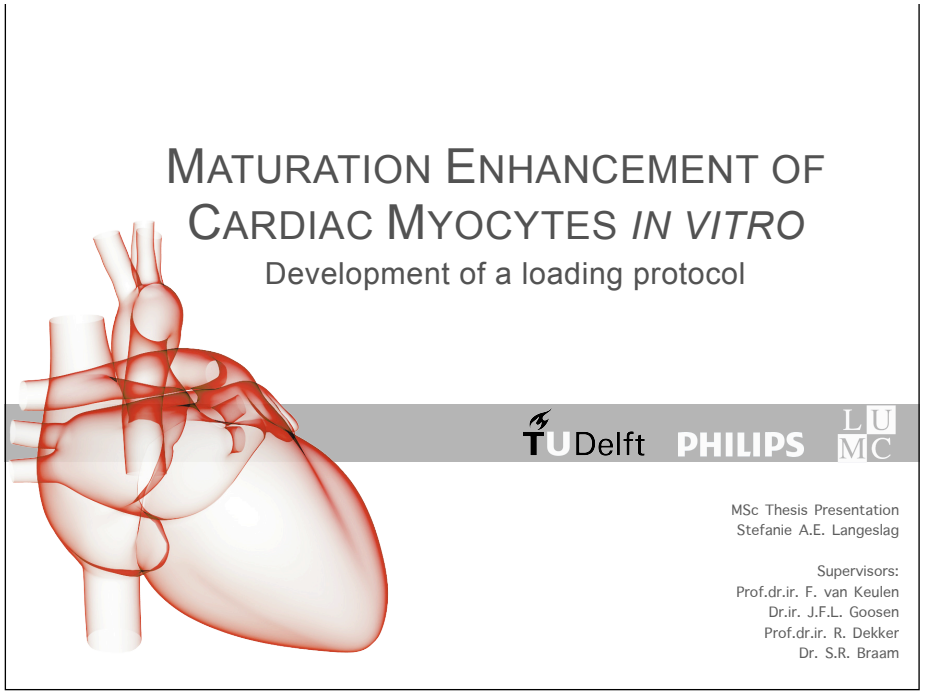
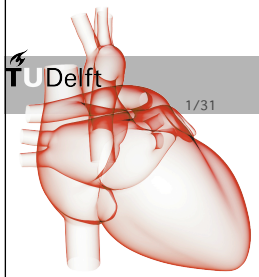
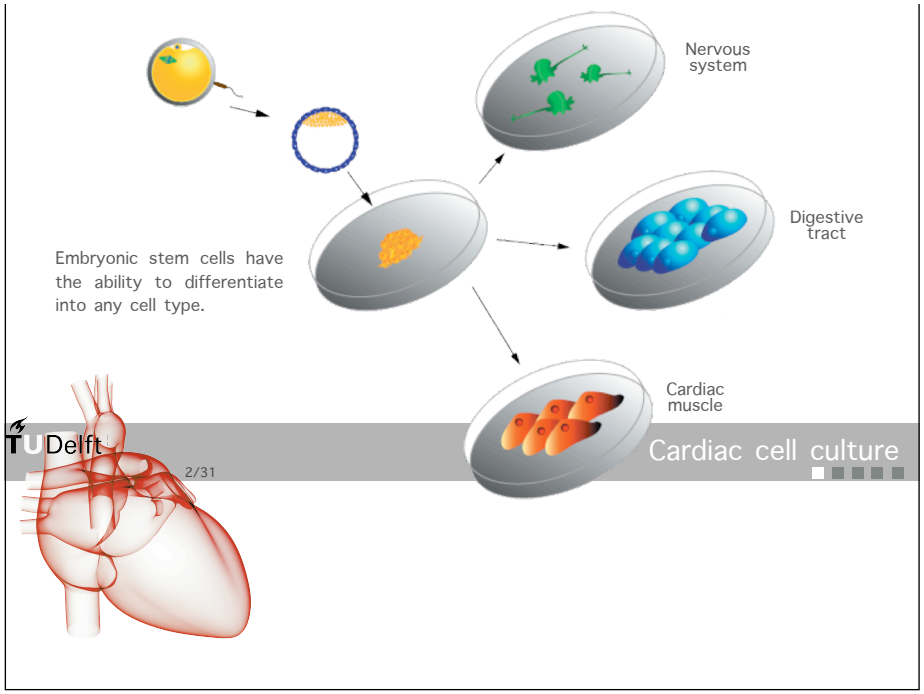



Developed a training-schedule for heart muscle cells, grown outside the body to get them to further mature.

