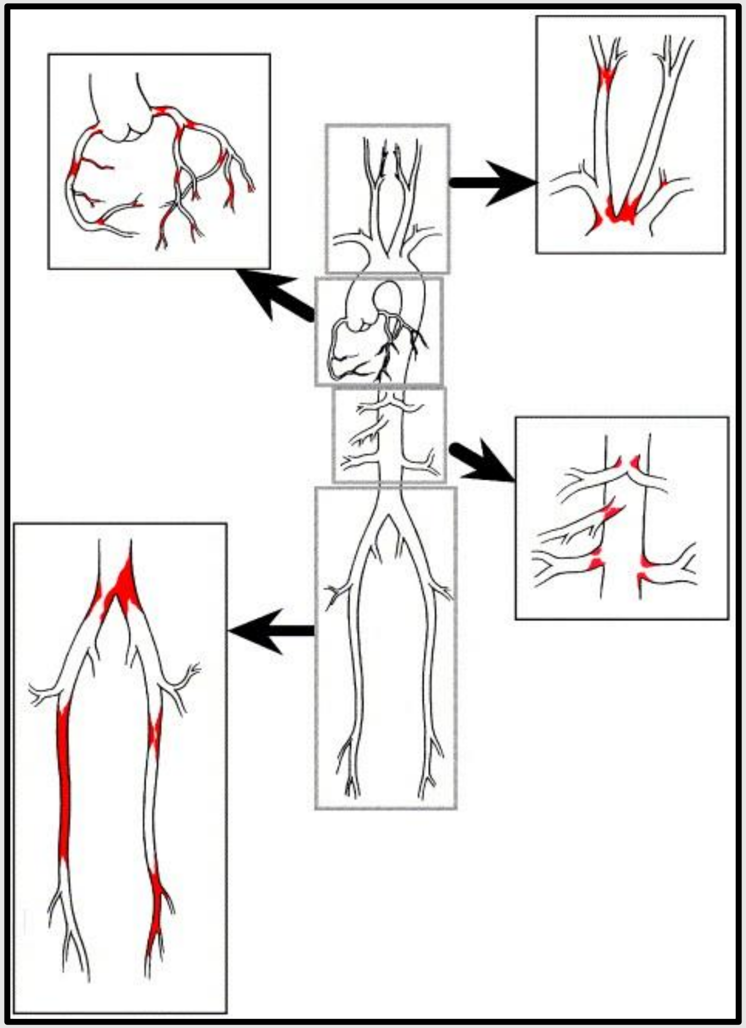
Understanding atherosclerosis :
How to study the effects of shear stress *in vitro* ?

Damien Calay, PhD

Cardiovascular BSc
16th November 2012

Why studying the effects of shear stress ?



DeBarkey et al., 1985

The well-known risk factors are :

- High LDL/HDL ratio
- Hypertension
- Smoking
- Diabetes
- Obesity
- Lack of physical exercise
- Age and gender
- Family history of early heart disease

But none of them explain the focal characteristic of atherosclerosis.

The role of blood flow in the focal development of atherosclerosis : Colin Caro

“Fluid dynamics has long been thought to play a role in this process of atherosclerosis. In 1969, a team led by Colin Caro, a physiologist at Imperial College in London, proposed that the locations where plaque usually develops were "dead spots" in the bloodstream, analogous to stagnant pools in a creek. They were broadly right, but only recently has the endothelium's sensitivity to fluid dynamics come to light, complicating the story.”

The role of blood flow in the focal development of atherosclerosis : Colin Caro

“Fluid dynamics has long been thought to play a role in this process of atherosclerosis. In 1969, a team led by Colin Caro, a physiologist at Imperial College in London, proposed that the locations where plaque usually develops were "dead spots" in the bloodstream, analogous to stagnant pools in a creek. They were broadly right, but only recently has the endothelium's sensitivity to fluid dynamics come to light, complicating the story.”

“Fluid mechanics has a controlling and an inhibiting effect on atherogenesis.”

Caro *et al.*, 1969. Nature ; 223(5211) Arterial wall shear and distribution of early atheroma in man

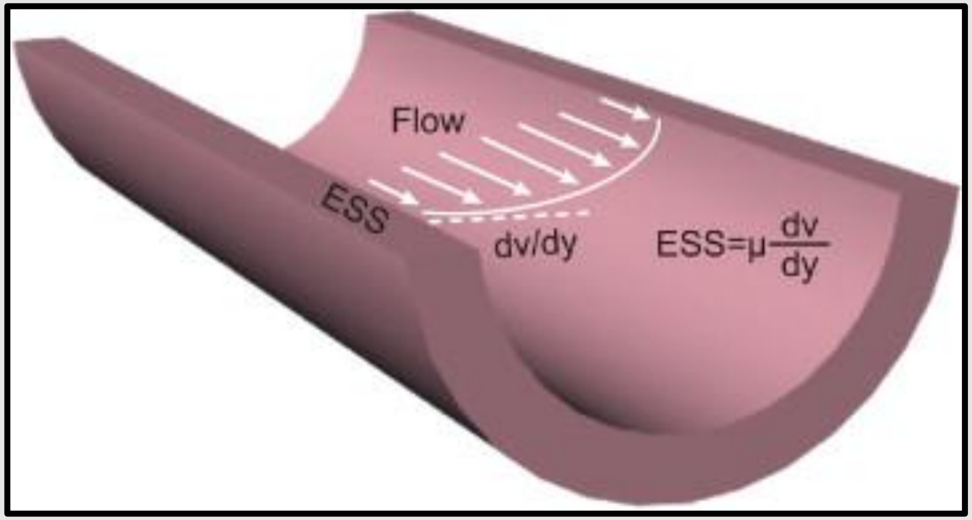
What is the shear stress ?

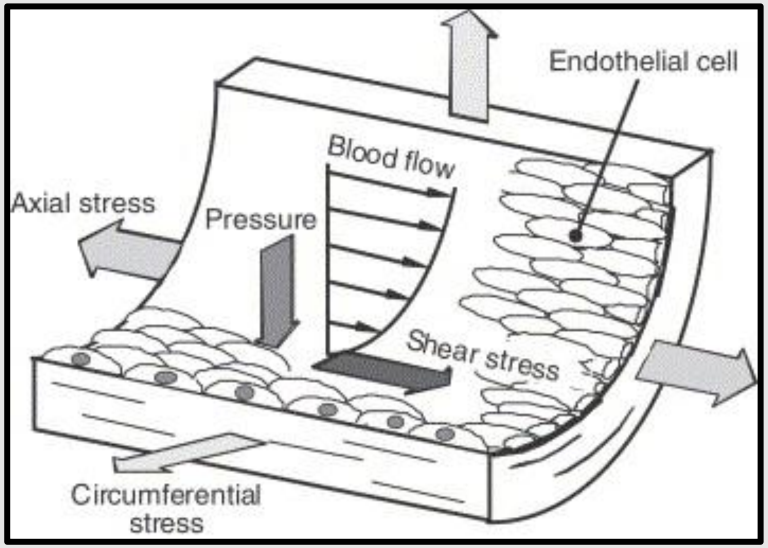
Endothelial shear stress :

- is the tangential force derived from the friction of the flowing blood on the endothelial surface of the arterial wall

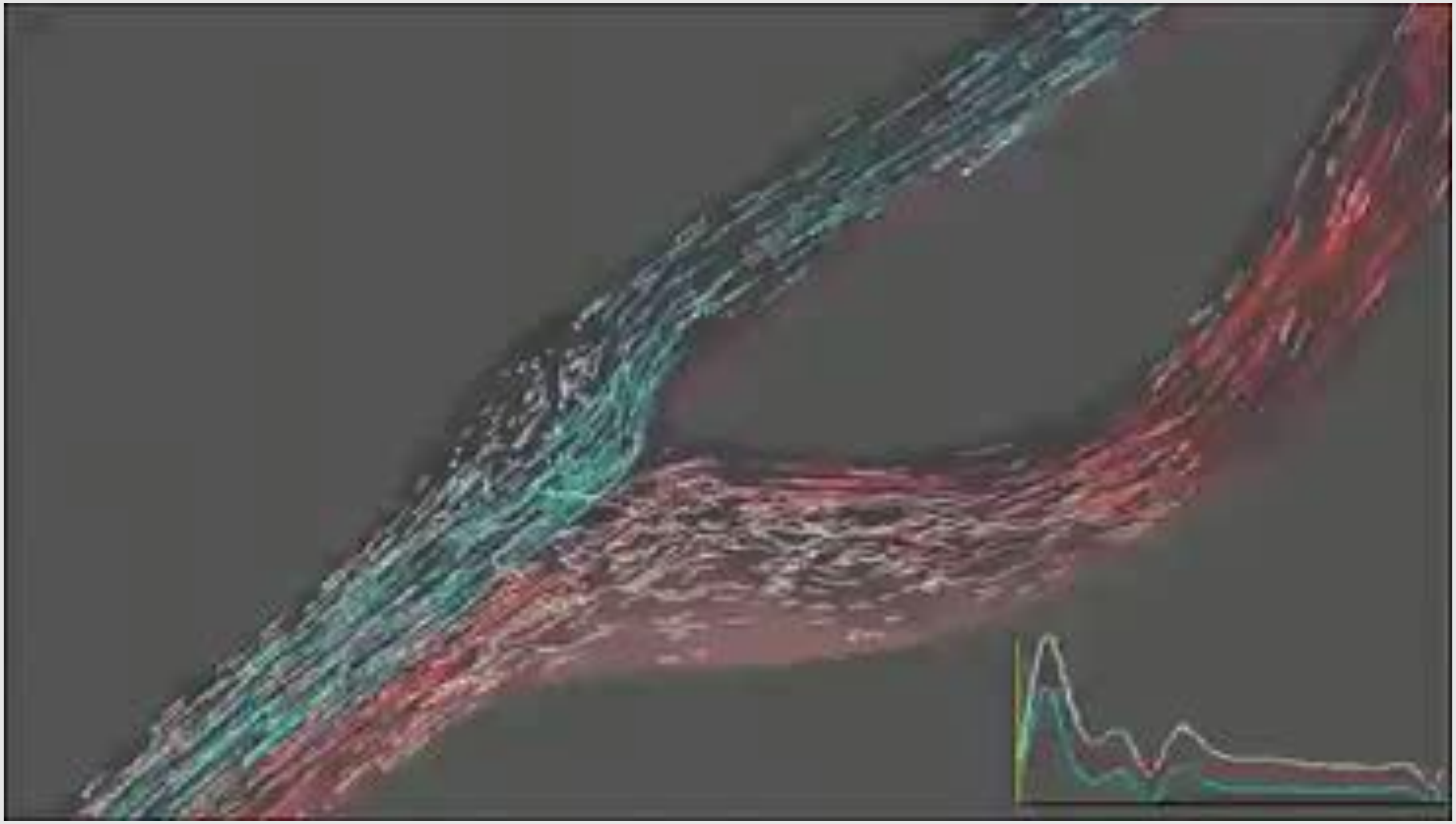
- proportional to the product of the blood viscosity (μ) and the spatial gradient of blood velocity at the wall (ESS = $\mu \times dv/dy$)

- is expressed in units of force / unit area (N/m^2 or Pascal [Pa] or $dyne/cm^2$; $1\ N/m^2 = 1\ Pa = 10\ dyne/cm^2$)





