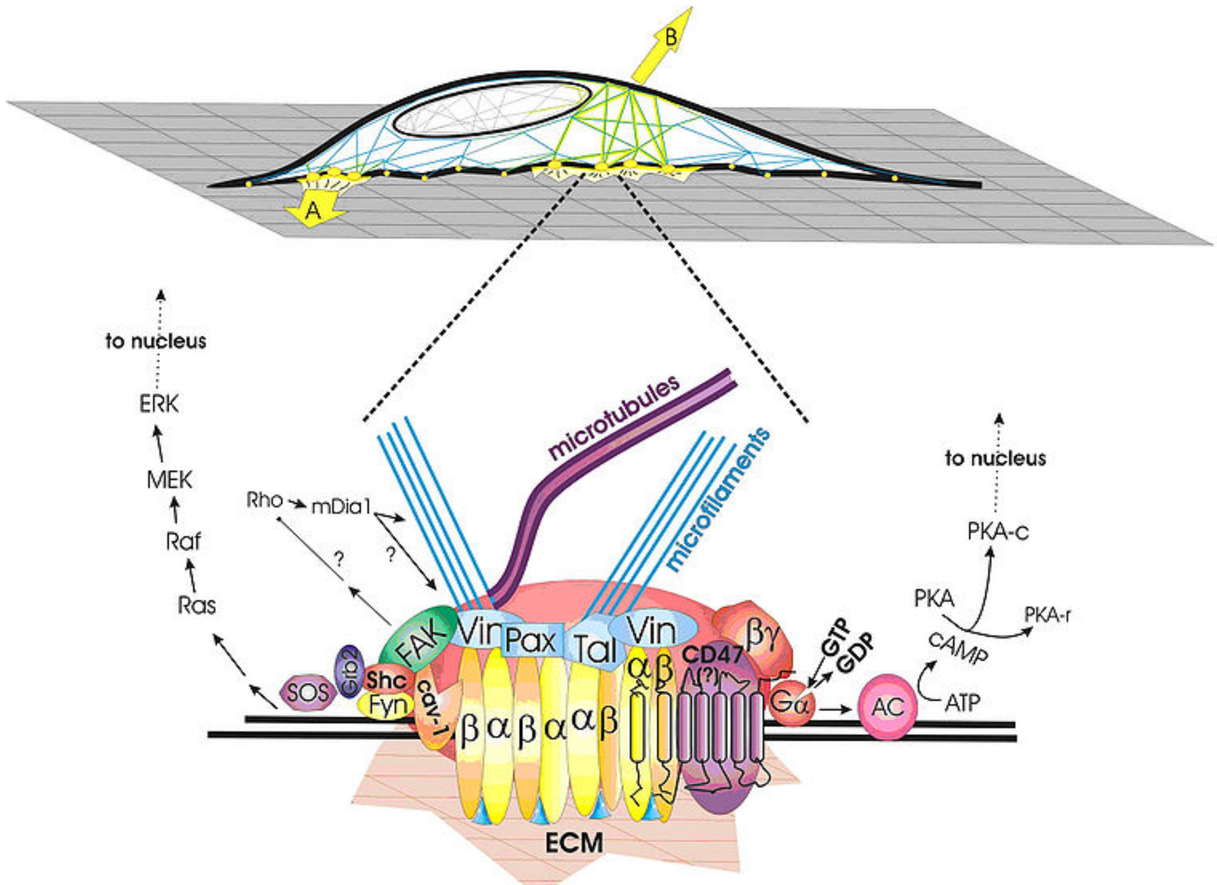
FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

Effect of 10%, 1Hz uniaxial cyclic strain, 2000rph and $10^3 s^{-1}$ shear rate on ECs culture