- Lack in drug-screening models
- Delayed drug development




Nervous system
Digestive tract
Cardiac muscle
Embryonic stem cells have the ability to differentiate into any cell type.
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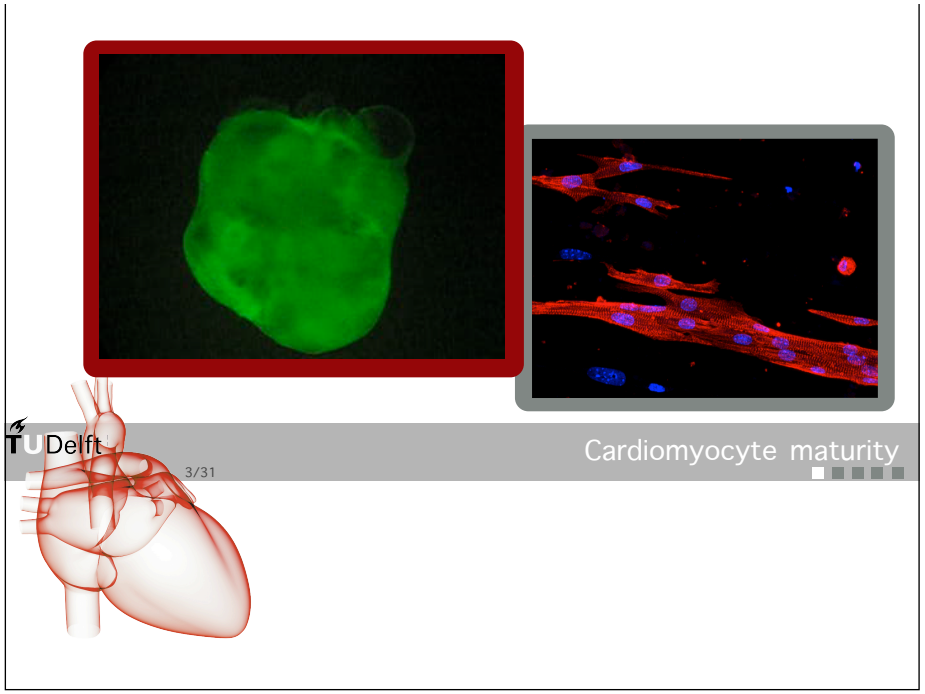
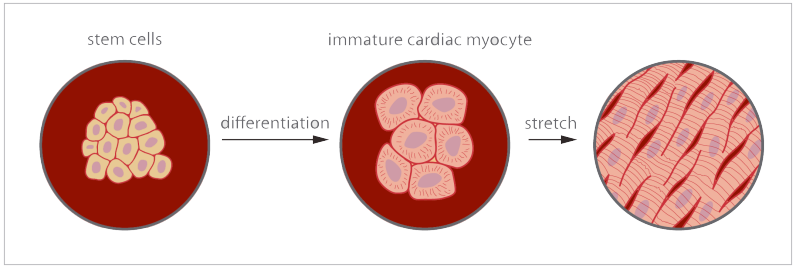
Cardiac cell culture

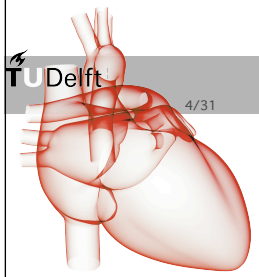



Earlier studies have showed that cardiac constructs exposed to cyclic stretch show enhanced maturation.