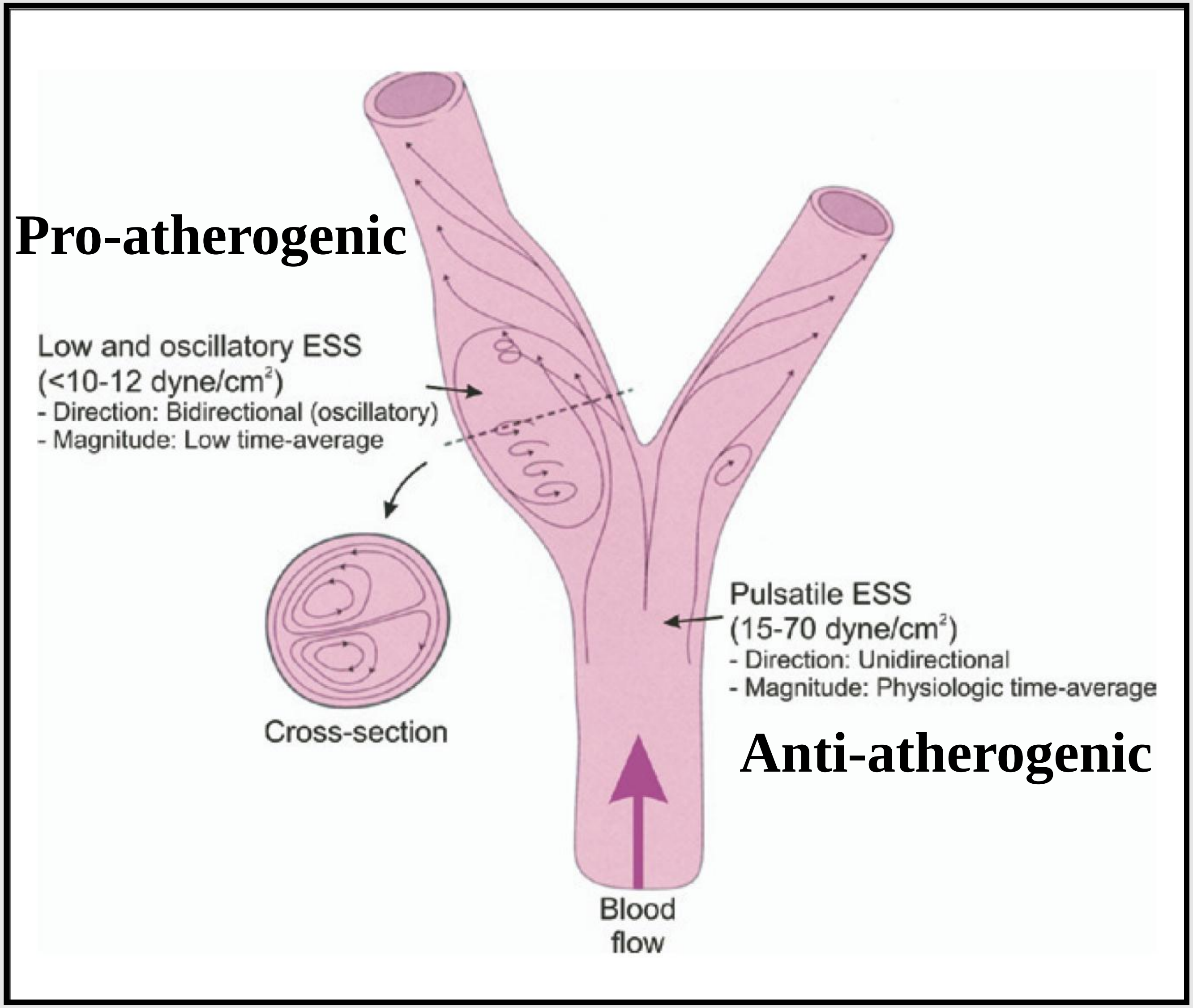
Regions of low and high shear stress in the vasculature



Blood flow in a normal carotid bifurcation

http://www.youtube.com/watch?feature=player_detailpage&v=Y3GQiBllgeY

Regions of low and high shear stress in the vasculature



Pro-atherogenic

Anti-atherogenic

In relatively **straight arterial segments**, ESS is pulsatile and **unidirectional** with a magnitude that varies within a range of **15 to 70 dyne/cm²** over the cardiac cycle.

In contrast, in **geometrically irregular regions**, where disturbed laminar flow occurs, pulsatile flow generates low and/or oscillatory ESS. Low ESS refers to ESS that is unidirectional at any given point but has a periodically fluctuating magnitude (**<10 to 12 dyne/cm²**)

Chatzizisis *et al.*, J Am Coll Cardiol. 2007 ; 49(25)

How to study the effects of shear stress ?

In vivo

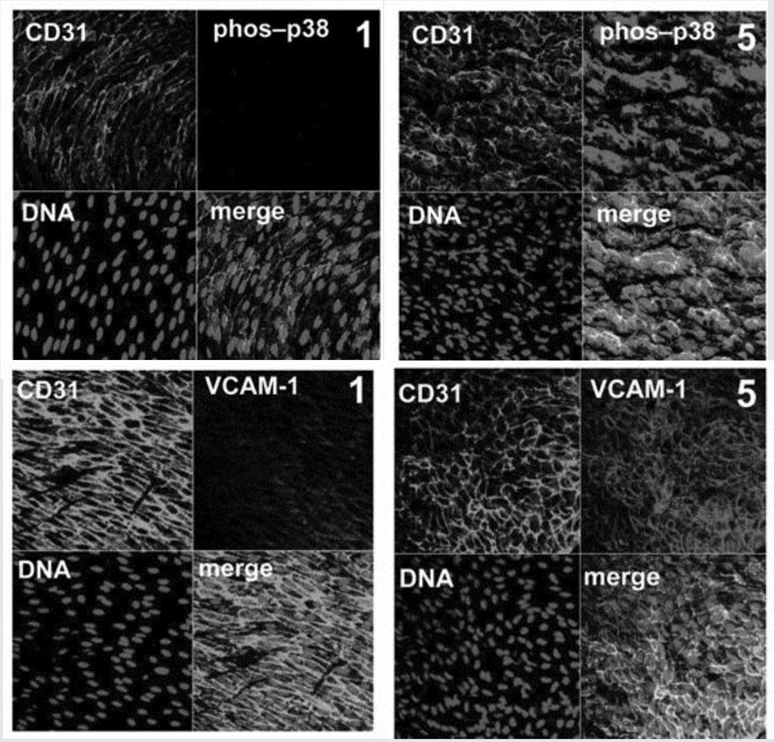
Murine model of atherosclerosis

WT mouse ApoE$^{-/-}$ mouse

Protected Susceptible

P-p38

VCAM-1



En face staining of areas of high and low probability of plaque development (aortic arch).

Zakkar M *et al.* Arterioscler Thromb Vasc Biol 2009;29.

How to study the effects of shear stress ?

In vivo

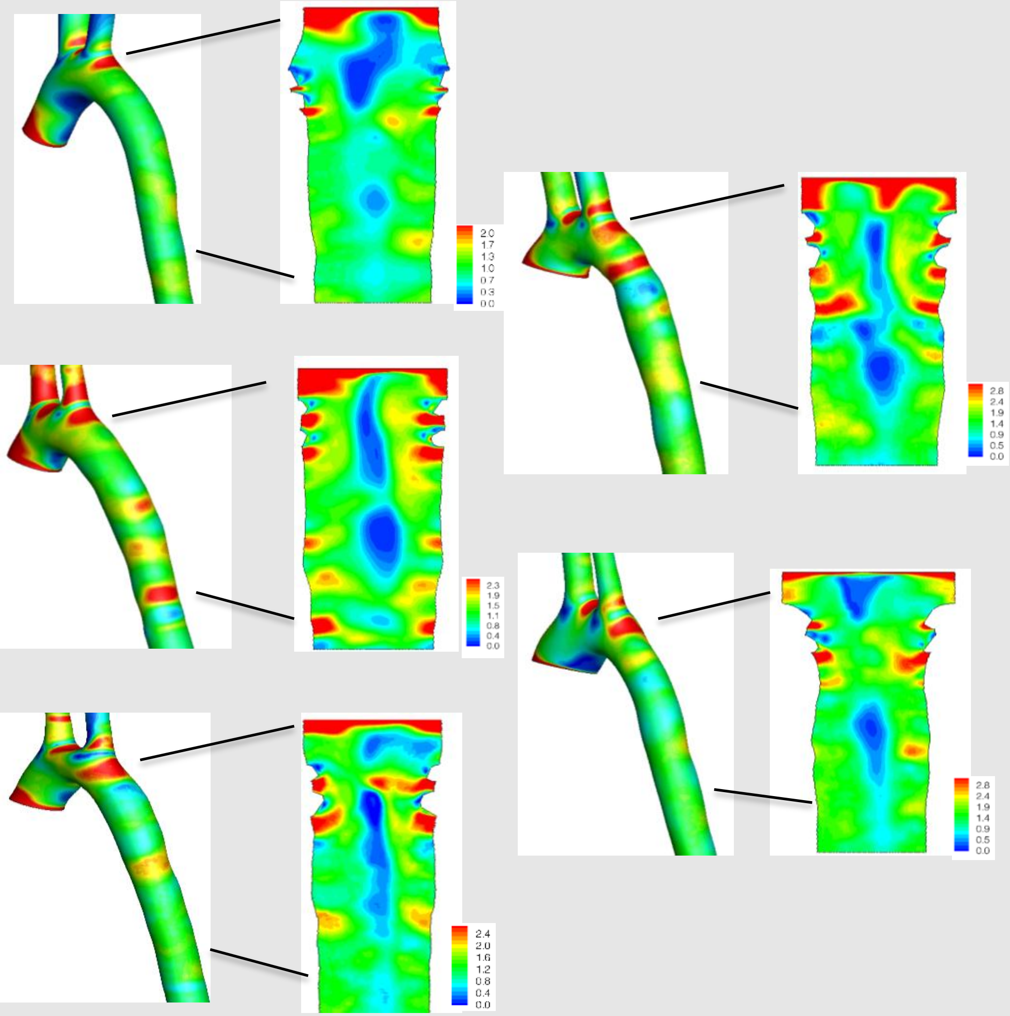
Pig

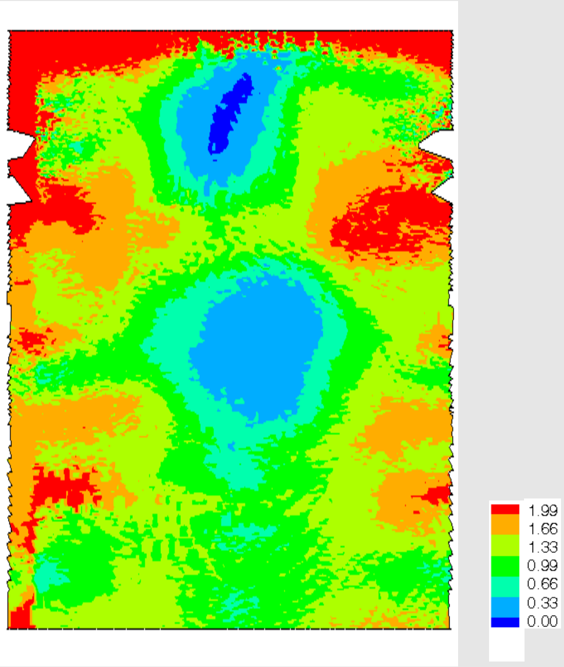
Porcine aorta

How to study the effects of shear stress ?

In vivo

<u>Pig</u>



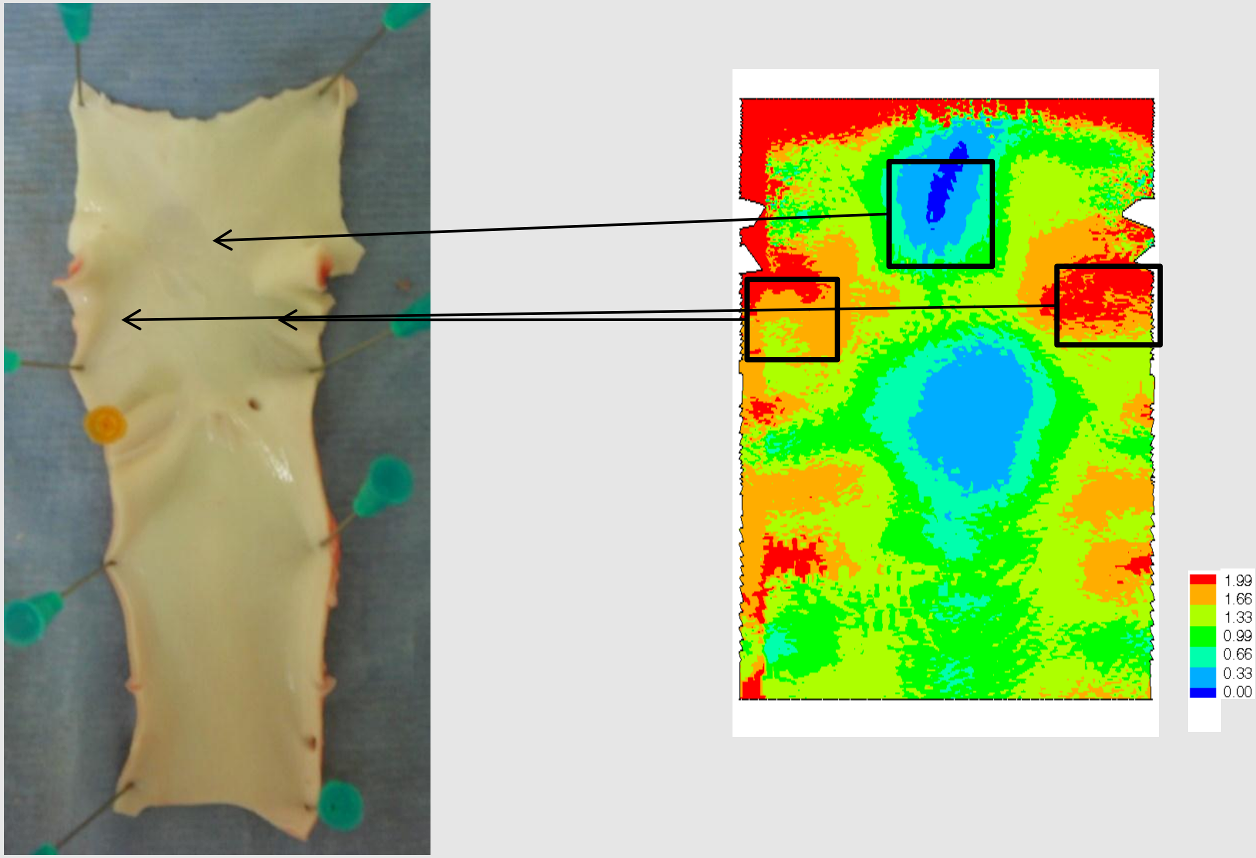


Amalia De Luca

How to study the effects of shear stress ?