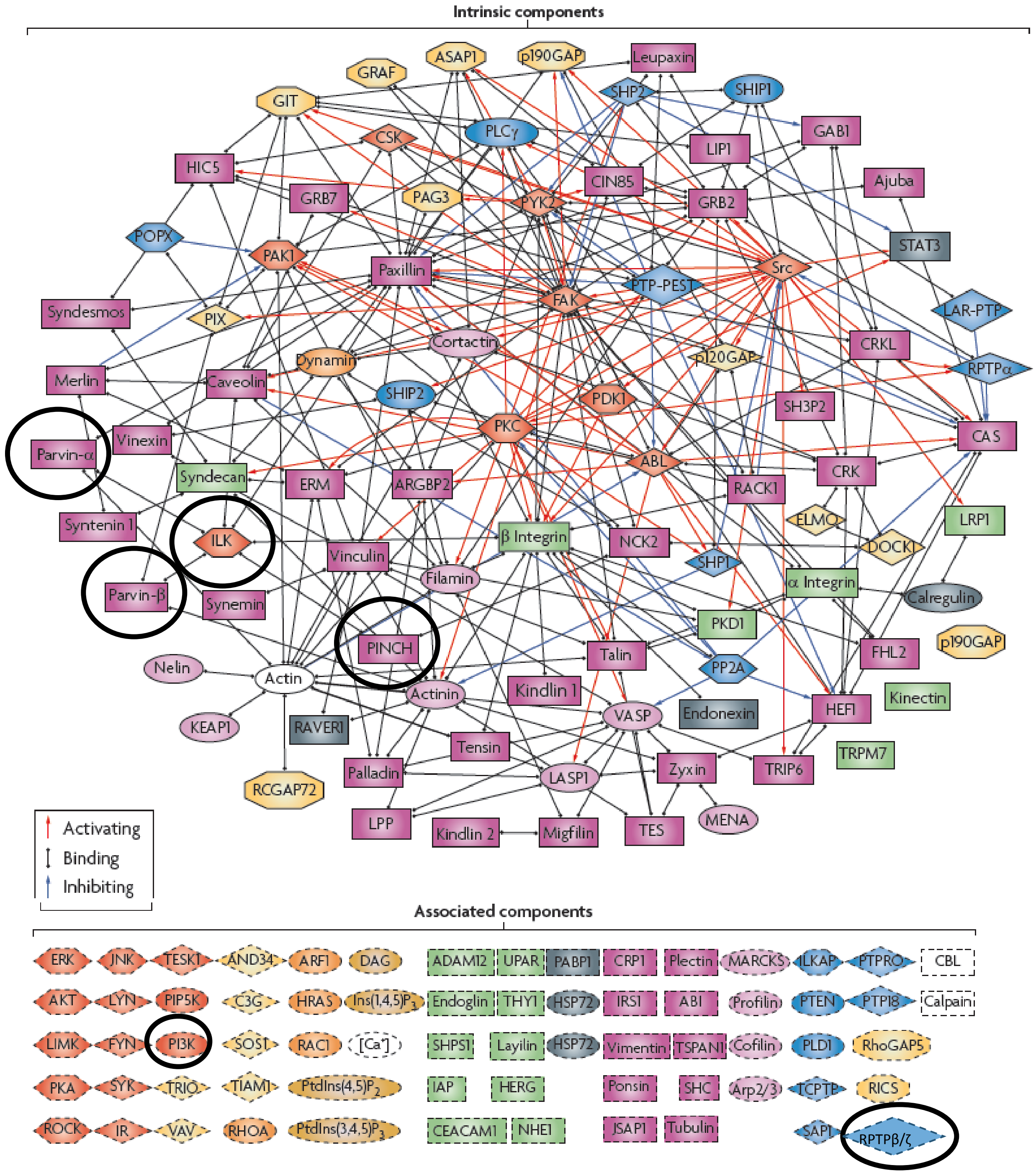




FDA/PI staining
Magnification: 10X
Field of view:
1,27mmX1,27mm

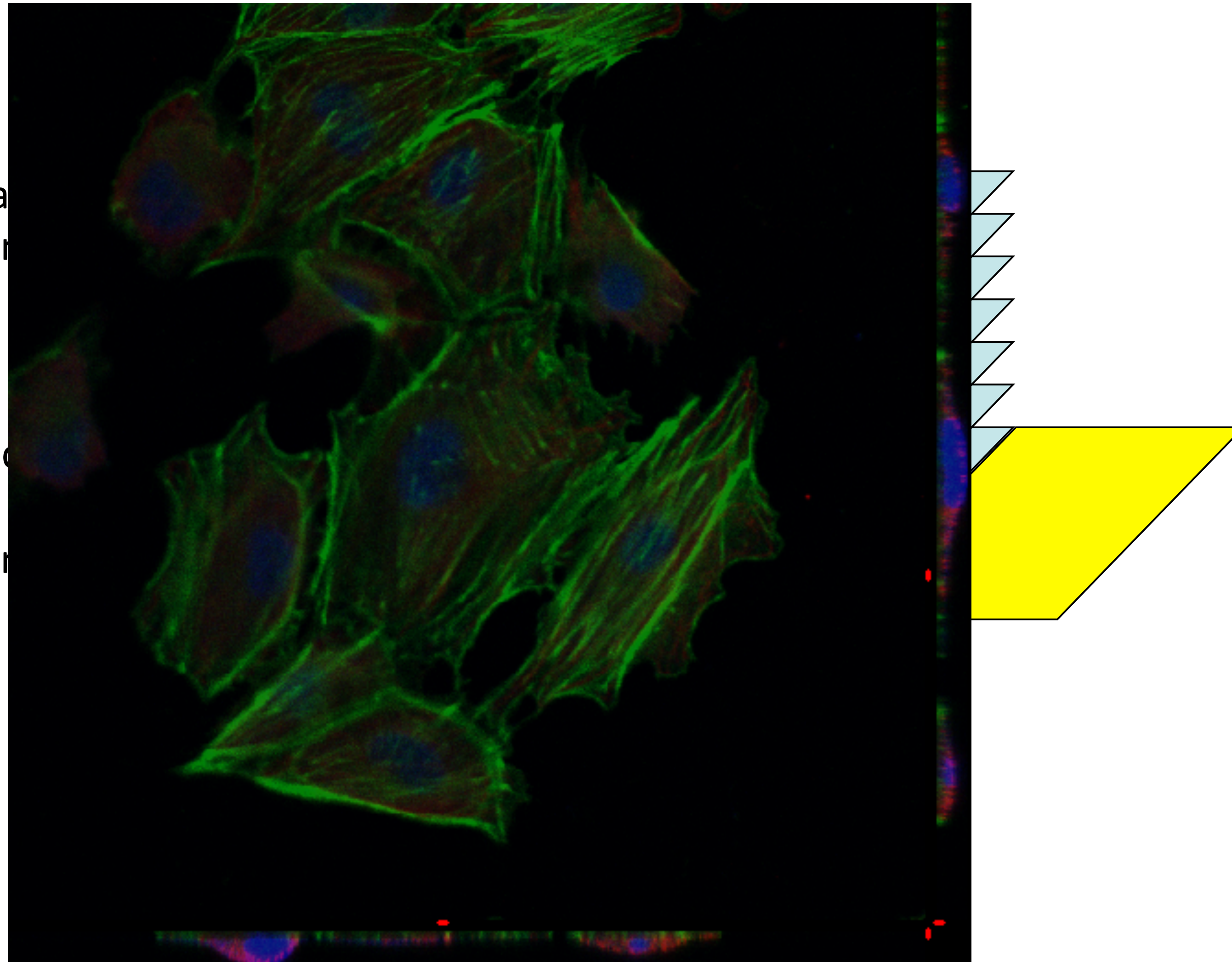


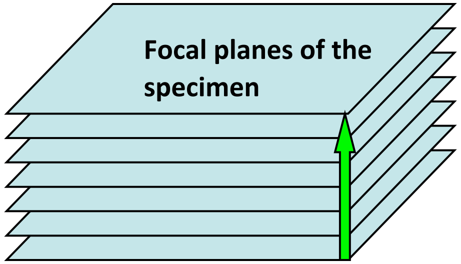
B
A
to nucleus
ERK
MEK
Raf
Ras
Rho
mDia1
?
?
microtubules
microfilaments
to nucleus
PKA-c
PKA
PKA-r
cAMP
ATP
AC
GTP
GDP
Gα
βγ
CD47
(?)
Vin
Tal
Pax
Vin
FAK
Grb2
SOS
Shc
Fyn
cav-1
β α β α α β
α β
ECM


Intrinsic components
ASAP1
p190GAP
Leupaxin
GRAF
SHP2
SHIP1
GIT
CSK
PLCγ
GAB1
LIP1
HIC5
CIN85
Ajuba
GRB7
PAG3
PYK2
GRB2
POPX
STAT3
PAK1
Paxillin
Src
PTP-PEST
FAK
LAR-PTP
Syndesmos
PIX
Cortactin
CRKL
p120GAP
RPTPα
Merlin
Dynamin
Caveolin
SHIP2
PDK1
SH3P2
PKC
CAS
Parvin-α
Vinexin
ABL
CRK
Syndecan
ERM
ARGBP2
RACK1
ELMO
LRP1
Syntenin 1
ILK
β Integrin
NCK2
DOCK1
Vinculin
SHP1
Parvin-β
Filamin
α Integrin
Calregulin
Synemin
PKD1
PINCH
FHL2
p190GAP
Talin
Nelin
Actin
PP2A
Kindlin 1
Actinin
Kinectin
VASP
Endonexin
HEF1
KEAP1
RAVER1
Tensin
TRPM7
Palladin
LASP1
Zyxin
TRIP6
RCGAP72
LPP
MENA
Kindlin 2
Migfilin
TES
Activating
Binding
Inhibiting
Associated components
ERK
JNK
TESK1
AND34
ARF1
DAG
ADAM12
UPAR
PABP1
CRP1
Plectin
MARCKS
ILKAP
PTPRO
CBL
AKT
LYN
PIP5K
C3G
HRAS
Ins(1,4,5)P3
Endoglin
THY1
HSP72
IRS1
ABI
Profilin
PTEN
PTP18
Calpain
LIMK
FYN
PI3K
SOS1
RAC1
[Ca+]
SHPS1
Layilin
HSP72
Vimentin
TSPAN1
Cofilin
PLD1
RhoGAP5
PKA
SYK
TRIO
TIAM1
PtdIns(4,5)P2
IAP
HERG
Ponsin
SHC
Arp2/3
TCPTP
RICS
ROCK
IR
VAV
RHOA
PtdIns(3,4,5)P3
CEACAM1
NHE1
JSAP1
Tubulin
SAP1
RPTPβ/ζ