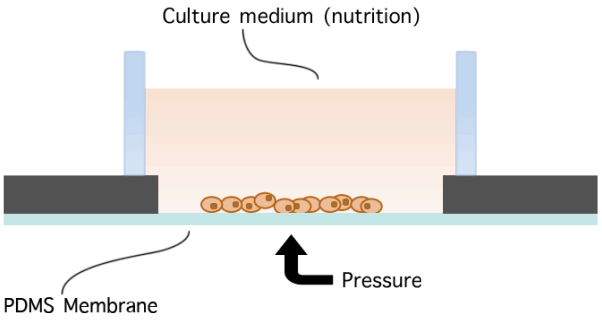


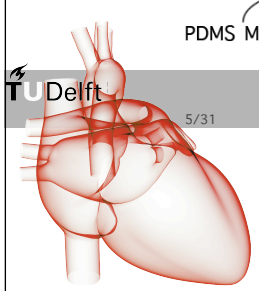


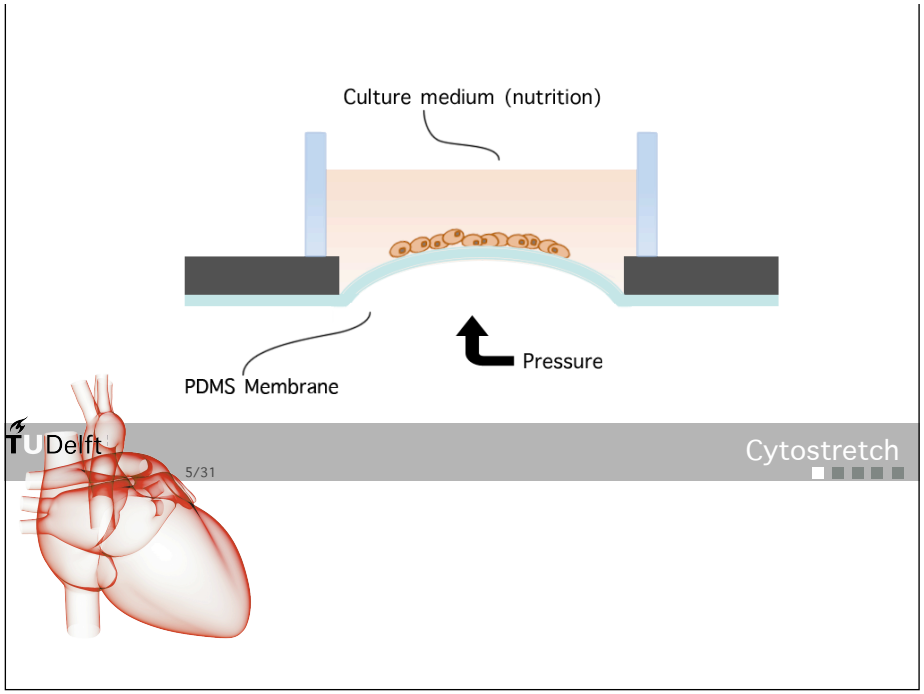

## Maturation enhancement

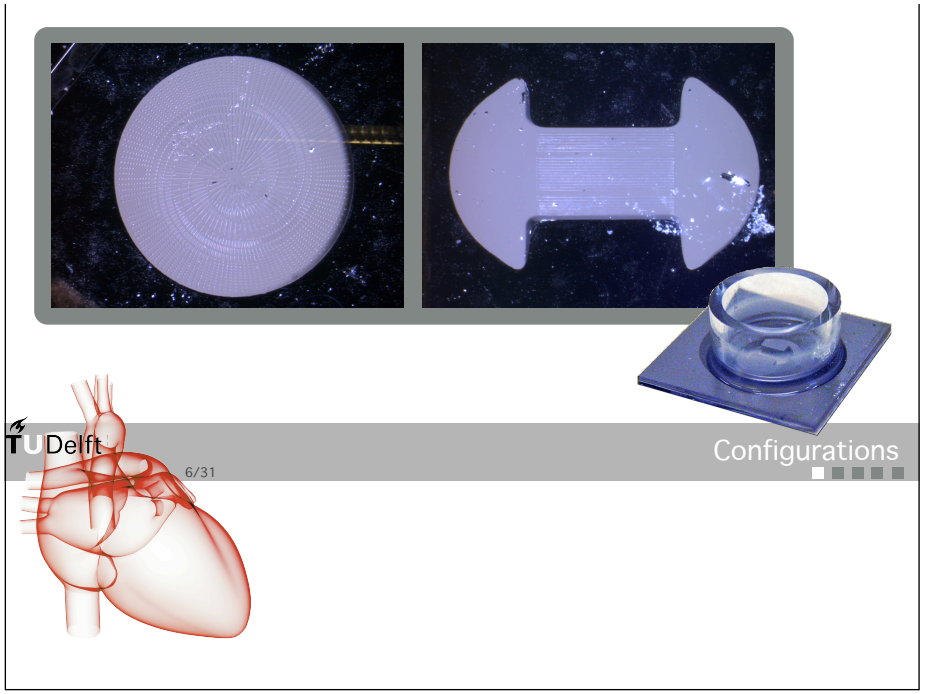


References: Salameh, A., *et al.*, 2010
Zimmermann, W.-H., *et al.*, 2002

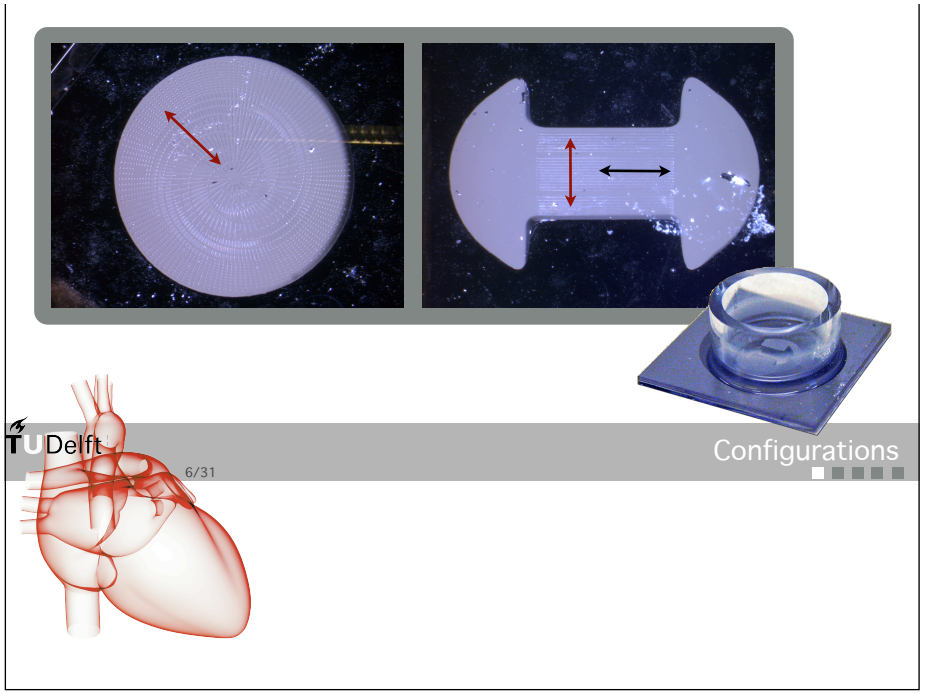

Culture medium (nutrition)
PDMS Membrane
Pressure




Culture medium (nutrition)
Pressure
PDMS Membrane




Two Cytostretch configurations have been developed. The circular configuration ensures a multi-directional cell-stretch. The dogbone configuration ensures a uni-directional cell-stretch.