In vivo

Pig

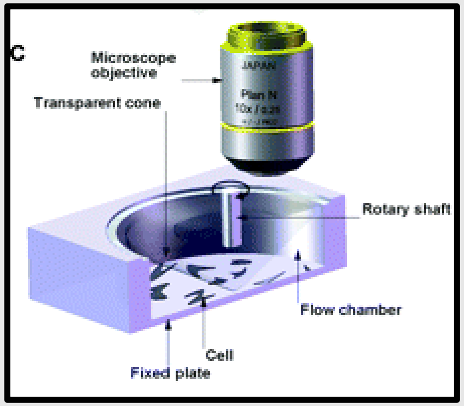


Amalia De Luca
Christina Warboys

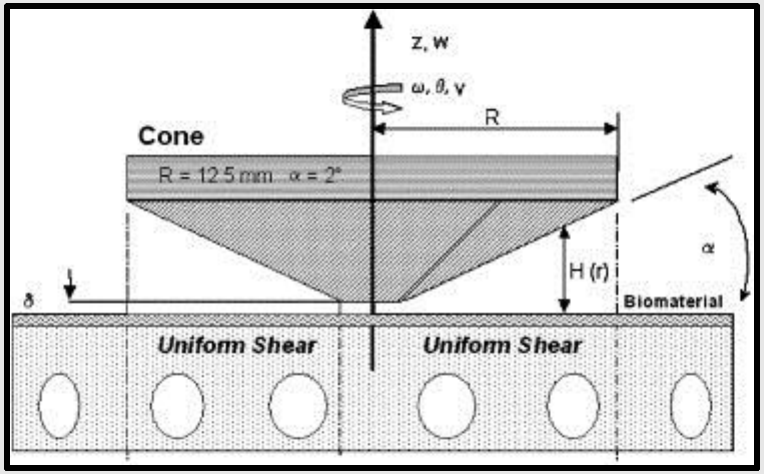
How to study the effects of shear stress ?

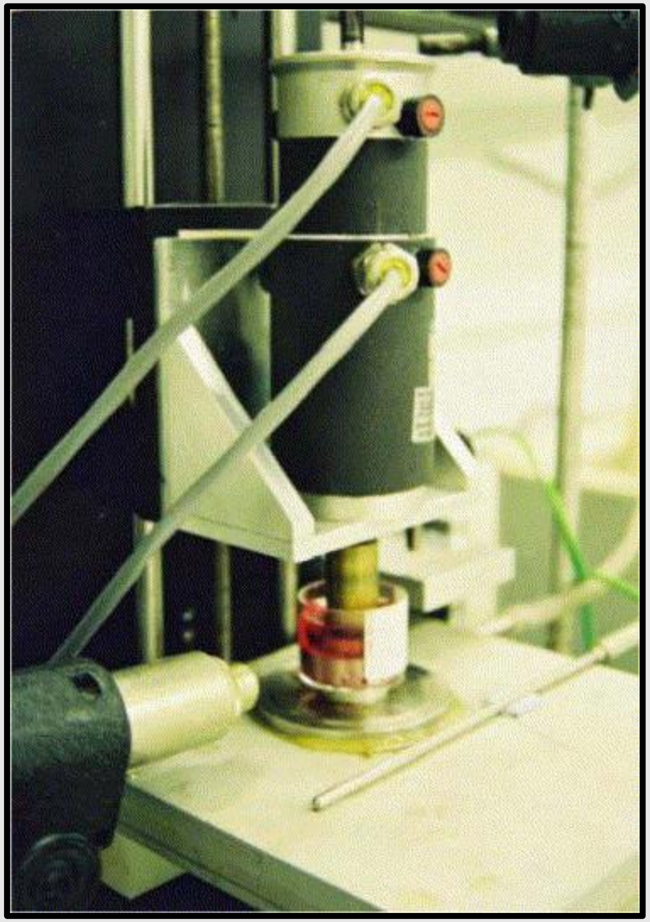
In vitro

The flow chambers : the cone-plate flow chamber



Kweku *et al.*, EBM 2008



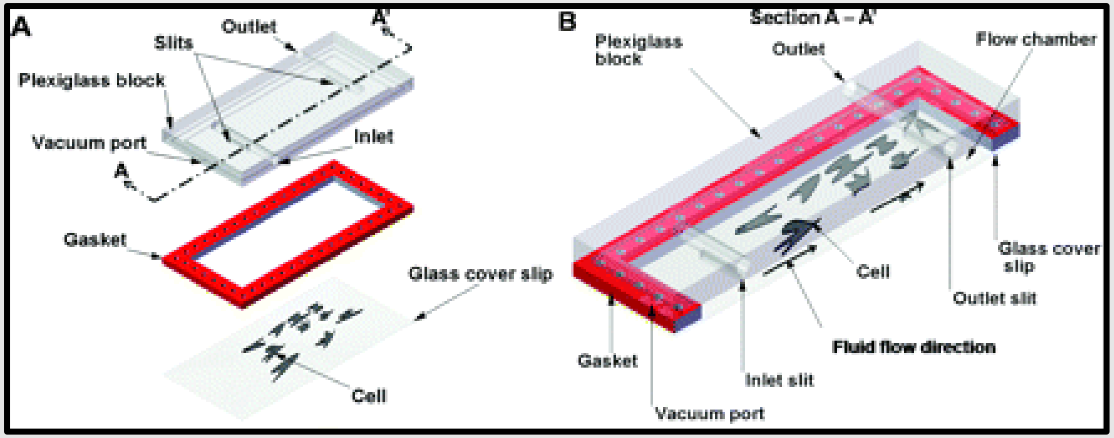


Feugier *et al.*, Biomaterials 2005, 26 (13).

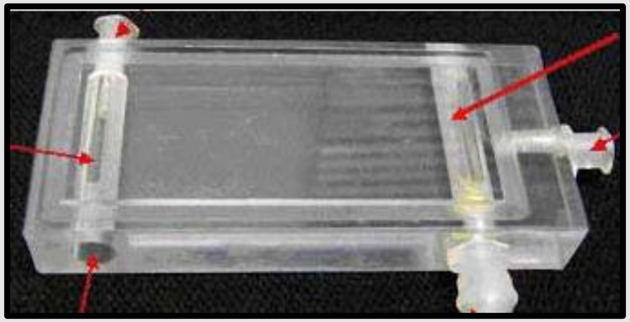
How to study the effects of shear stress ?

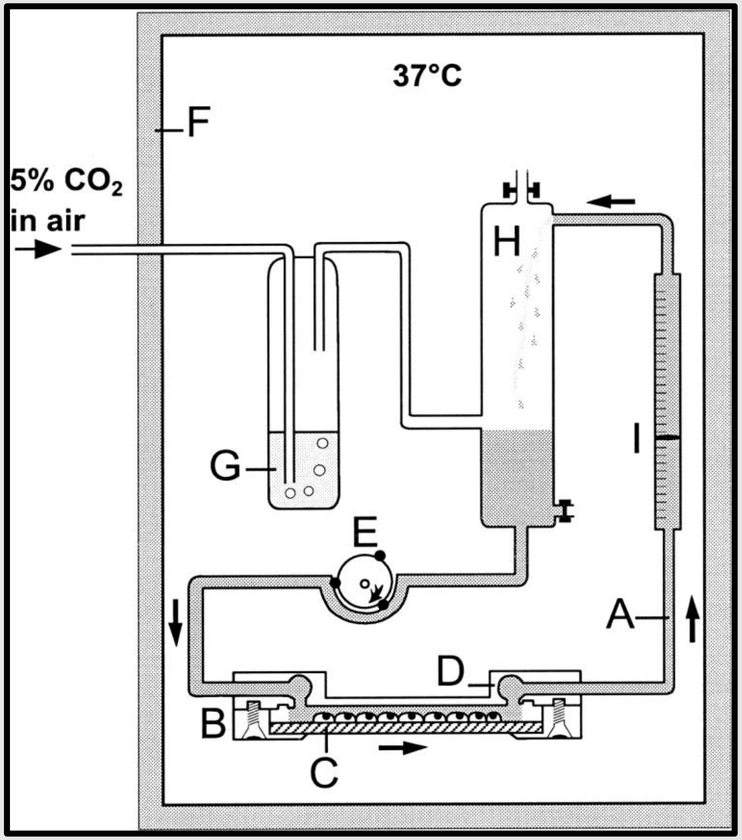
In vitro

The flow chambers : the parallel-plate flow chamber



Kweku *et al.*, EBM 2008



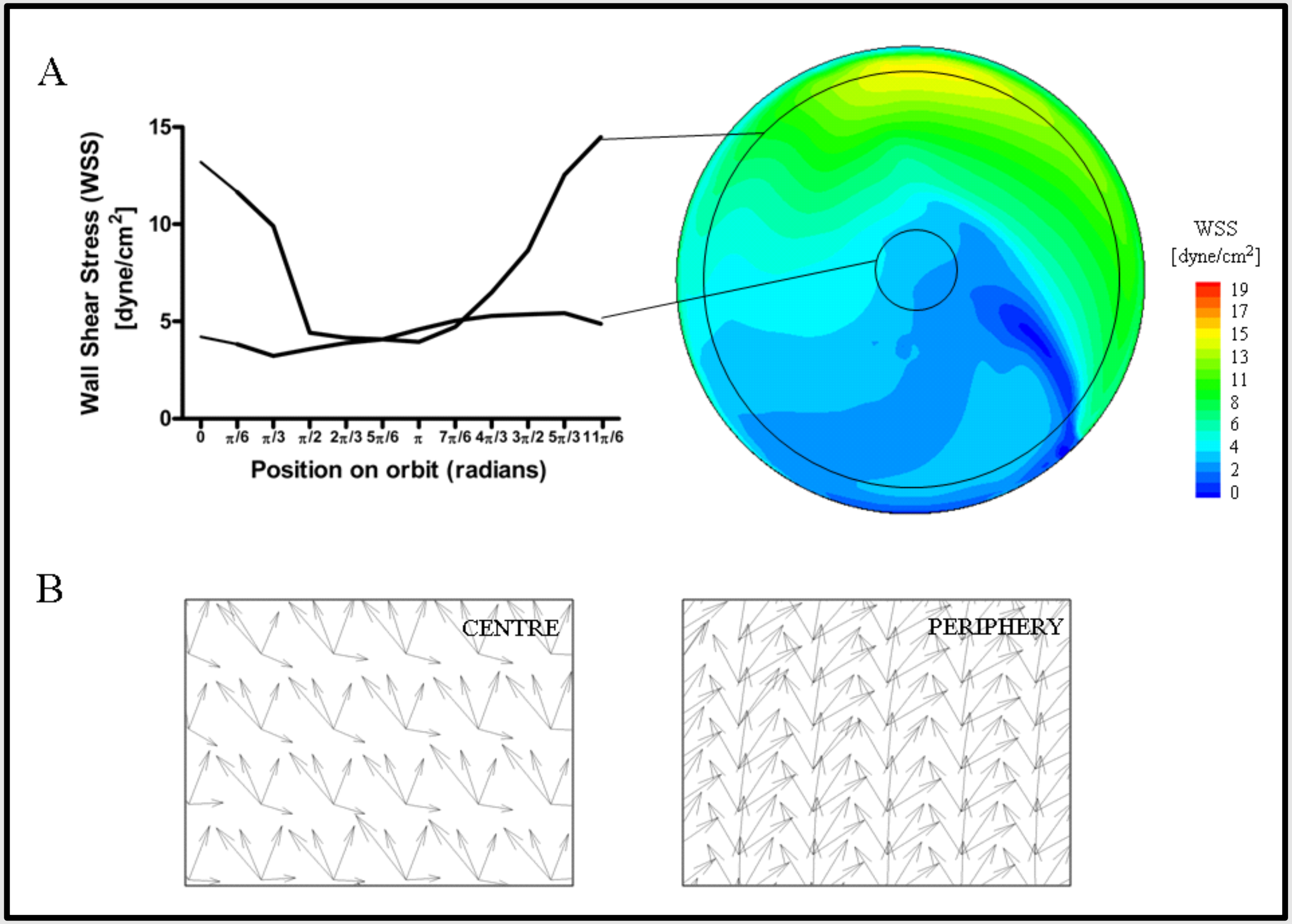


Sterck J G H *et al.* Am J Physiol Endocrinol Metab 1998

How to study the effects of shear stress ?