Focal pla
specime

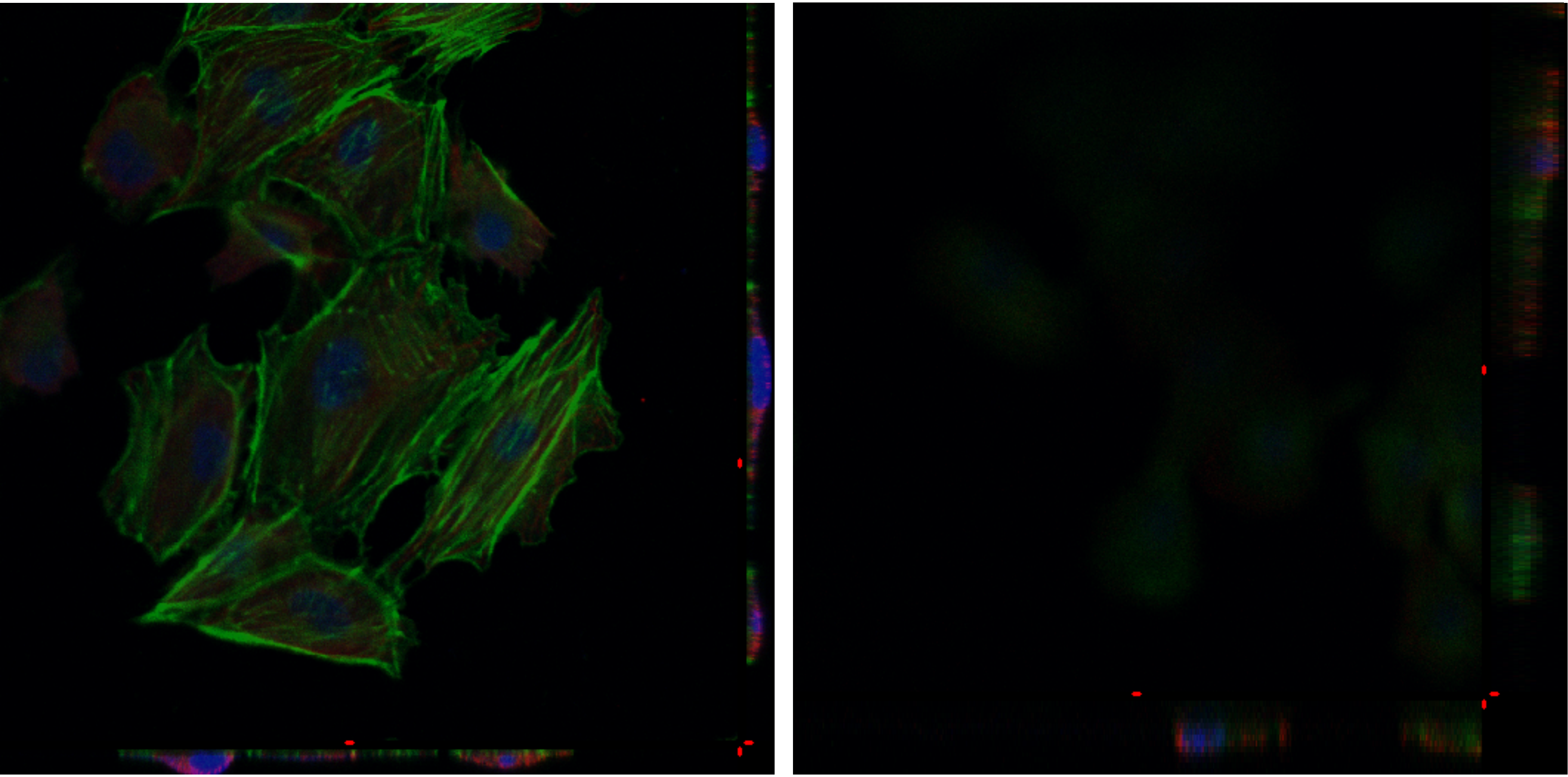
Projecte
sections
specime





F-actin
tubulin
nucleus

60X objective lens



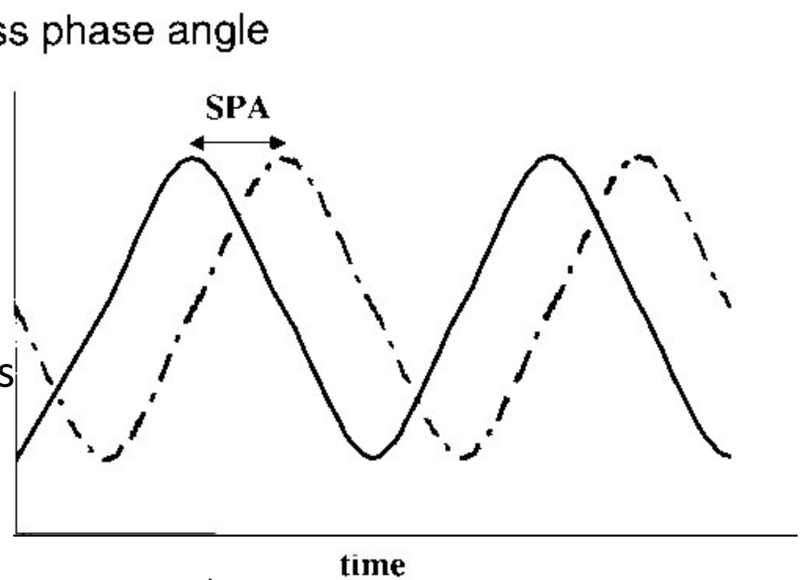
Work in progress : investigation of remodeling and spatial distribution of cytoskeleton elements (F-actin, tubulin)

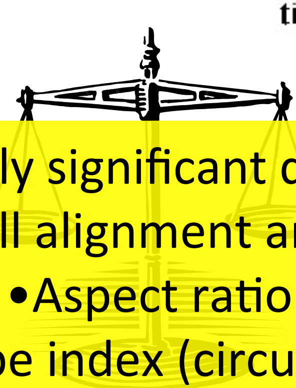
"Stretch and Shear Interactions Affect Intercellular Junction Protein Expression and Turnover in Endothelial Cells"*

Wall Shear Stress ——

......

Circumferential strain/stress





SPA = 0^0

Tight Junction proteins ↓

Apoptosis ↓

No leaky junctions

ATHEROPROTECTIVE

SPA = -180^0

Tight Junction proteins ↓

Apoptosis ↑

Leaky junctions

ATHEROGENIC

NO statistically significant differences in:

- Cell alignment angle
- Aspect ratio
- Shape index (circularity)

*Berardi D., Tarbell J., Cellular and Molecular Bioengineering, Vol. 2, No. 3, September 2009, p.320-331

The most abundant proteins in our cells are there to generate mechanical forces.