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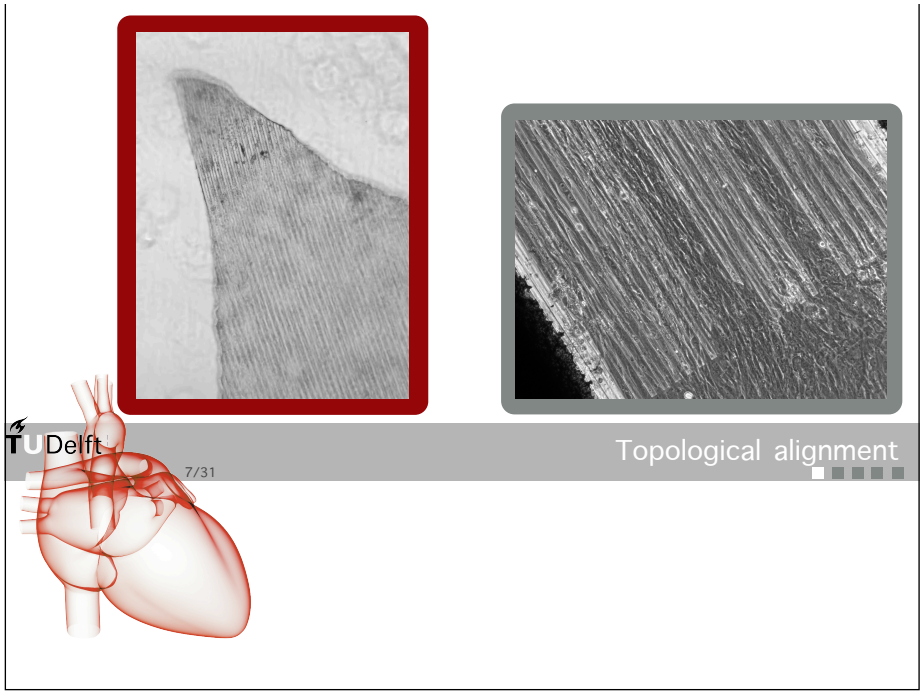
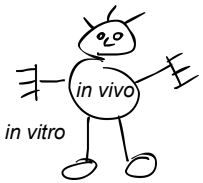


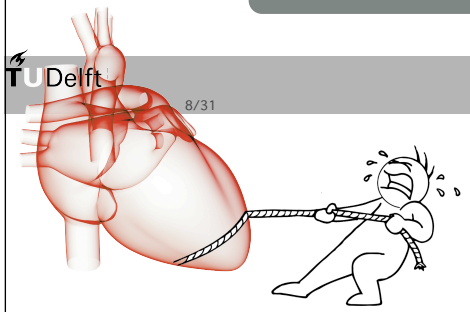

Cells react to geometrical cues. The cells appear to prefer to anchor in corners, where the mechanical stress on the cells (anchorage) is higher. Which means that we are able to lead cells in a certain direction.

Hypothesis

"Cardiac myocytes subjected to mechanical stimuli, comparable to *in vivo* stimuli, will show enhanced maturation"

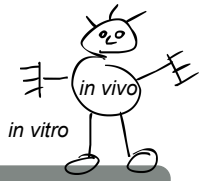



In order to stretch cardiac myocytes *in vitro* the development of an *in vivo* mimicking loading protocol is essential.



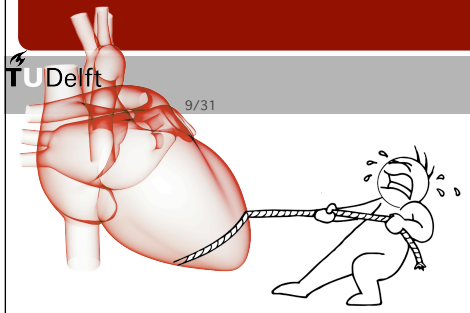
## The aim of the study presented

Development of a proper loading protocol for the stretching of cardiac myocytes *in vitro*




## Two main objectives have to be fullfilled

- Gain insight in the strain cardiac myocytes endure *in vivo*
- Determine membrane behavior of both Cytostretch configurations



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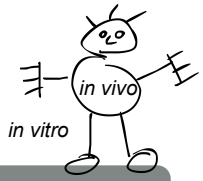


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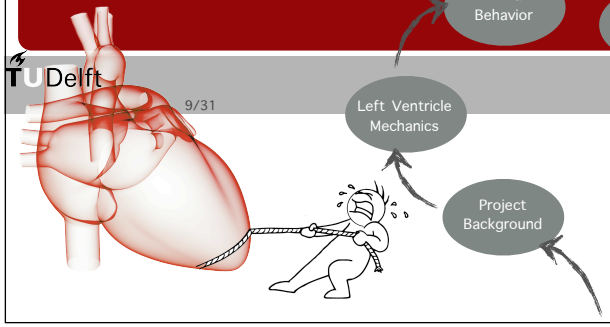