In vitro

The orbital plate shaker

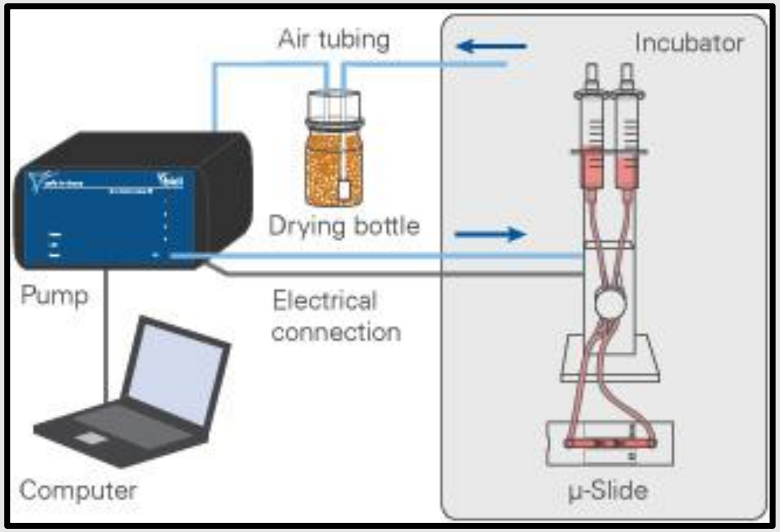


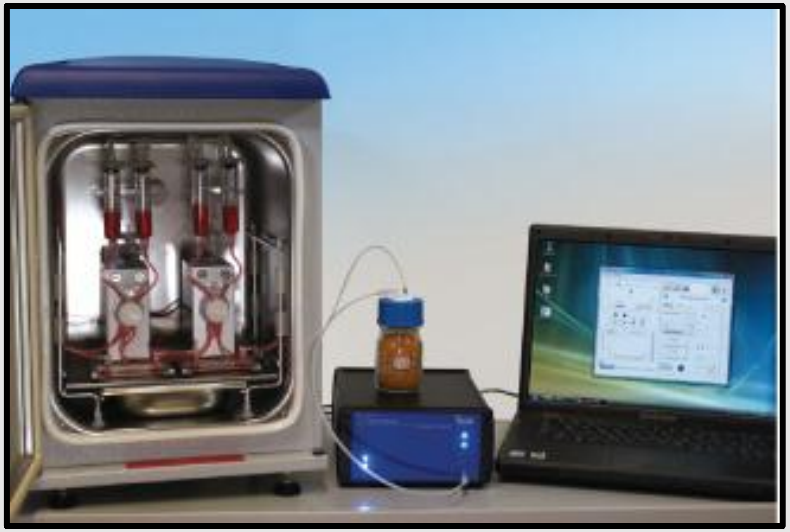
Centre: low, multidirectional shear
Periphery: higher magnitude with temporal oscillations and unidirectional

How to study the effects of shear stress ?

In vitro

The Ibidi® system



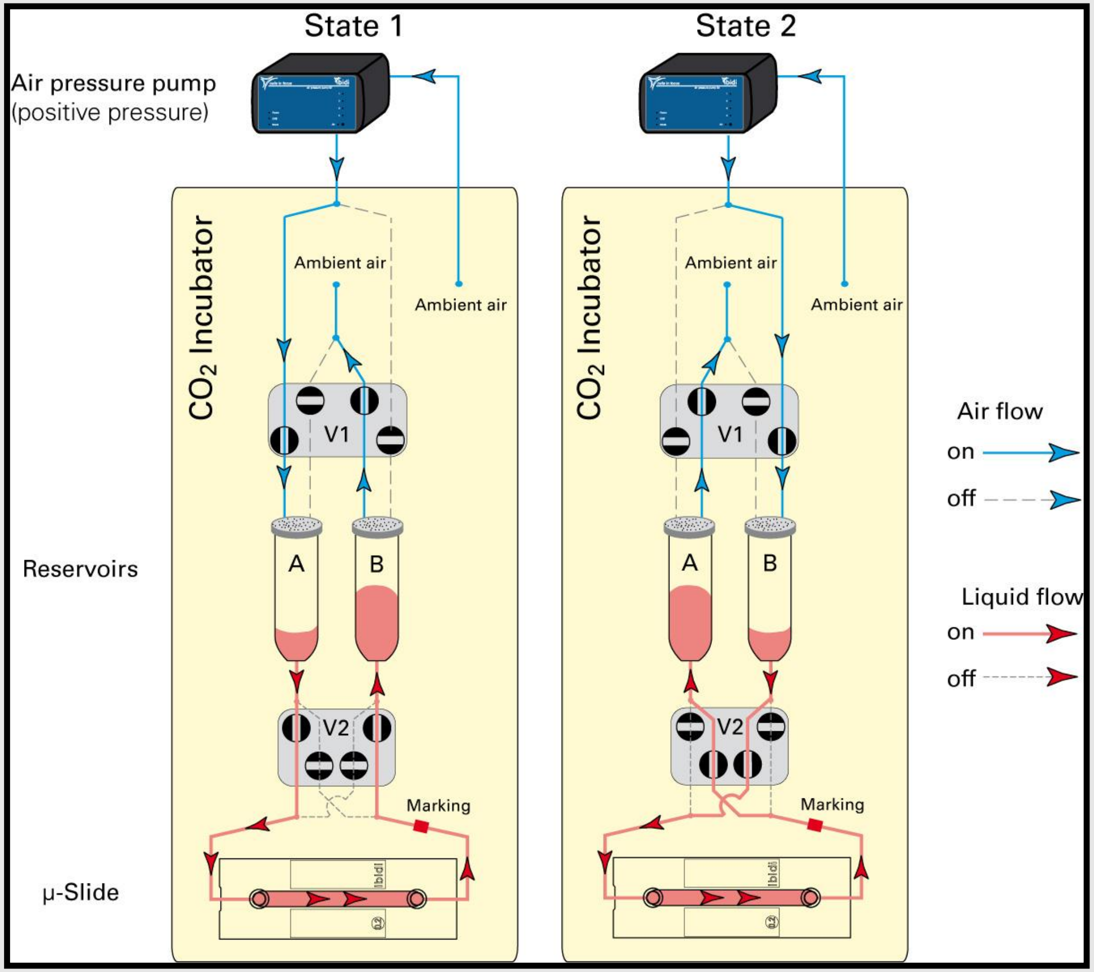


µ-slides connected to fluidic units, linked to an air-pressure pump controlled by a computer

How to study the effects of shear stress ?

In vitro

The Ibidi® system



Working principle of the Ibidi® pump system using positive pressure

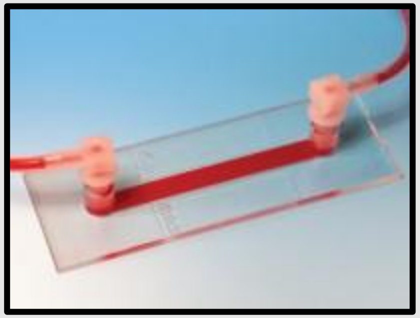
http://www.ibidiusa.com/product/ibidi-pump-system/

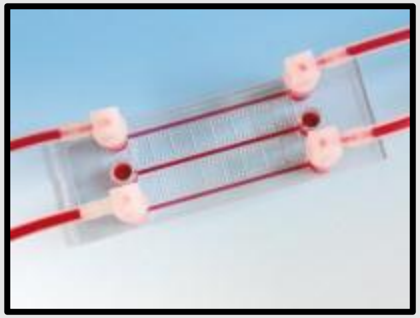
www.ibidi.com

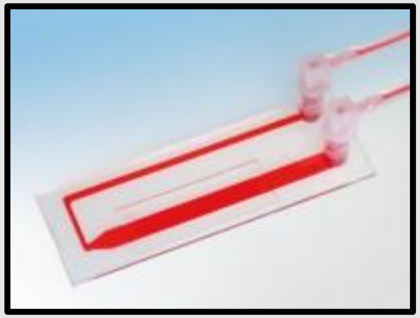
How to study the effects of shear stress ?

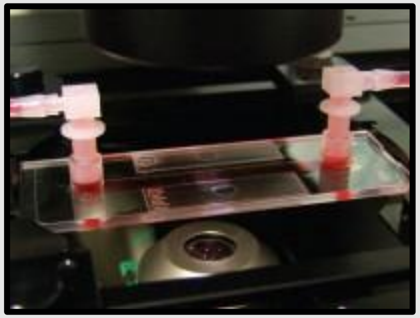
In vitro

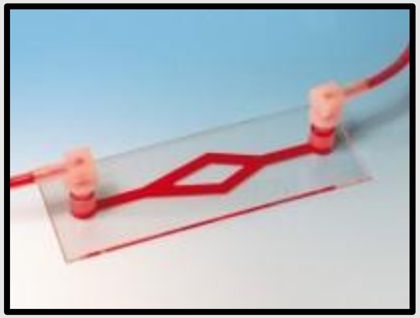
<u>The Ibidi® system</u>

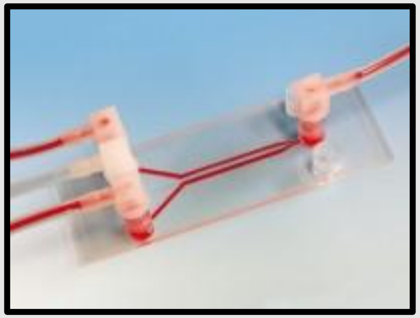

μ-slide I Luer

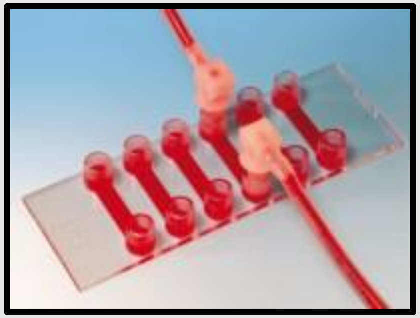

μ-slide III

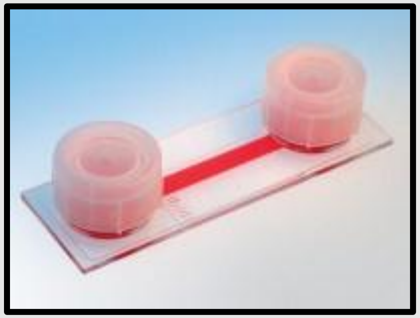

μ-slide upright




μ-slide y-shaped


μ-slide III $^{3\text{ in }1}$

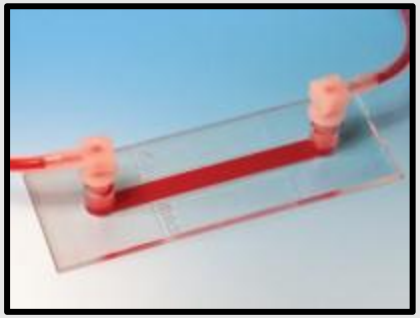

μ-slide VI


μ-slide I

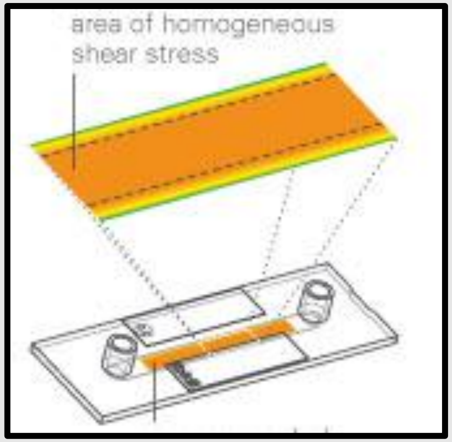
How to study the effects of shear stress ?