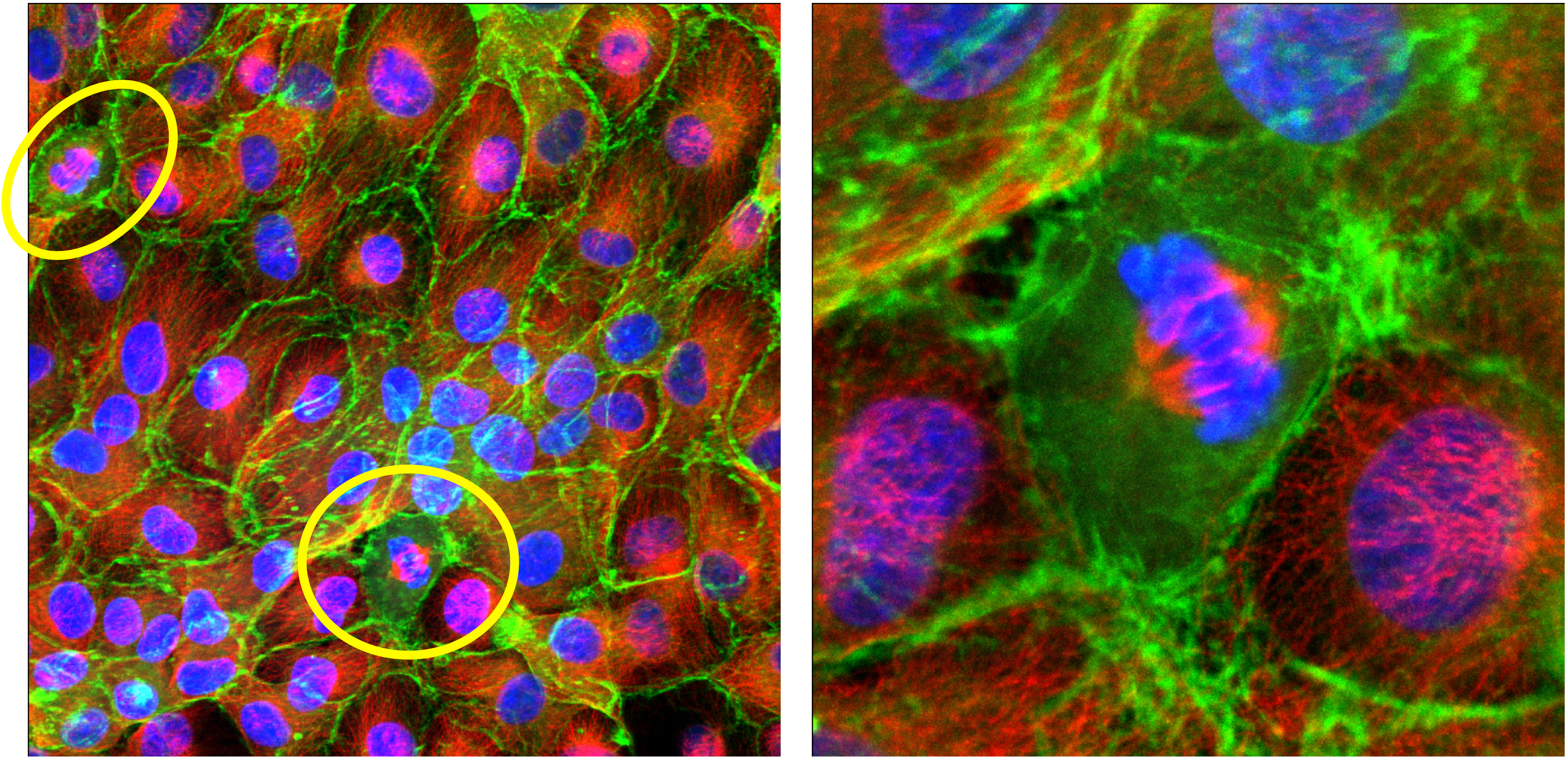
If some of the over-riding principles of mechanobiology at the organ level are now known, then our understanding of underlying mechanisms at the cellular level still remains unclear.

For example, how does the individual cell manage to sense physical forces locally in its three-dimensional microenvironment, respond to those forces or even generate those forces?

More specifically, how do physical forces in three dimensions modulate cell adhesion, cytoskeletal tension, the rate of cytoskeletal remodeling as well as chemotaxis, durotaxis and cellular responses to tissue stretch?

Do local physical forces guide cellular migrations, or are forces a by-product of those migrations?

Nature Methods 7, 963–965 (2010)



Cultivation under **fast (2000rph) rotation** for 15hrs after seeding

Increased number of dividing cells

Study of the multiparametric phenomena at the biomaterial-tissue interface under dynamic conditions

L. McIntire (1987!) : Culture HUVECs

Shear stress	rate of prostacyclin production
0	20 pg/10^6 cells/min
(steady) 24 dyn/cm^2	130 pg/10^6 cells/min
(pulsatile) 24 dyn/cm^2	310 pg/10^6 cells/min

Chemical and Mechanical signals

- Chemical signals propagate through **diffusion** of molecules in the cytoplasm and their speed is limited by the chemical reaction rates (f.ex. phosphorylation), binding and/or diffusion rates.
- D = 0.01 – 100 $\mu m^2 s^{-1}$: a molecule needs 1-100 s to travel 10 microns by diffusion (*Luo and Robinson, in "Mechanosensitivity and Mechanotransduction", Springer e-book, 2010*).
- Mechanical signals are transmitted through the **deformation** of the cytoskeleton of the cell along actin filaments, microtubules, and intermediate filaments.
- The speed of the mechanical signals depend on the elasticity of the cytoskeleton.
- They travel the 10 micron distance in sub-second timescales (*G.Forgacs, J.Cell Sci., 1995*).

- Mechanical signals travel FASTER than chemical ones.
- Signals can be transmitted over **long distances** and broad areas, and to ***different organelles*** through the network of elastic actin cytoskeleton (f. ex. Nucleus-nesprin, plasma membrane-ERM proteins, mitochondria-ABPs, stretch activated channels…).

- Already A.Grinnell (*Science, 1995*) showed that motor nerve terminals release neurotransmitters within 10 msec. after cell-surf. integrins are mechanically stressed.

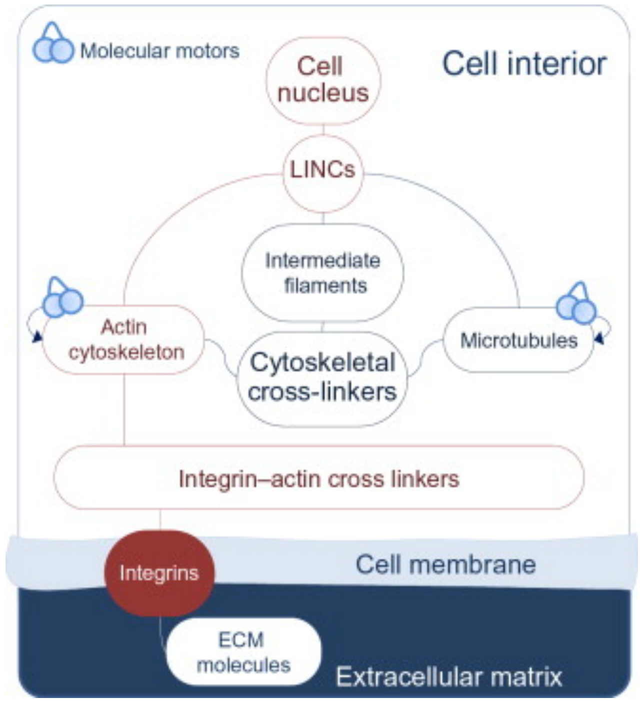


Figure 5.1 The cell mechanical network: The cell is mechanically wired from the ECM to the cell nucleus through a network of molecules. At the exterior, extracellular matrix molecules are mechanically coupled to transmembrane receptor proteins, integrins. ...