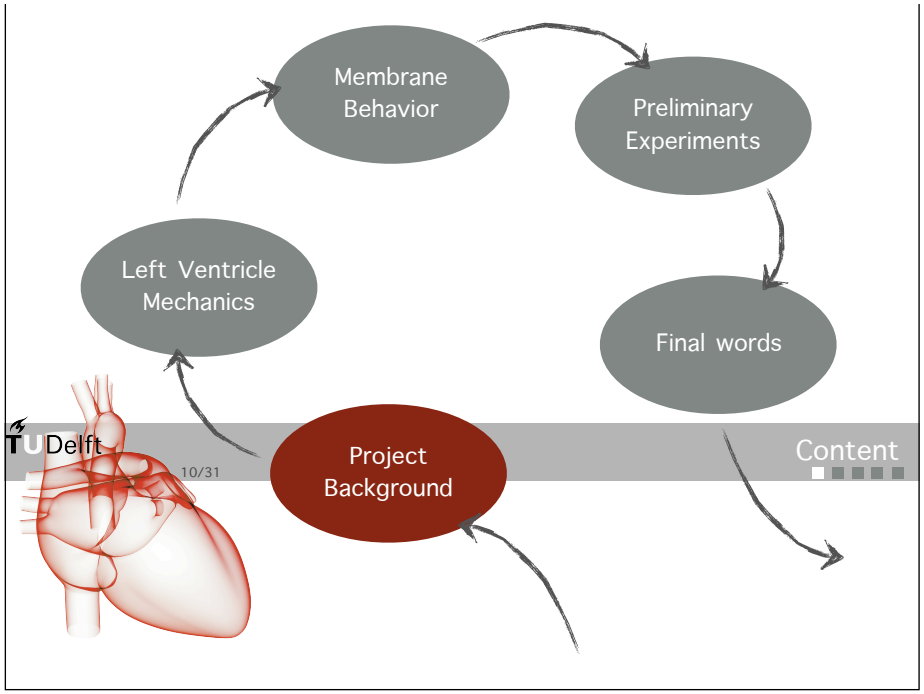
Membrane Behavior
Preliminary Experiments
Left Ventricle Mechanics
Final words
Project Background
TU Delft
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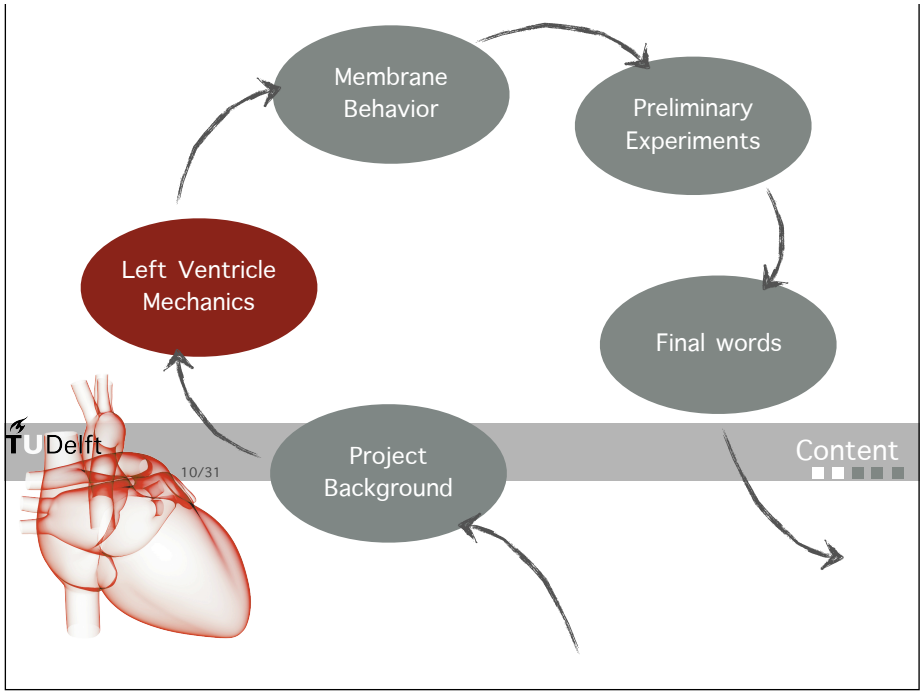