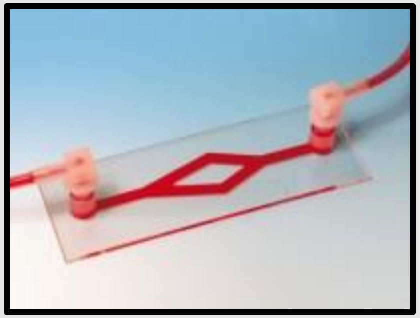
In vitro

<u>The Ibidi® system</u>

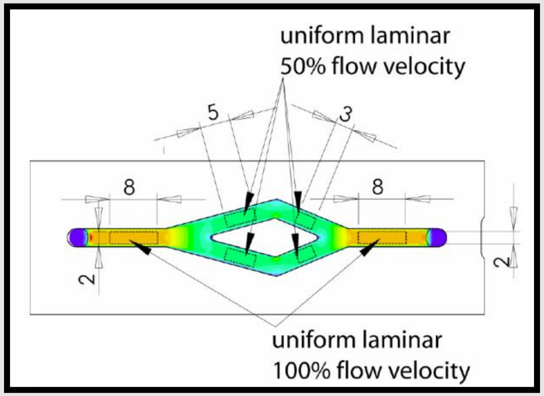


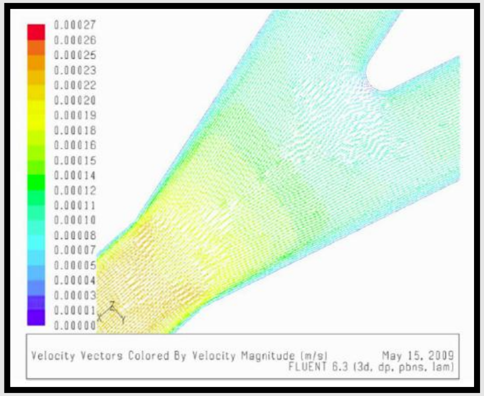
μ-slide I Luer





μ-slide y-shaped





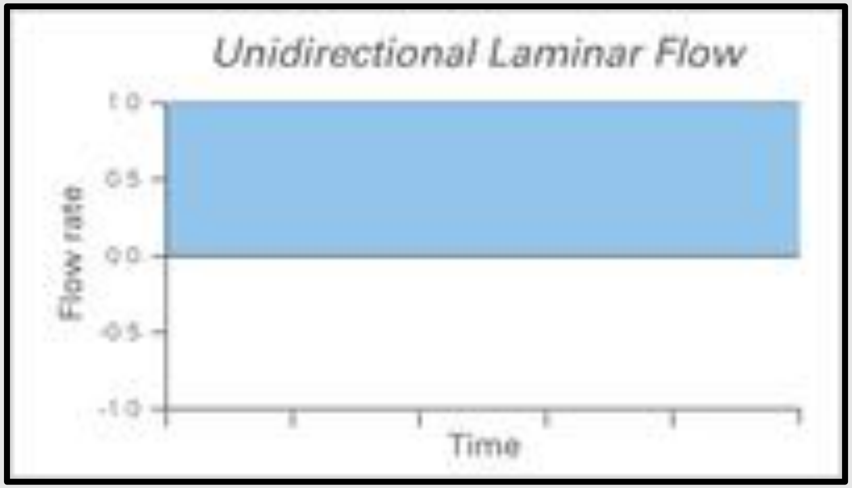
$$\tau[\frac{\text{dyne}}{\text{cm}^2}] = \frac{\text{velocity from color [m/sec]}}{\text{velocity 100 \%} = 0.00024\ \text{m/sec}} \cdot 2.274\frac{\text{dyne / cm}^2}{1\ \text{ml/min}} \cdot \Phi[\frac{\text{ml}}{\text{min}}]$$

How to study the effects of shear stress ?

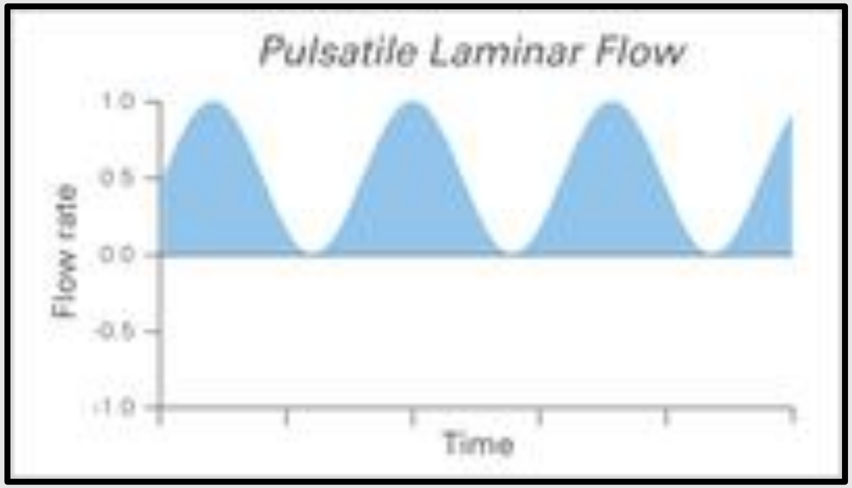
In vitro

The Ibidi® system

Unidirectional Laminar Flow
is encountered in most small healthy biological vessels, such as small arteries and veins. This is achieved by perfusing medium through low walled microchannels, and by keeping the flow constant over time for both direction and velocity.



Pulsatile Laminar Flow
is encountered in large arteries due to the fluctuations caused by the heartbeat. Experimentally, this type of flow can be mimicked by employing unidirectional flow with a periodically changing flow rate while keeping the flow direction constant.



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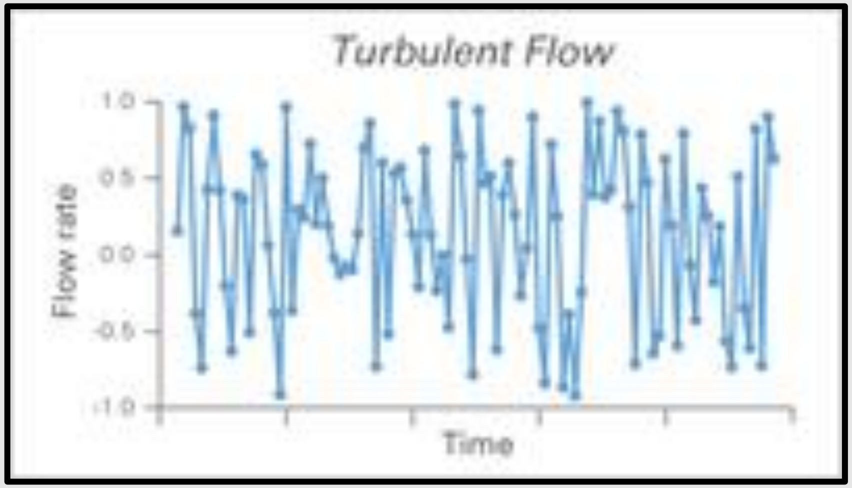
How to study the effects of shear stress ?

In vitro

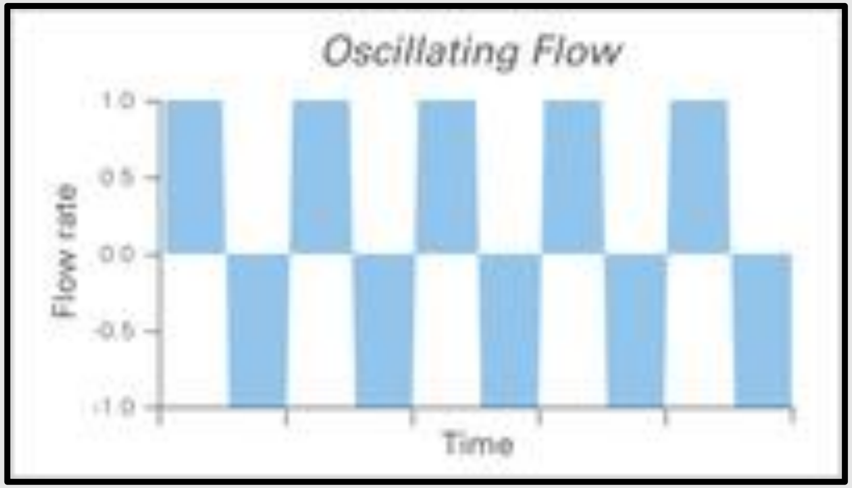
The Ibidi® system

Turbulent Flow
near surfaces is characterized by changes in flow rate and direction. Direction and velocity change over time, thus the flow profile is not constant. *In vivo*, turbulences are rare and can only be found during pathophysiological processes.



Note: *Due to physical reasons, turbulent flow cannot be achieved in microchannels using physiological flow regimes.*

Oscillating Flow
is accepted as a means of simulating turbulences when using microchannels. Although the flow is laminar, there is no main direction due to the fact that the direction of the flow is changed at regular intervals (every 0.5 s).



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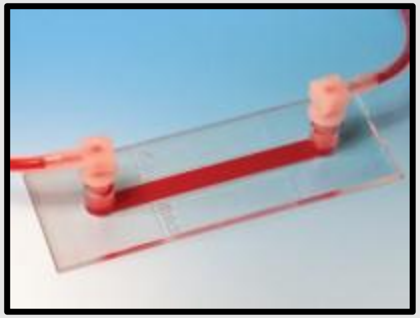
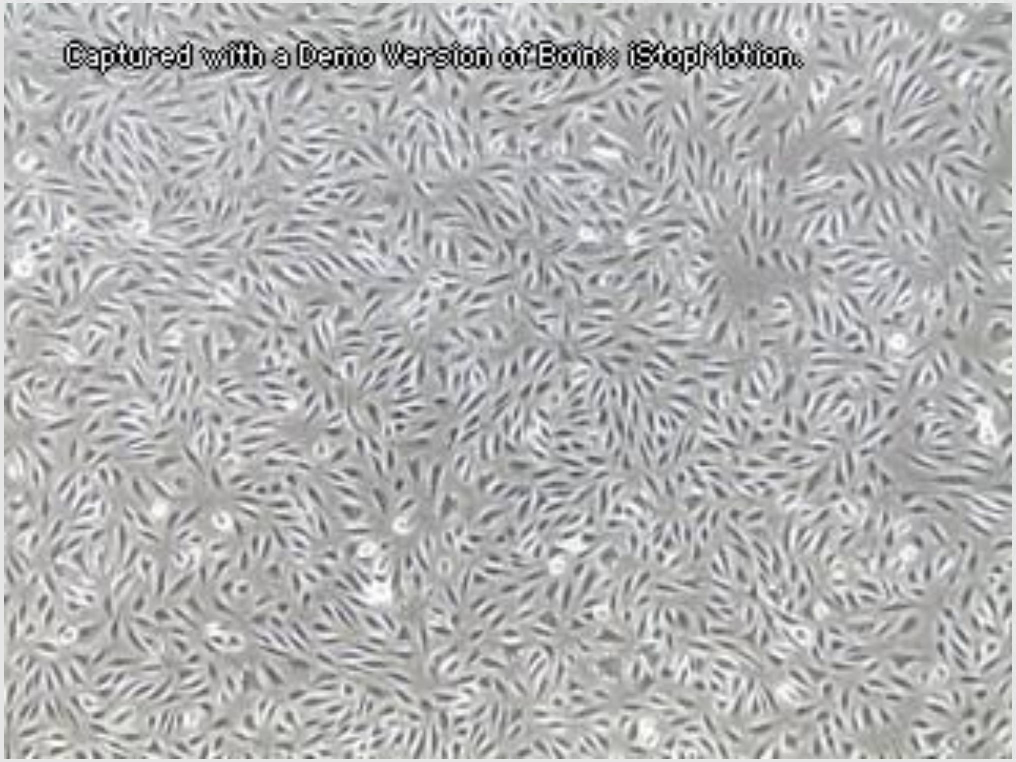
ECs behaviour under a unidirectional laminar shear stress of 50 dyn/cm^2 during 24 h