Zeinab Jahed , Hengameh Shams , Mehrdad Mehrbod , Mohammad R.K. Mofrad

Chapter Five - Mechanotransduction Pathways Linking the Extracellular Matrix to the Nucleus

International Review of Cell and Molecular Biology, Volume 310, 2014, 171 - 220

http://dx.doi.org/10.1016/B978-0-12-800180-6.00005-0

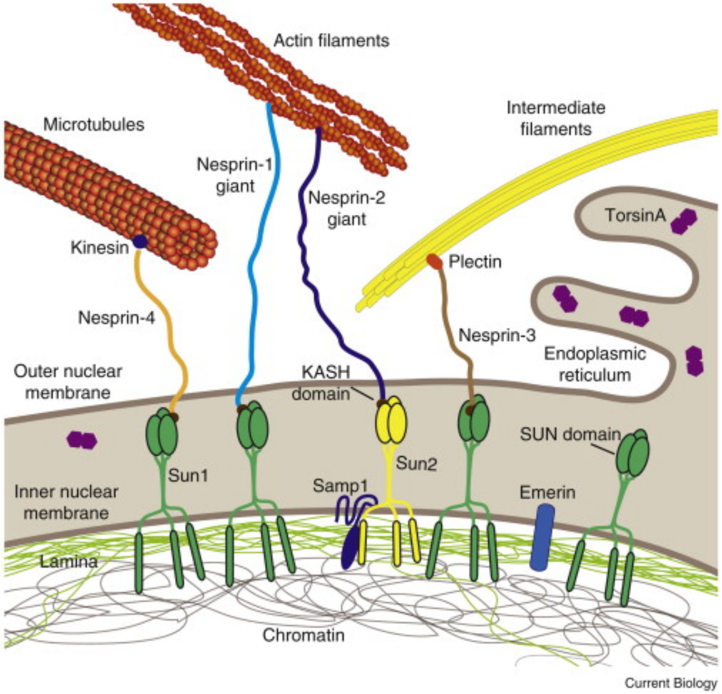


Figure 1 Schematic overview of LINC complex proteins and their connections to the cytoskeleton and nuclear interior. SUN proteins at the inner nuclear membrane bind to the nuclear lamina and other nucleoplasmic proteins while interacting with KASH domain ...

Philipp Isermann , Jan Lammerding

Nuclear Mechanics and Mechanotransduction in Health and Disease

Current Biology, Volume 23, Issue 24, 2013, R1113 - R1121

http://dx.doi.org/10.1016/j.cub.2013.11.009

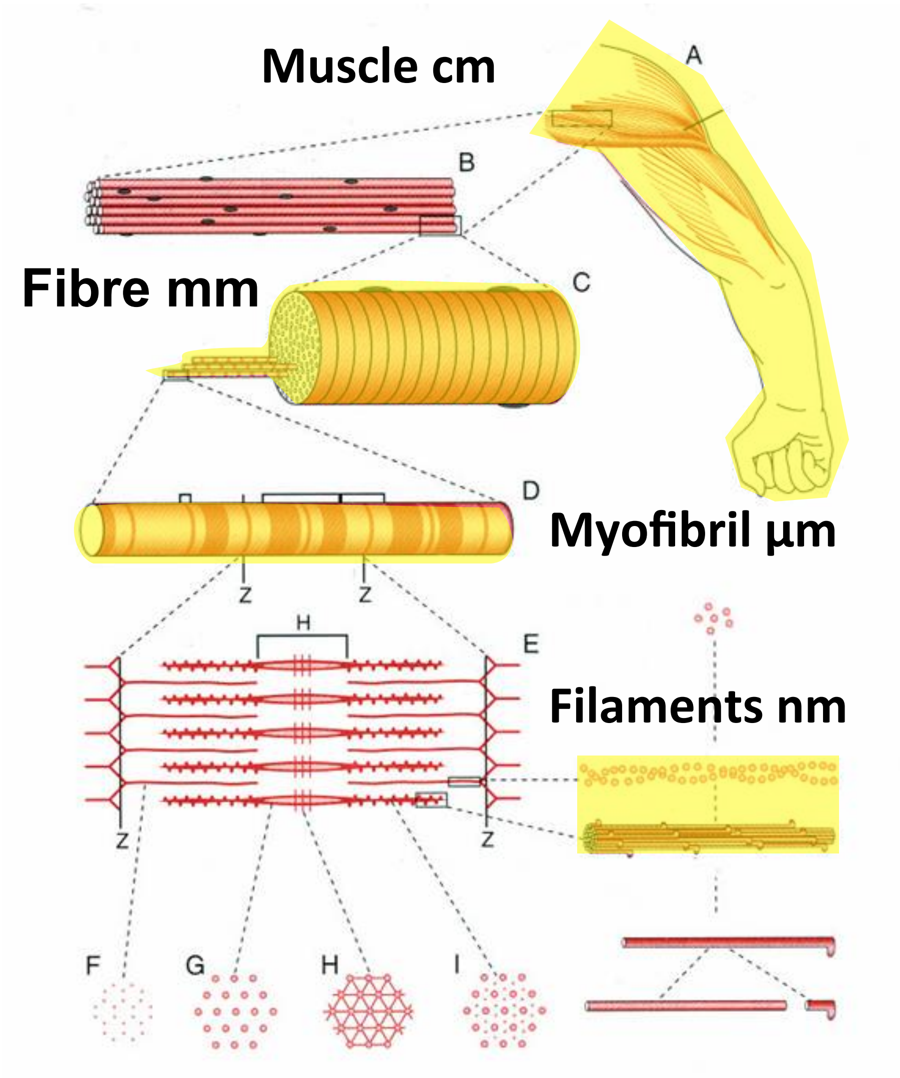
Mechanics

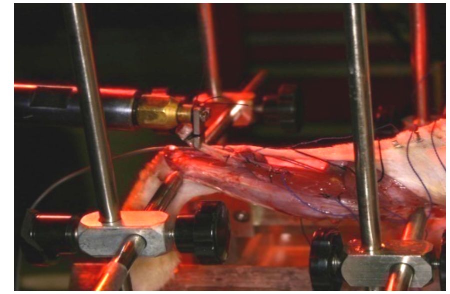
- Forces   (we cannot see them!)

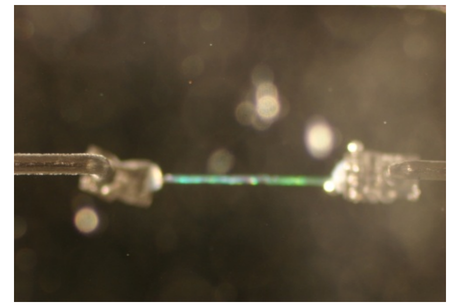
- Deformations (we see them)

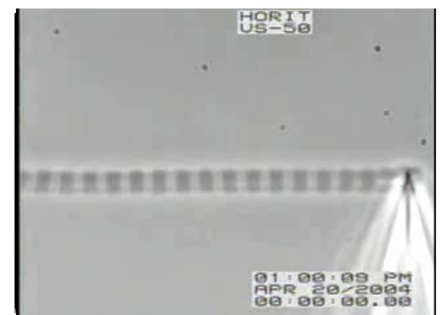
- Forces (stress) = K * Deformations (strain)