Content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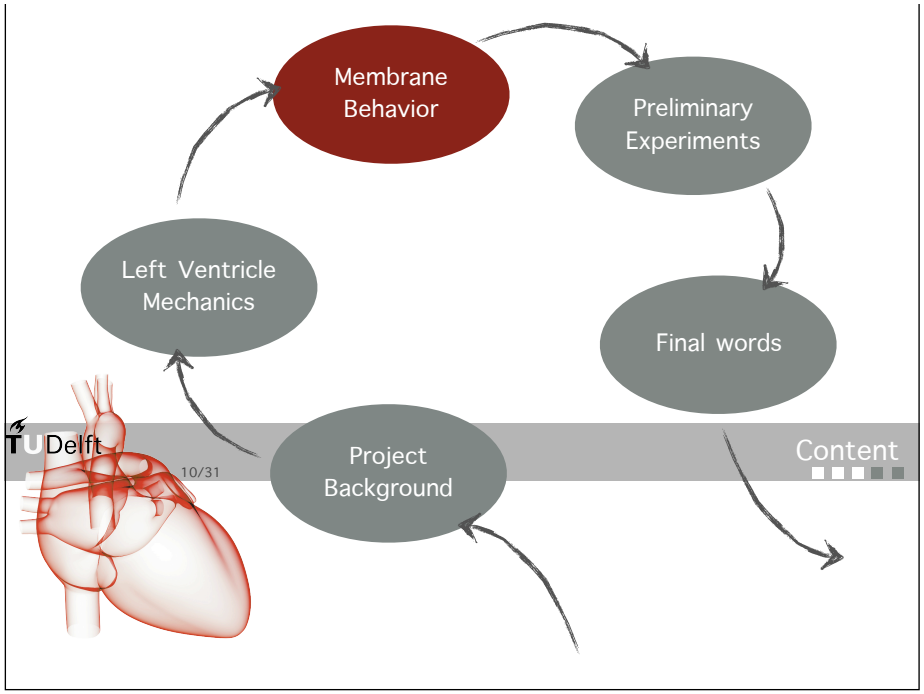
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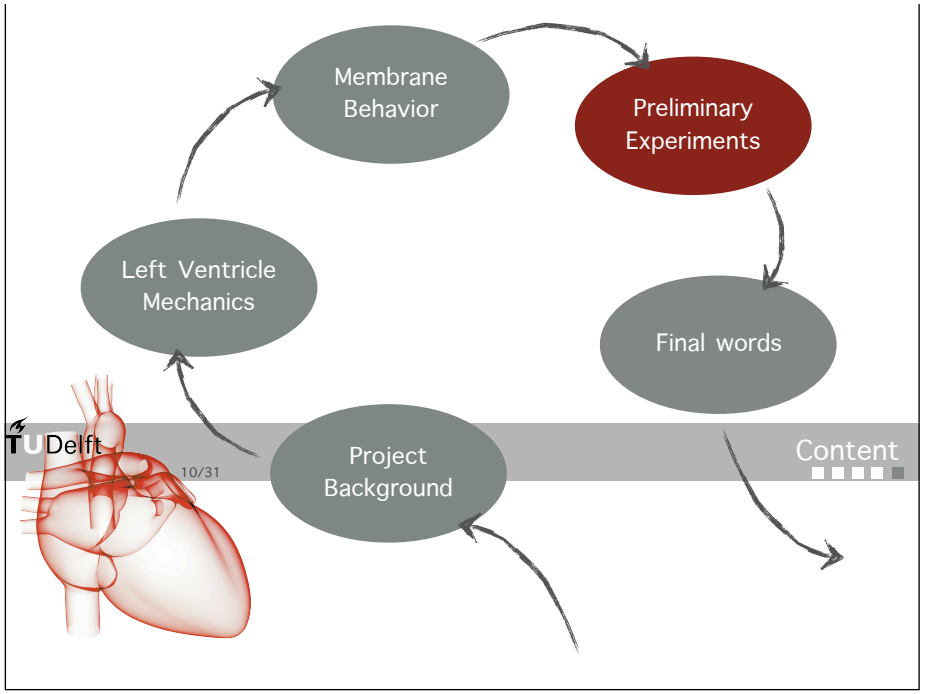
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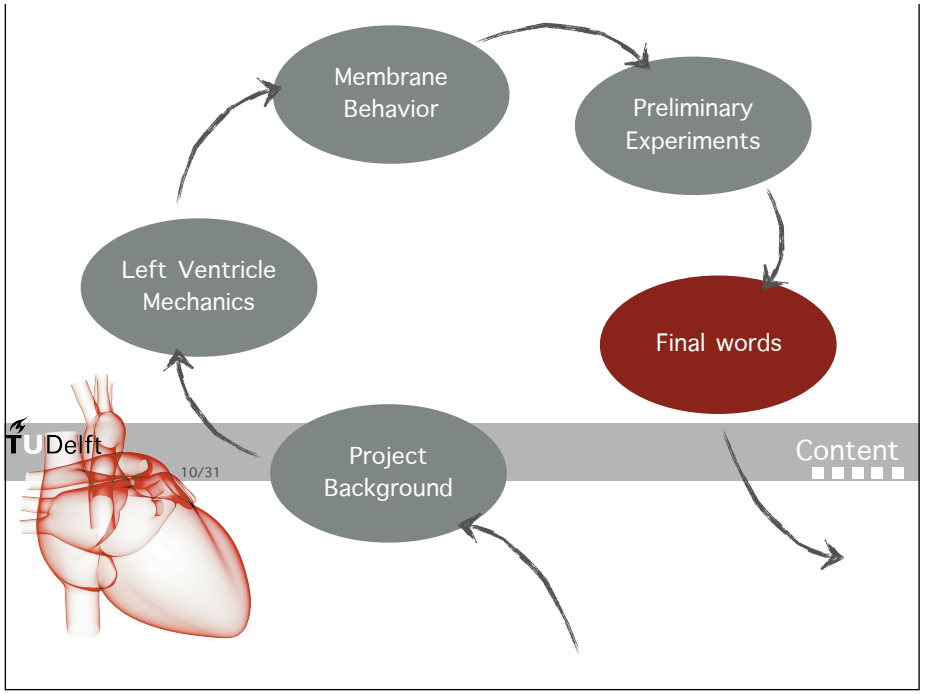