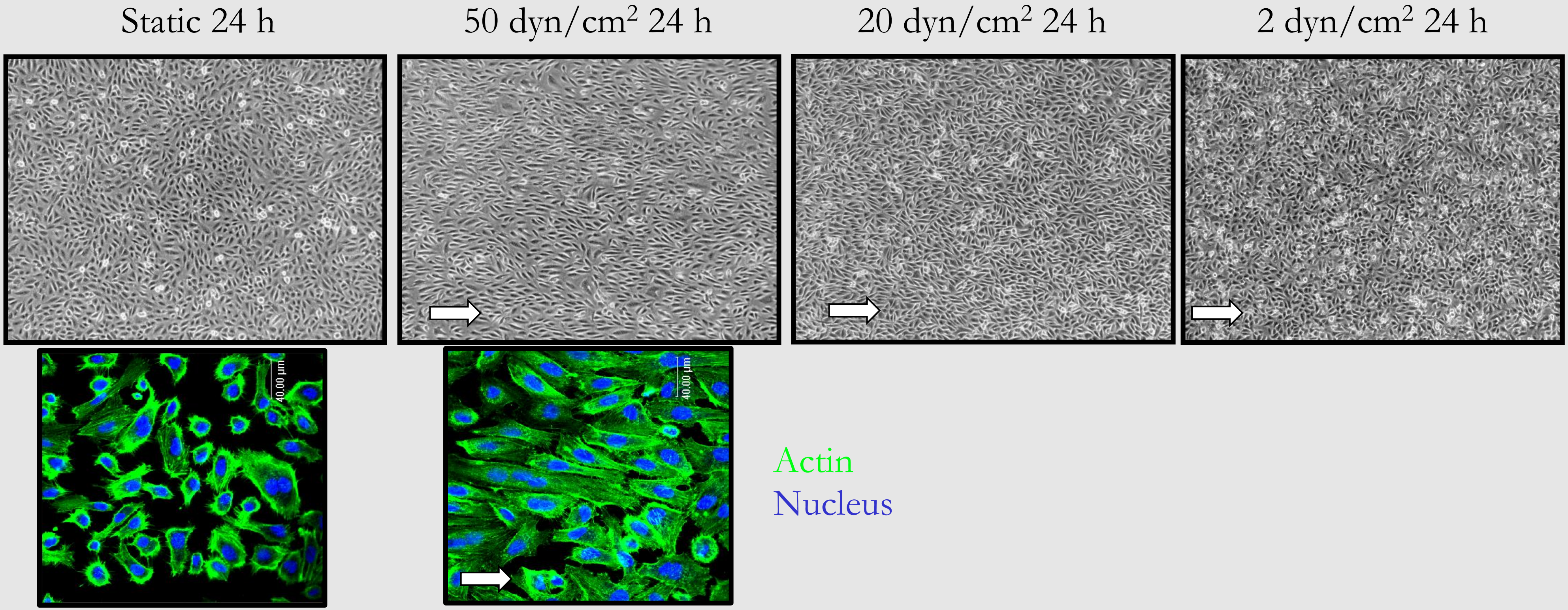
μ-slide I Luer



Eahy926 endothelial cells seeded onto a μ-slide I Luer 0.4 and exposed to a laminar flow of 50 dyn/cm^2 for 24 h

Calay D, PhD thesis

ECs morphology after exposure to a unidirectional laminar shear stress for 24 h



Static 24 h

50 dyn/cm^2 24 h

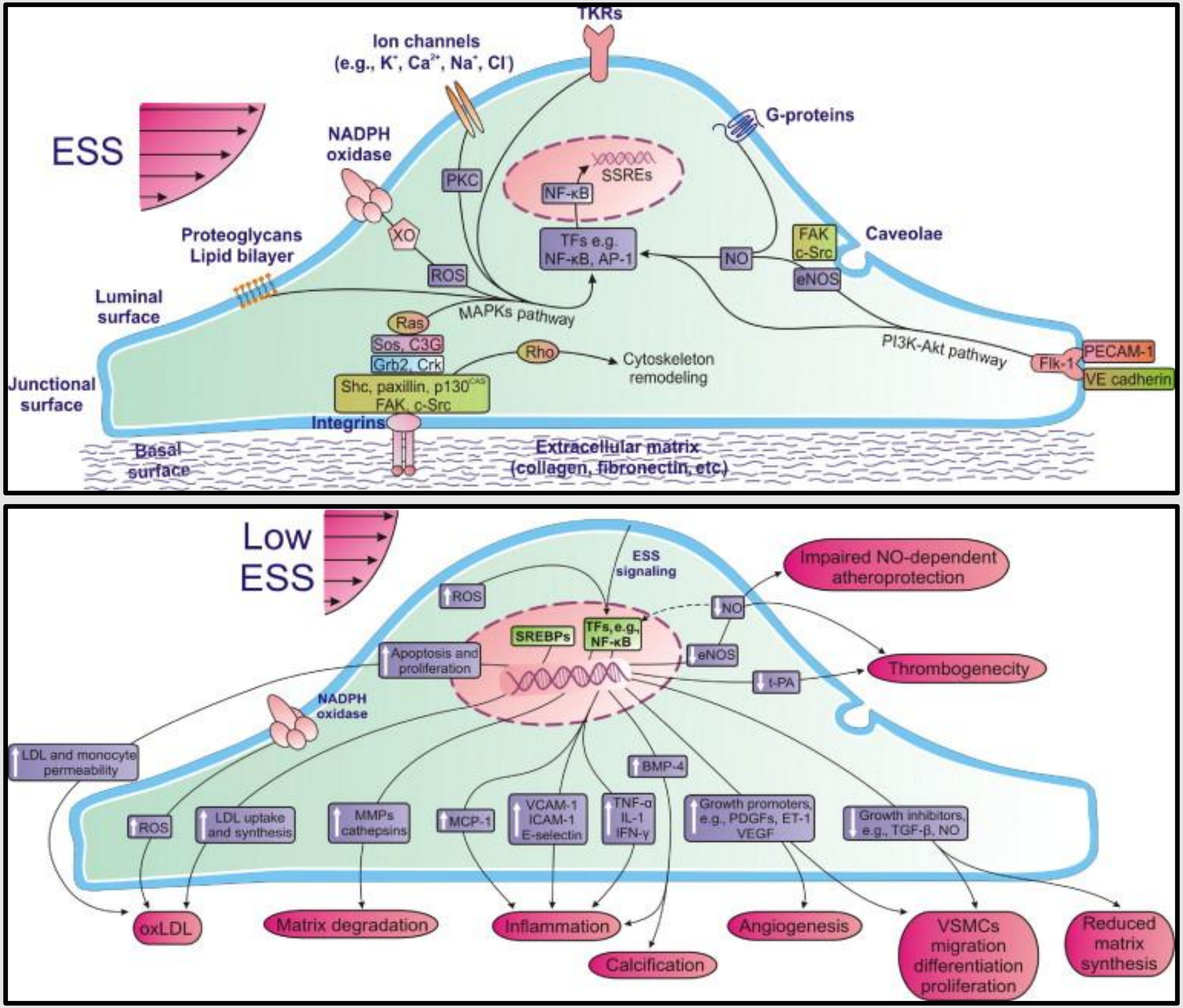
20 dyn/cm^2 24 h

2 dyn/cm^2 24 h

Eahy926 endothelial cells seeded onto a µ-slide I (static) or µ-slides I Luer 0.4 and exposed to a laminar flow at different shear stresses for 24 h

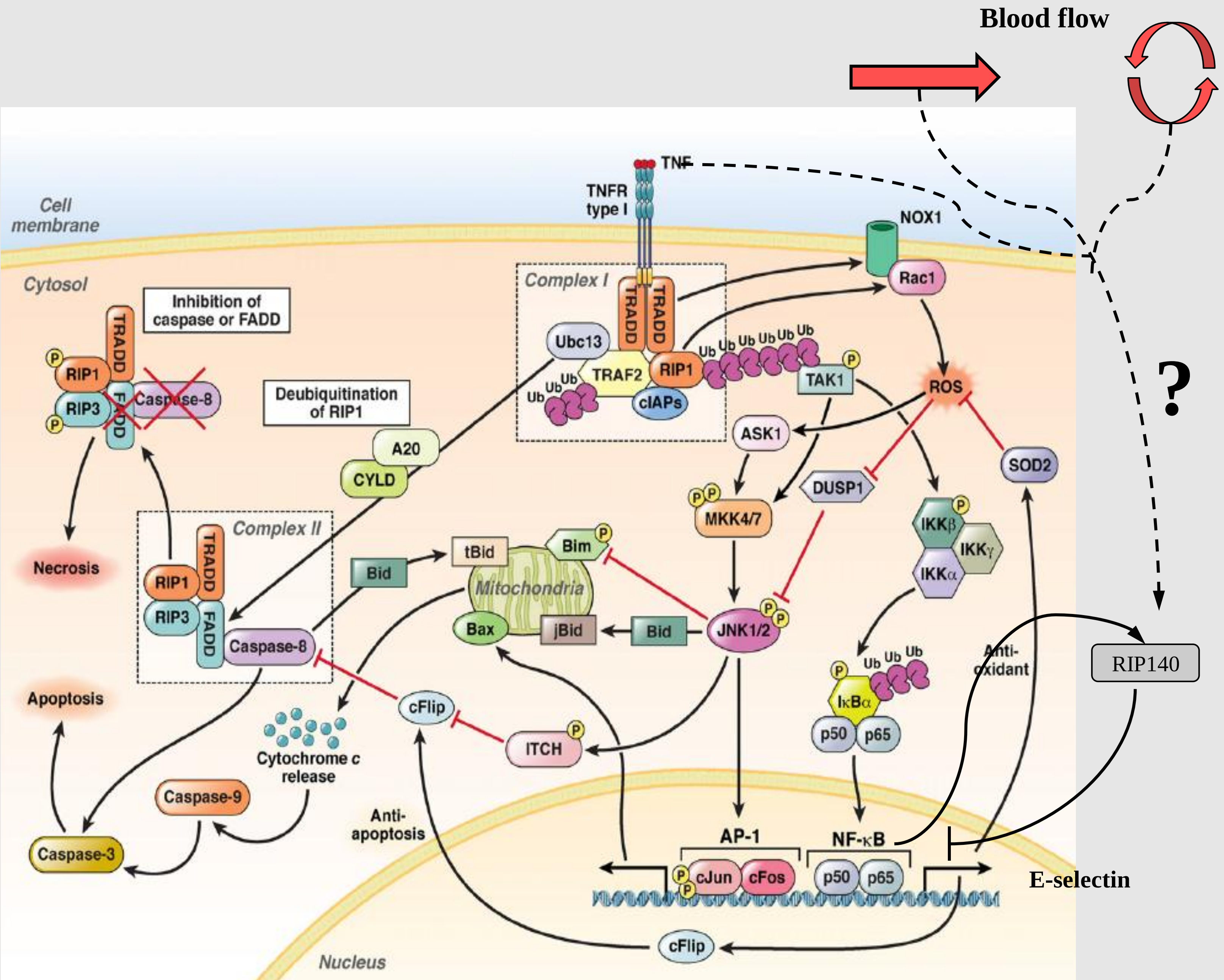
Calay D, PhD thesis

Some signalling pathways activated in ECs exposed to a laminar high or low shear stress



Chatzizisis *et al.*, J Am Coll Cardiol. 2007 ; 49(25)

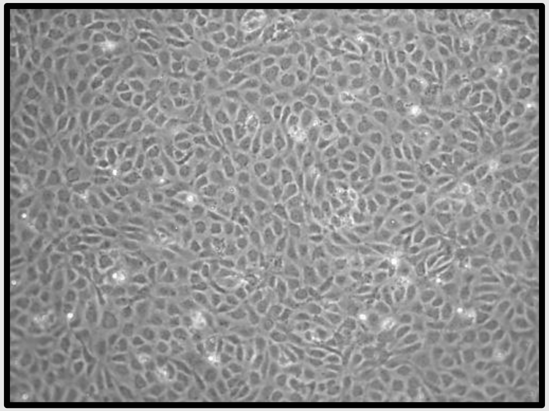
The influence of shear stress in the regulation of RIP140 by TNFα



Adapted from Seki *et al*. Gastroenterology. 2012.