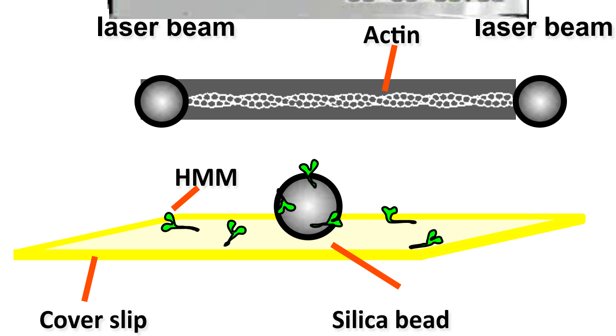
- ***G*** : natural phenomenon
- ***Rates (rhythms)***
- ***Differences in Potentials***









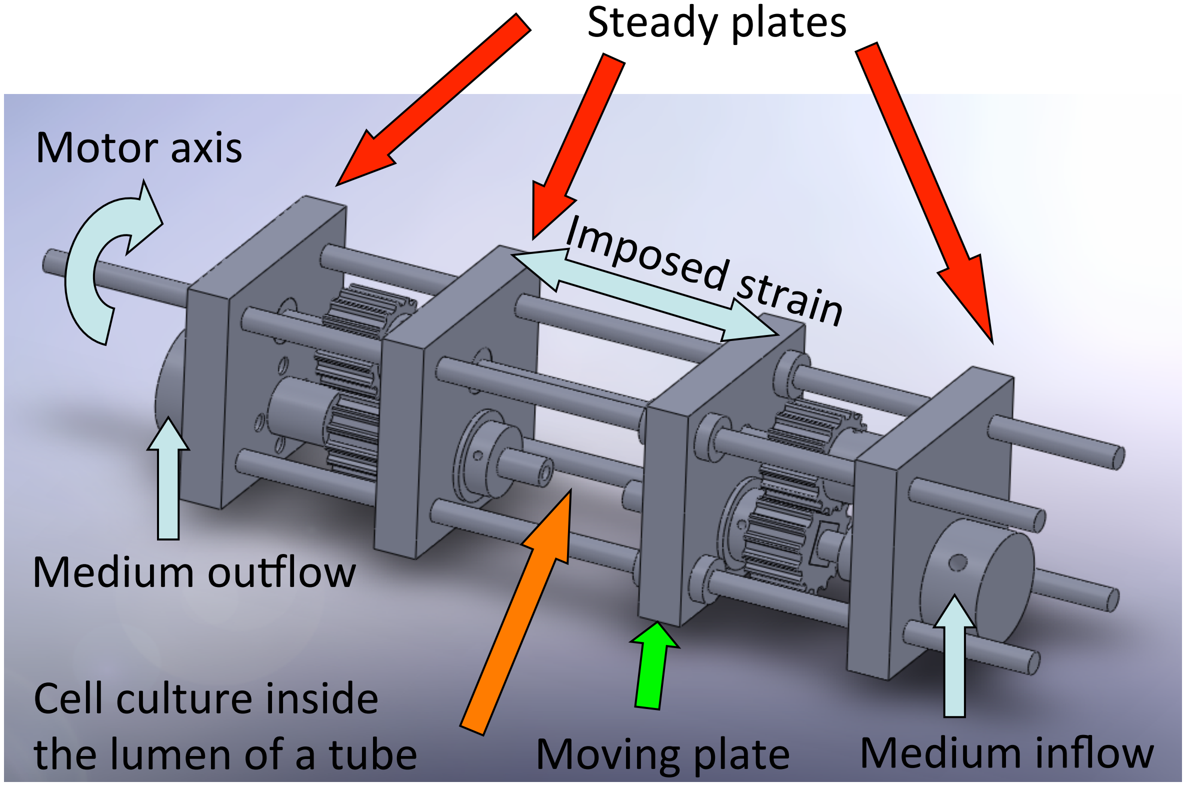


Muscle mechanics (after W. Herzog)

Humans in microgravity

- Bones (load bearing decrease, bone loss, calcium release, more brittle and weak, risk for bone fractures and kidney stone formation. Also radiation might impact bone loss).
- Muscles (leg and posture muscles might weaken or atrophy, exercise & nutritional intervention)
- Fluid shift (redistribution to upper part (puffy face, congestion), legs of smaller circumference)
- Cardiovascular system (heart becomes smaller, radiation might affect endothelial cells, initiating coronary disease)
- The spine- Taller in space (expansion of compressed vertebral discs, back pain)
- Inner ear and balance system (disorientation, nervous misinformation (f.ex. Otoliths: gravitation dependent function))
- Sleep and performance (24h cycle, body clocks)
- ***after : National Space Biomedical Research Institute***

Design and construction of a multipotent testing device

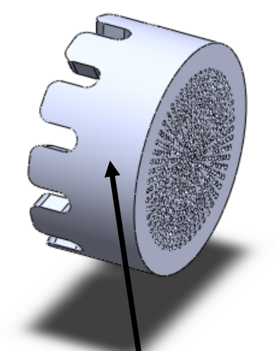


Drawing of the rotating wall bioreactor created by 3-D CAD software (Solidworks)

Functional modes of the bioractor :

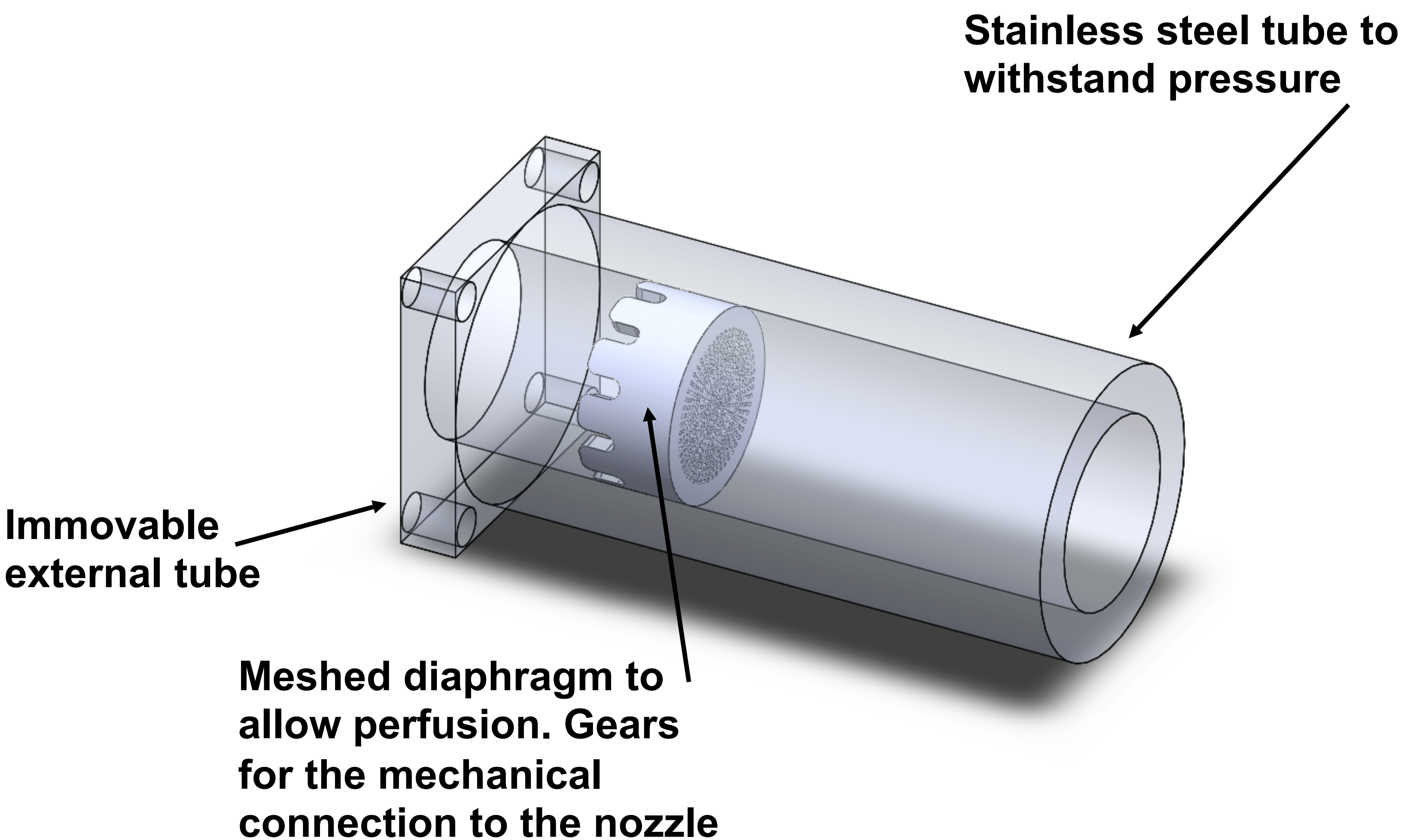
- shear stress, as a result of the medium flow,
- normal stress attributable to internal pressure,
- substrate strain due to vessel's wall compliance and
- gravitational forces (gravity orientation due to rotation)