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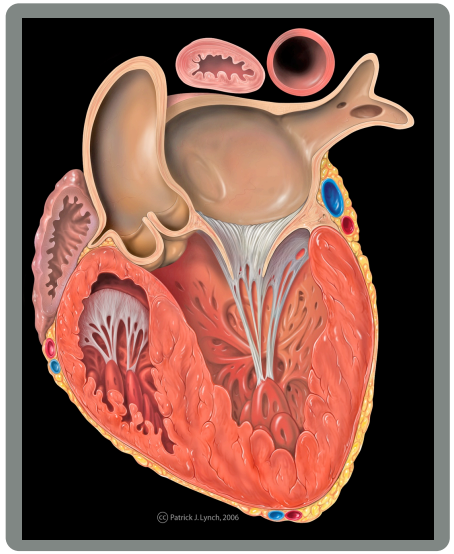
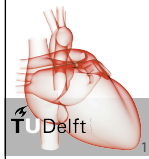
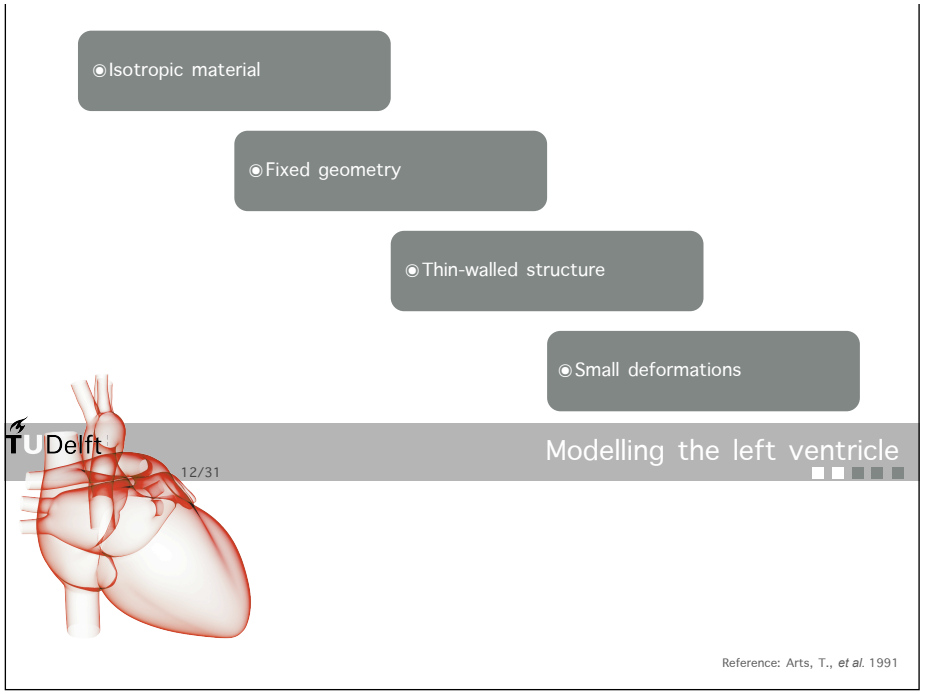
Each simplification is a comprimise between accuracy and calculation time.