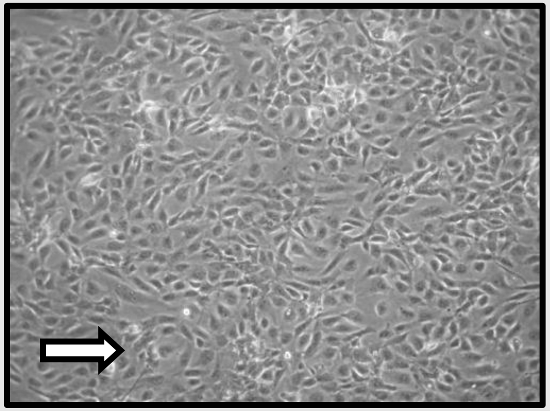
The influence of shear stress in the regulation of RIP140 by TNFα

Static condition : 24 h

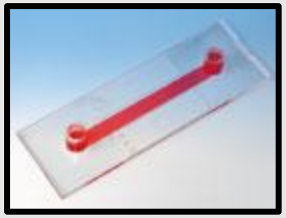


μ-slide I

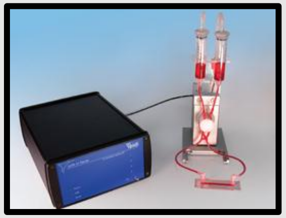
Laminar flow :
20 dyn/cm^2 ; 24 h



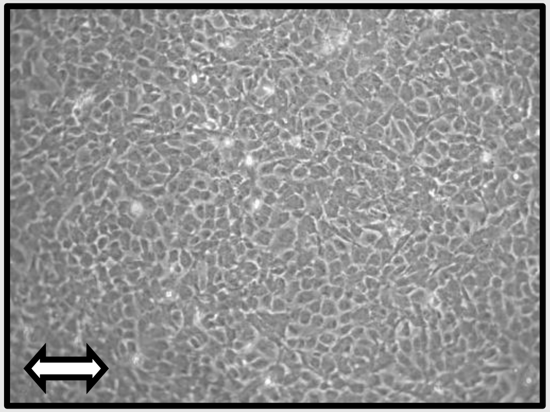
μ-slide I Luer 0.4



Fluidic unit and air pressure pump



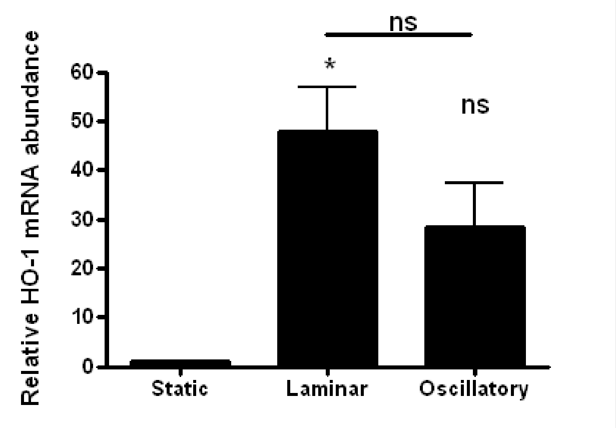
Oscillatory flow :
± 5 dyn/cm^2 ; 2 Hz ; 24 h



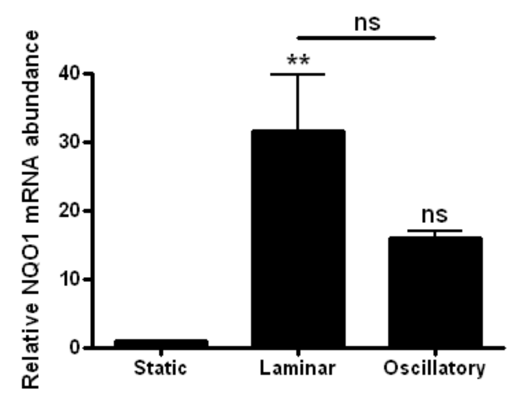
The influence of shear stress in the regulation of RIP140 by TNFα

Effect of shear stress on RIP140 expression

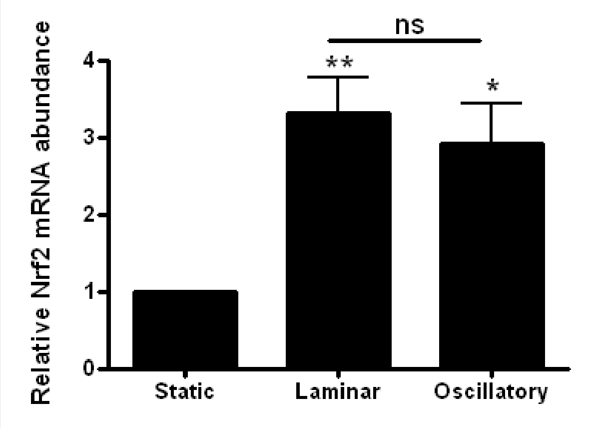
HO-1



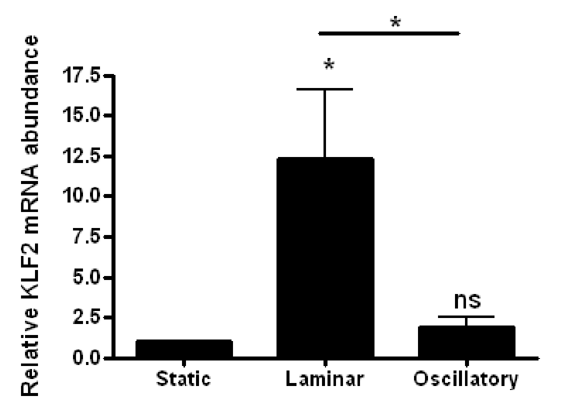
NQO1



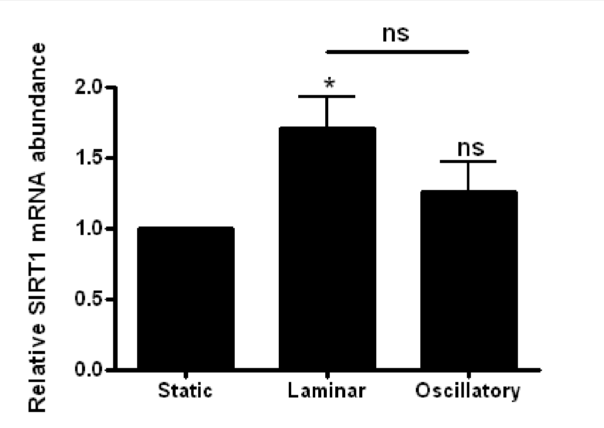
Nrf2



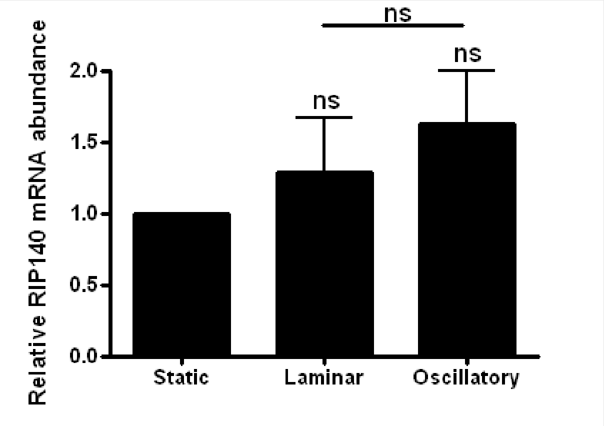
KLF2



SIRT1



RIP140

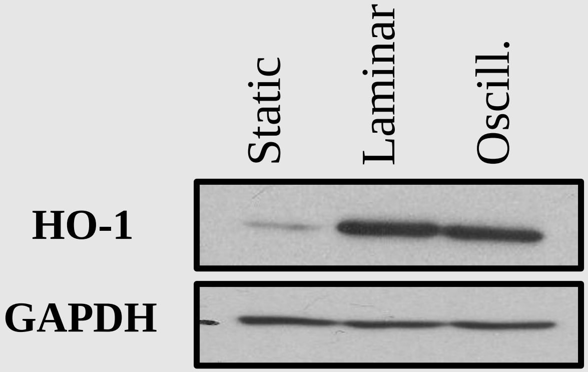


n=4

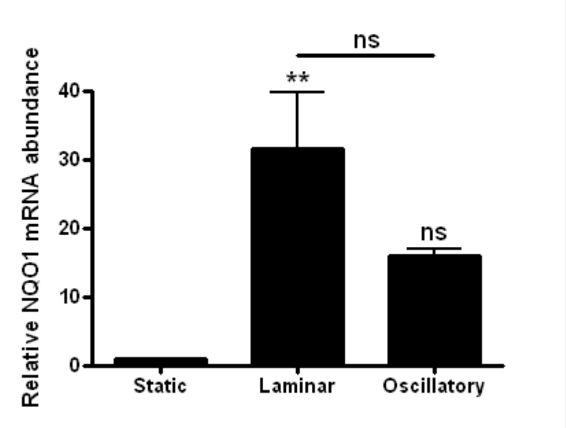
The influence of shear stress in the regulation of RIP140 by TNFα