Possible modification of the device's loading mechanism to test tissue engineered cartilage constructs

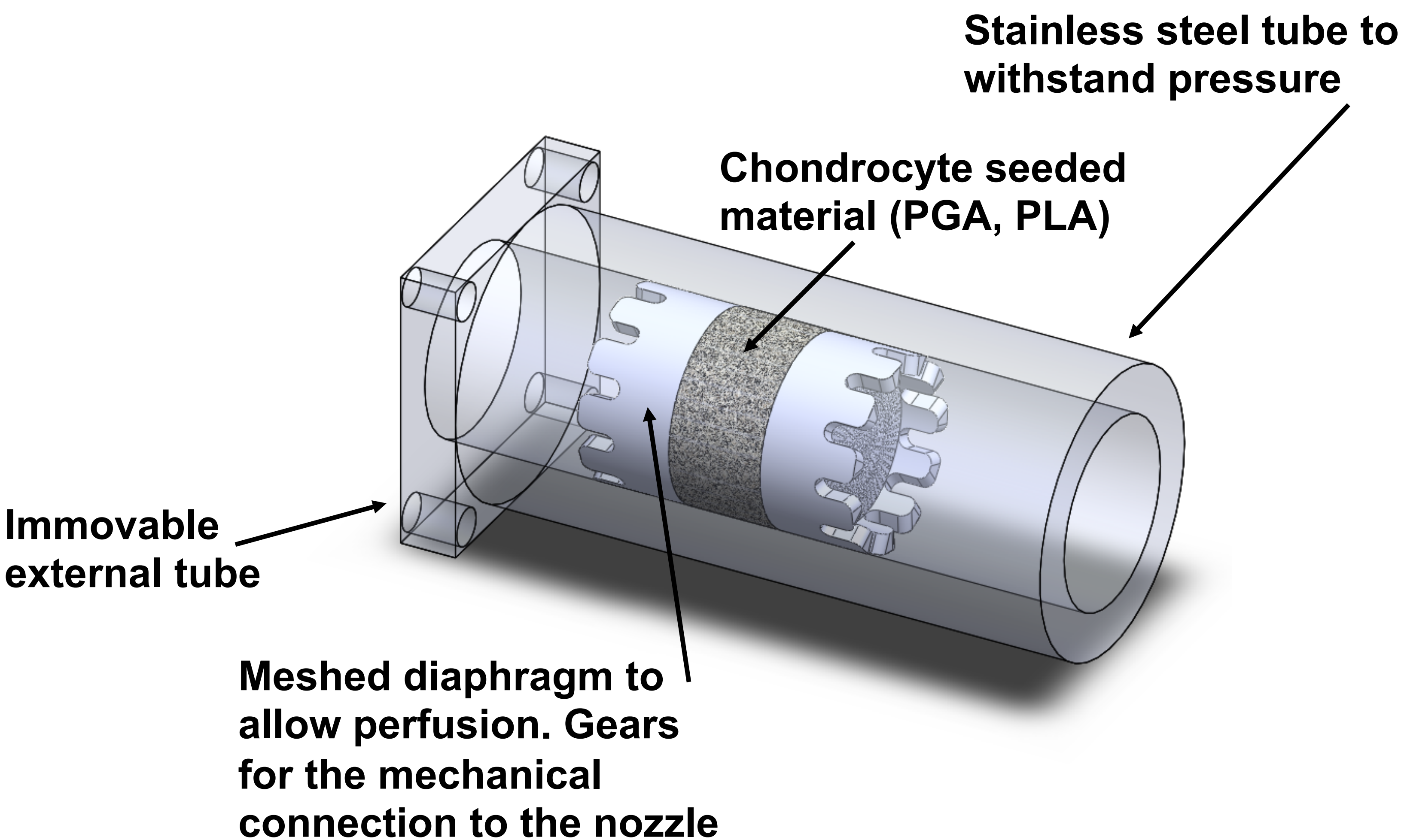


Meshed diaphragm to allow perfusion. Gears for the mechanical connection to the nozzle

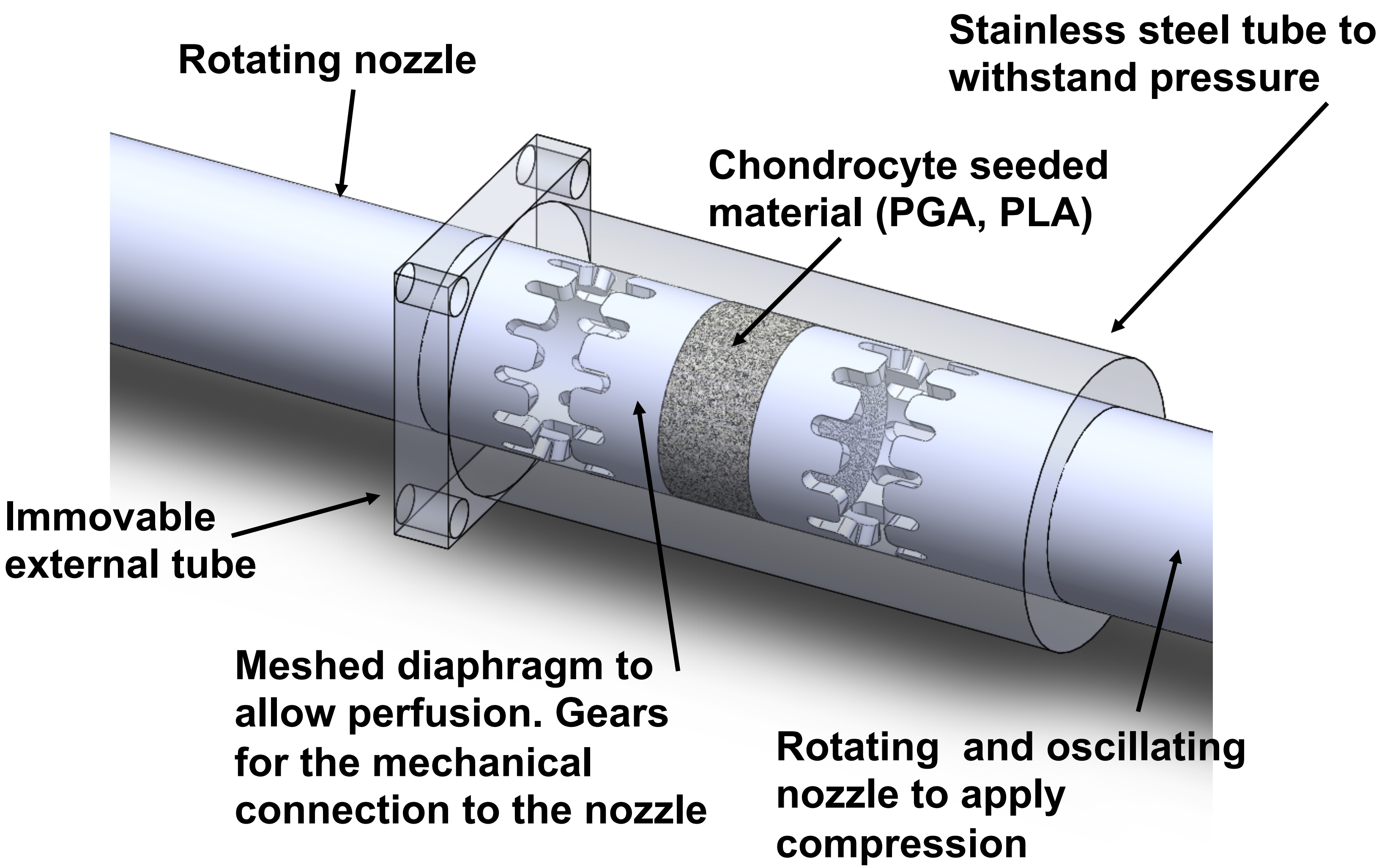
Possible modification of the device's loading mechanism to test tissue engineered cartilage constructs



Possible modification of the device's loading mechanism to test tissue engineered cartilage constructs



Possible modification of the device's loading mechanism to test tissue engineered cartilage constructs



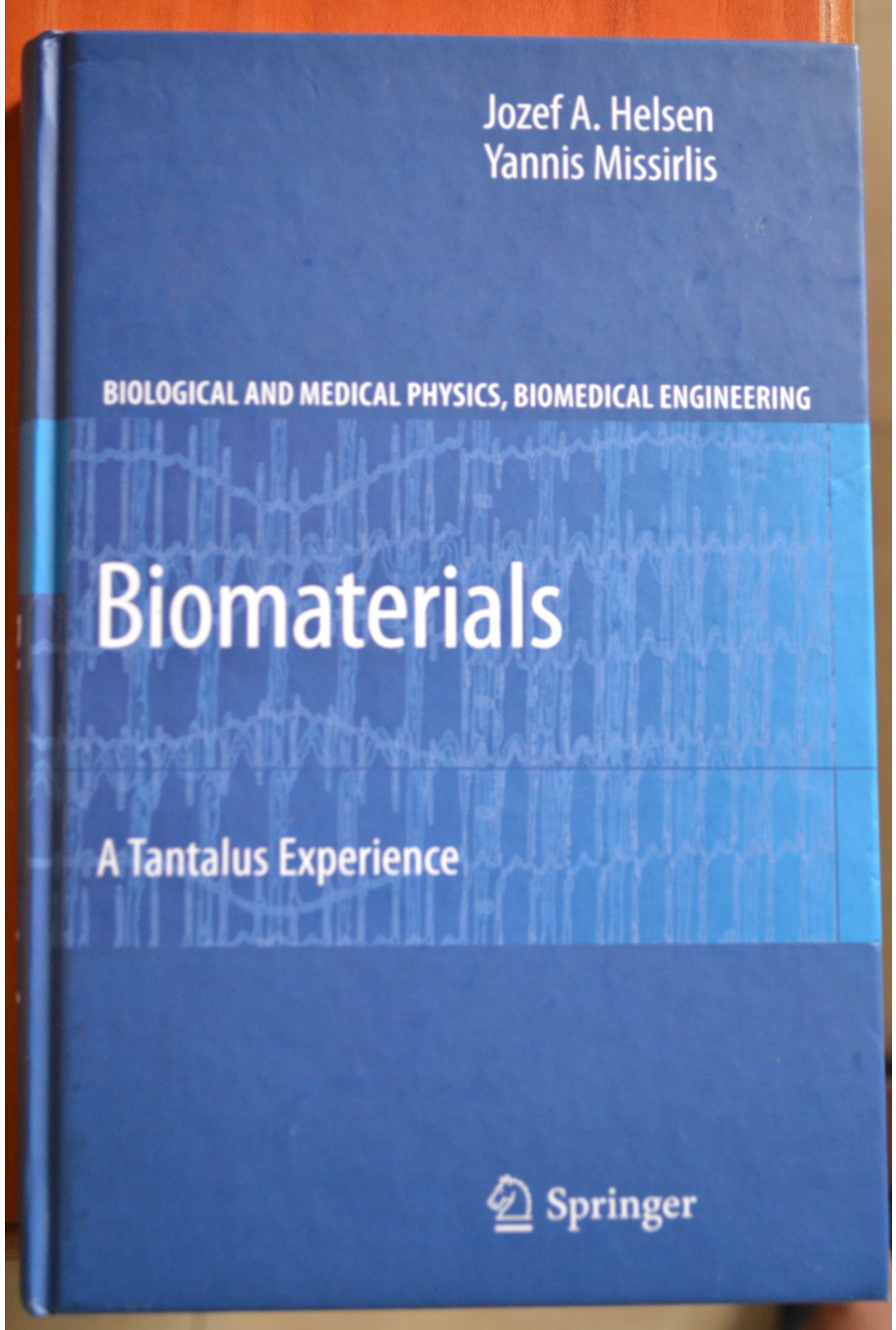
Bioreactor specialized for musculoskeletal tissue generation

Concluding discussion

- *Cells in-vitro already have lost part of their in-vivo information*
- *Culturing conditions "guide" somehow their fate*
- *There are many communication pathways between the cell nucleus and the cell's environment*
- *These pathways: biochemical, ECM related, mechanical etc. alone or in concert ALL give appropriate signals*
- *Fate/differentiation of stem cells is partly based on mechanical cues*
- *Influence of specific drugs/soluble factors on the fate of cells is strongly dependent on the appropriate mechanical environment*

Future Suggestion

- *Appropriate (?) combination of dynamic **mechanical signals** reach first the nucleus and rearrange the chromatin…*
- ***Epigenetic** changes might take place, including methylation, histone modifications and miRNA involvement*
- *The extent of epigenetic input is a result of the appropriate combination of **biochemical signals** that arrive at the site AFTER mechanics has set the stage…*
- *Studies on the **gene expression** of dynamically cultured cells in-vitro should take the above into account*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