Effect of shear stress on RIP140 expression

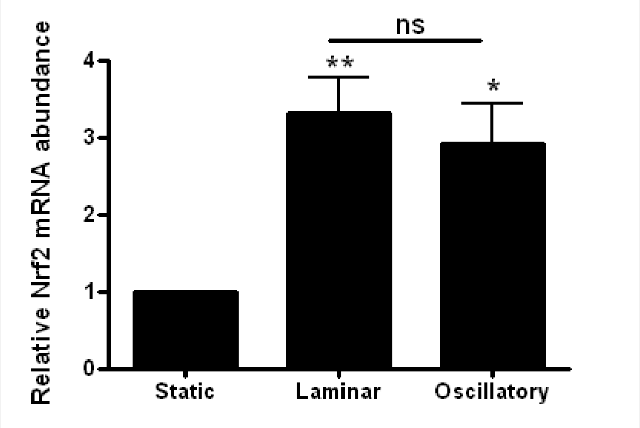
HO-1



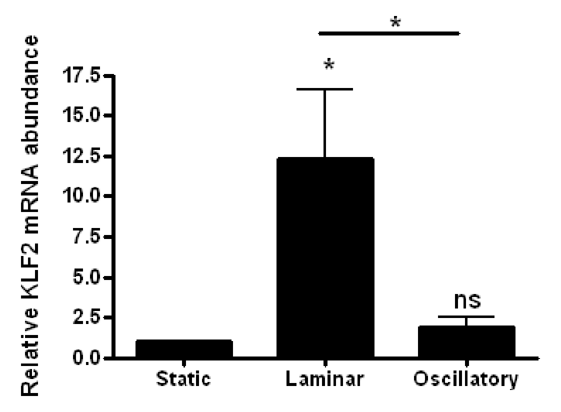
NQO1



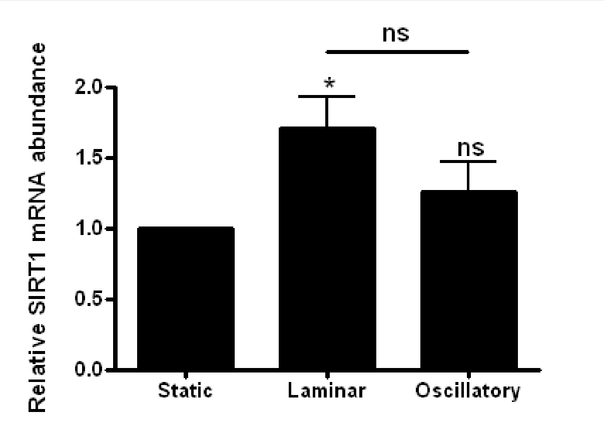
Nrf2



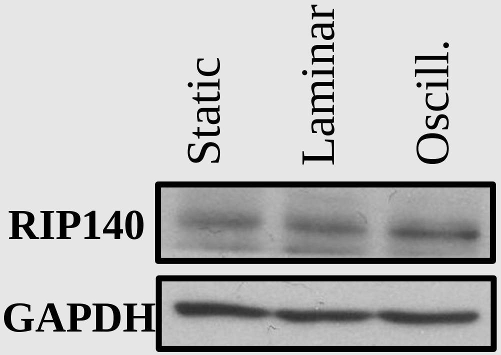
KLF2



SIRT1



RIP140

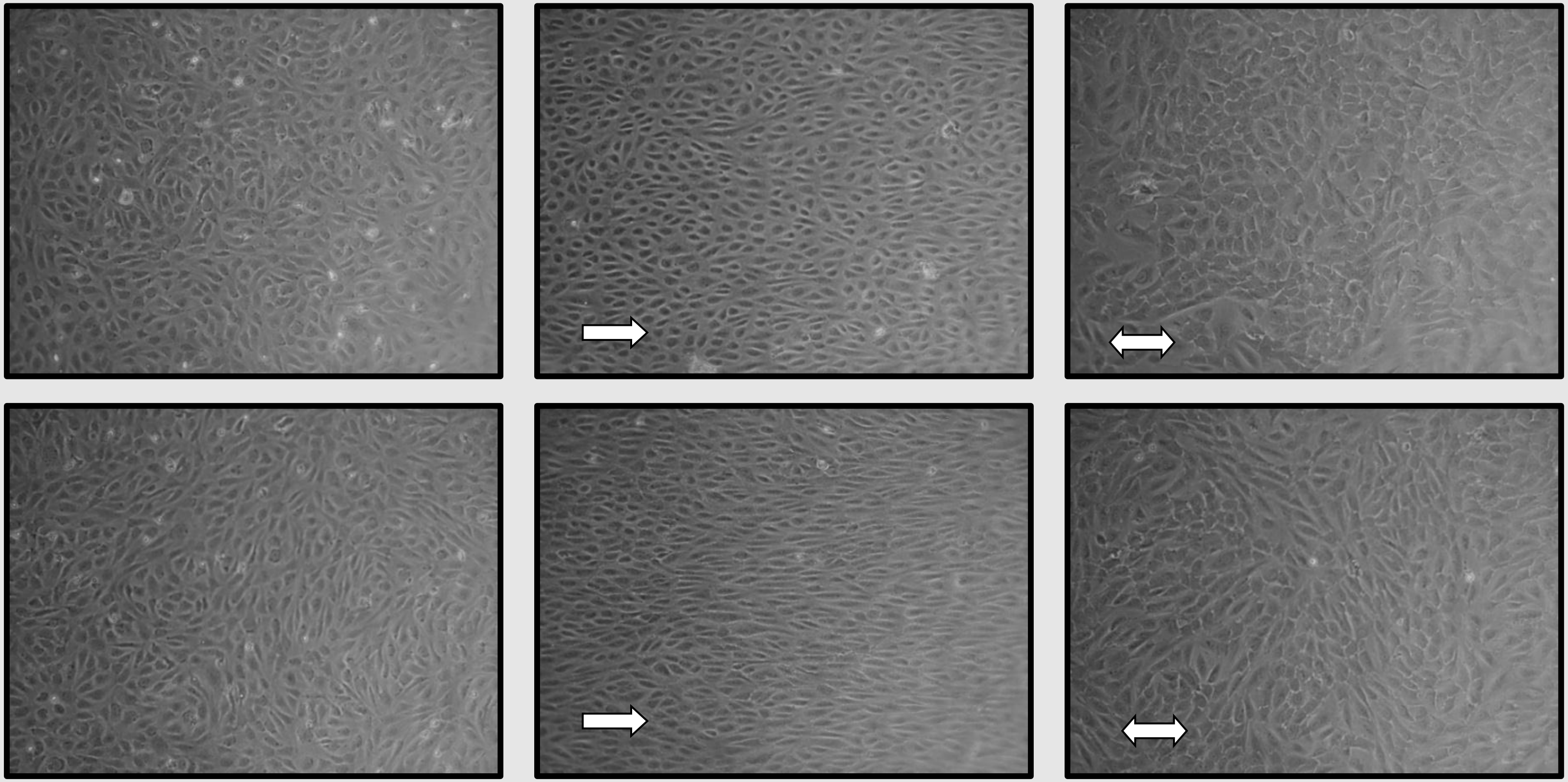


n=4

The influence of shear stress in the regulation of RIP140 by TNFα

Combined effect of shear stress and TNFα on RIP140 expression

Static, laminar flow, oscill.flow : 24 h → TNFα ↓ → Flow ± TNFα : 16 h → **Protein extraction**



Static condition

Laminar flow : 20 dyn/cm²

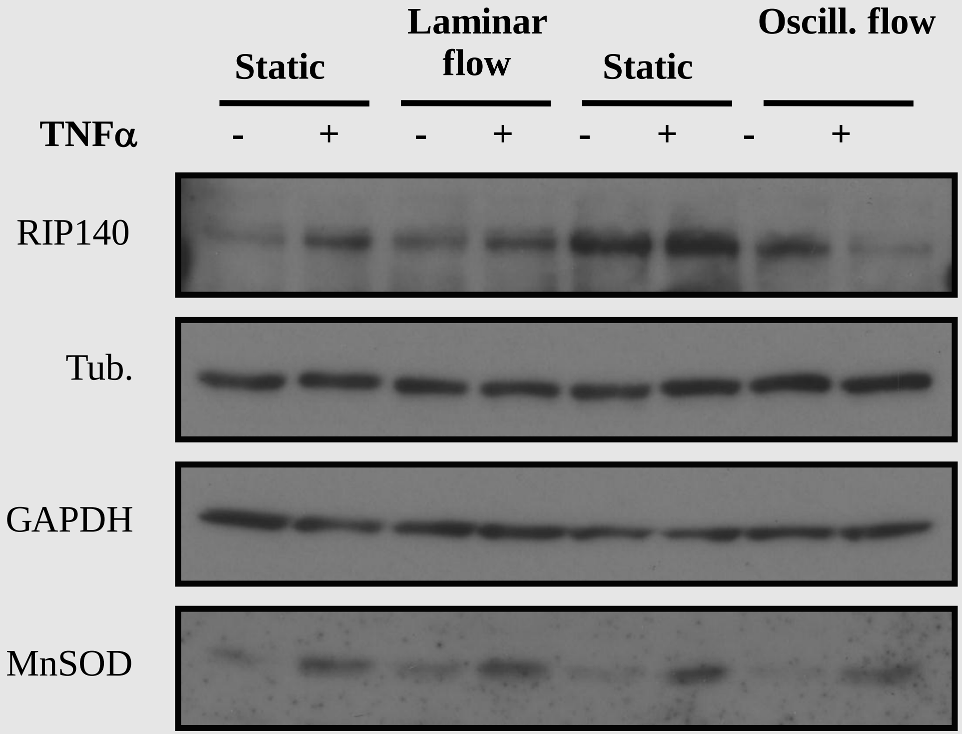
Oscillatory flow : ± 5 dyn/cm² ; 2 Hz

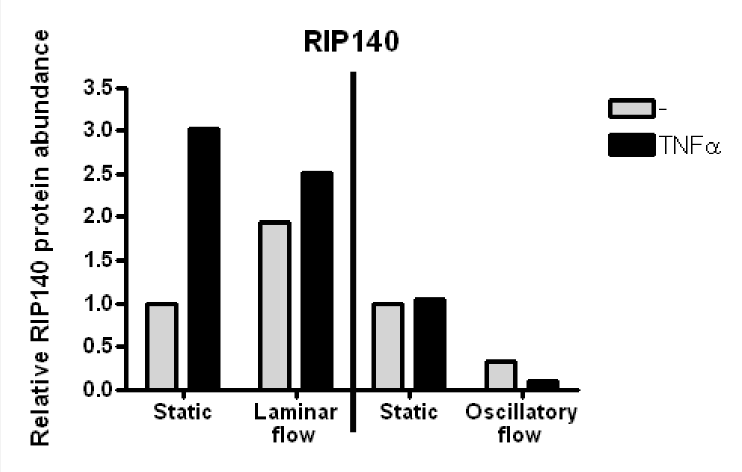
-

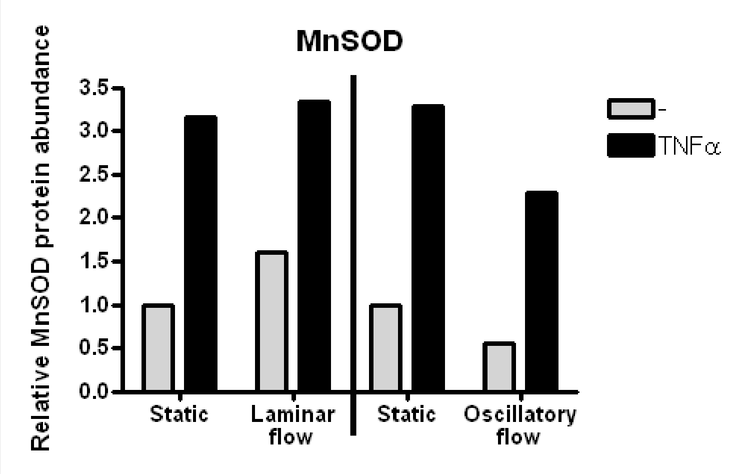
TNFα (1 ng/ml)

The influence of shear stress in the regulation of RIP140 by TNFα

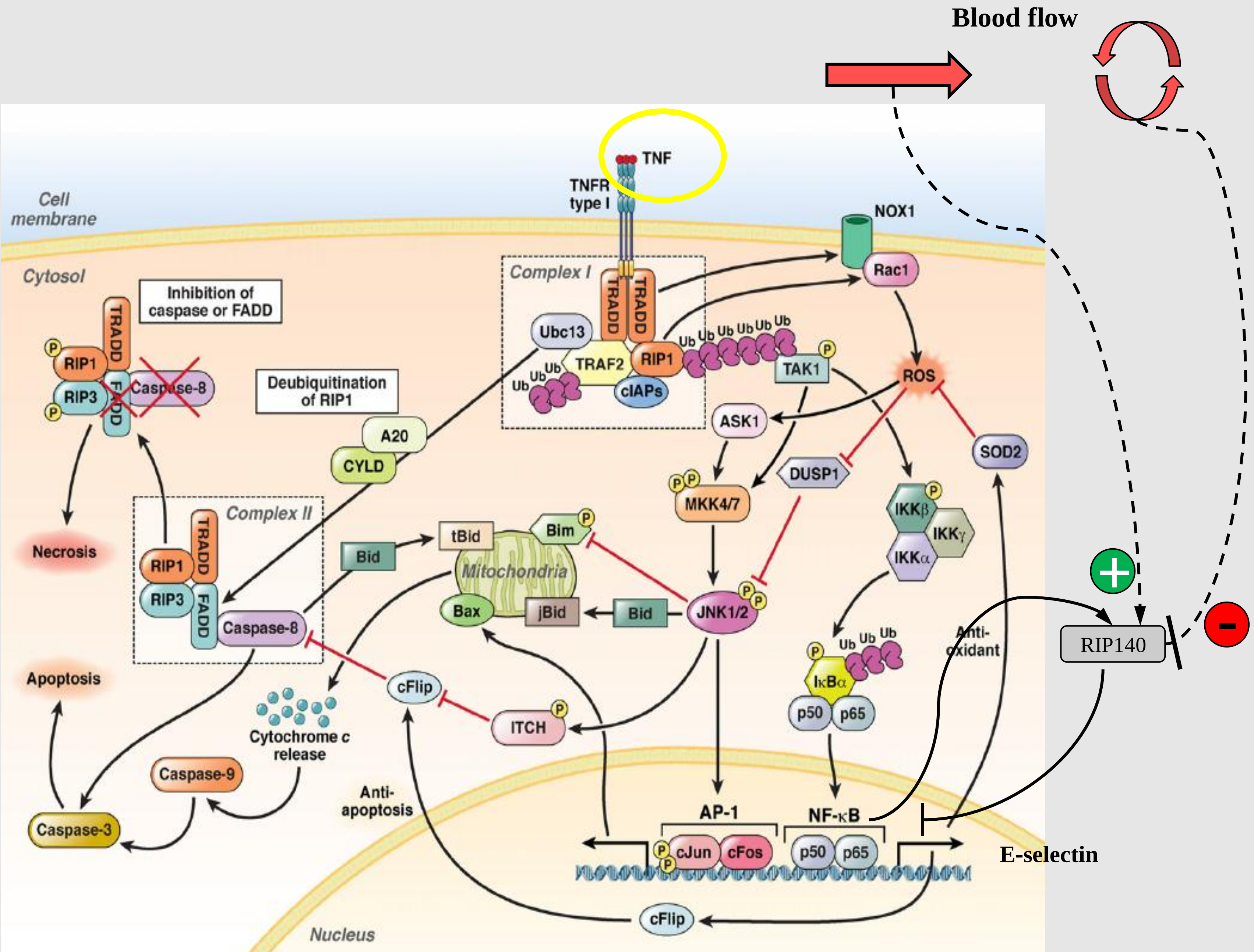
Combined effect of shear stress and TNFα on RIP140 expression







The influence of shear stress in the regulation of RIP140 by TNFα



Blood flow

RIP140

E-selectin

Adapted from Seki *et al*. Gastroenterology. 2012.

Thank you for your attention