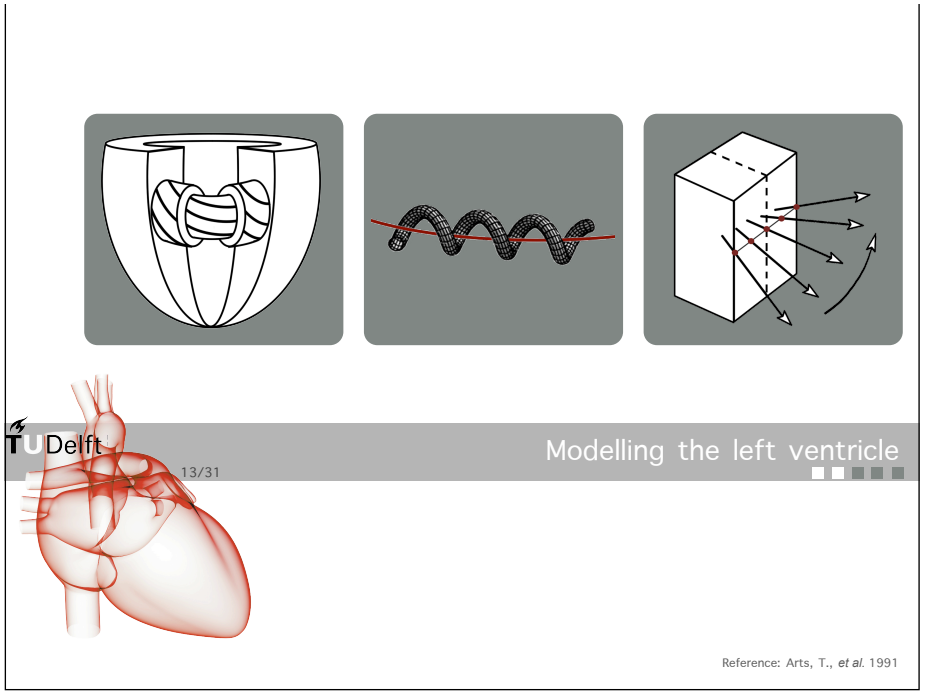





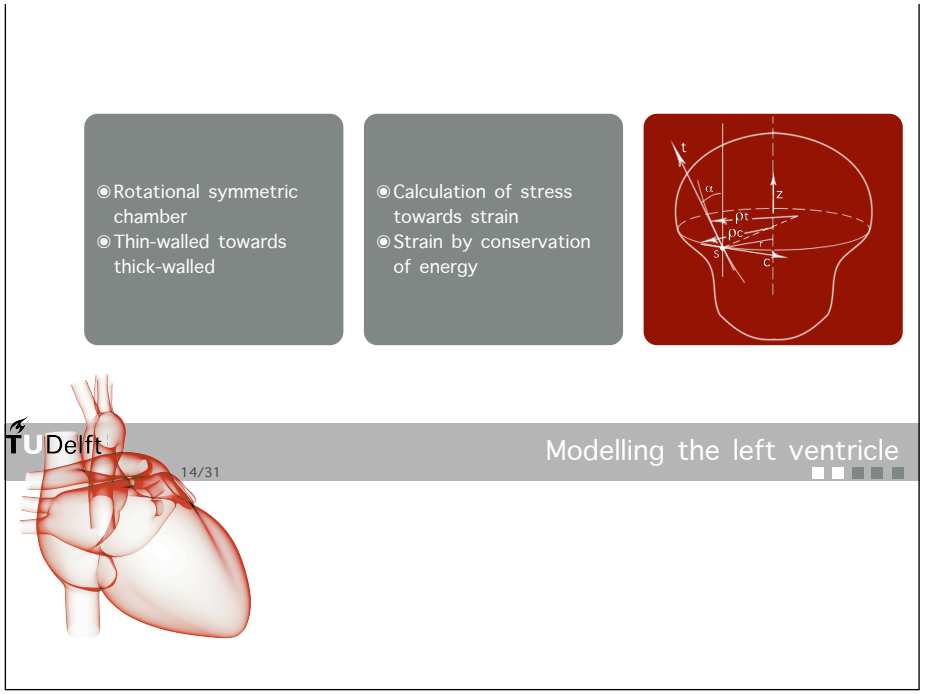
In vivo difficult: mathematically.



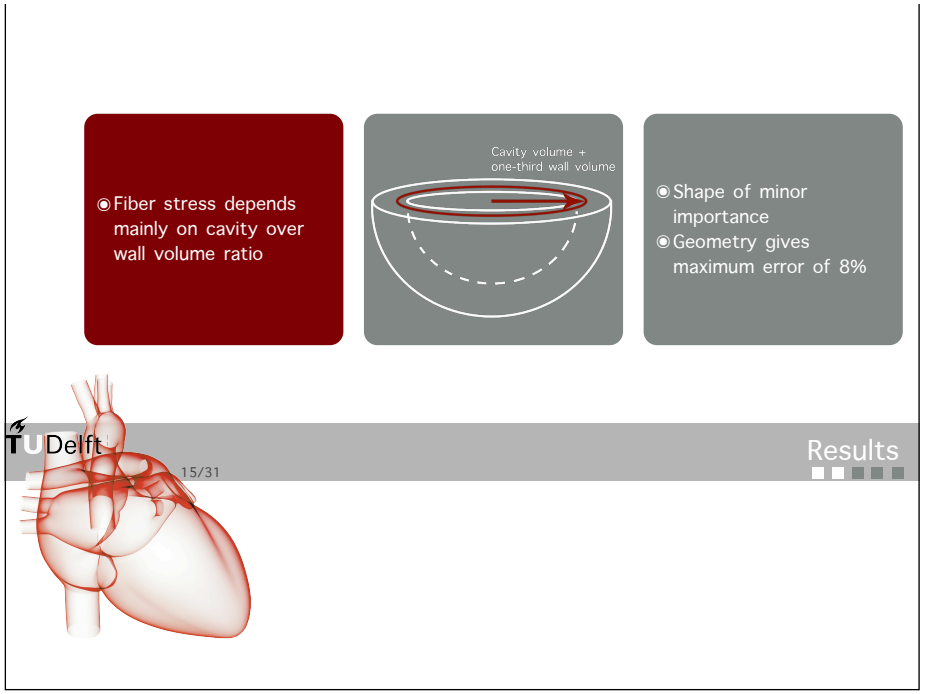

Earlier simplifications made (assumptions). Invalid. Chosen Arts, vd Bovendeerd.



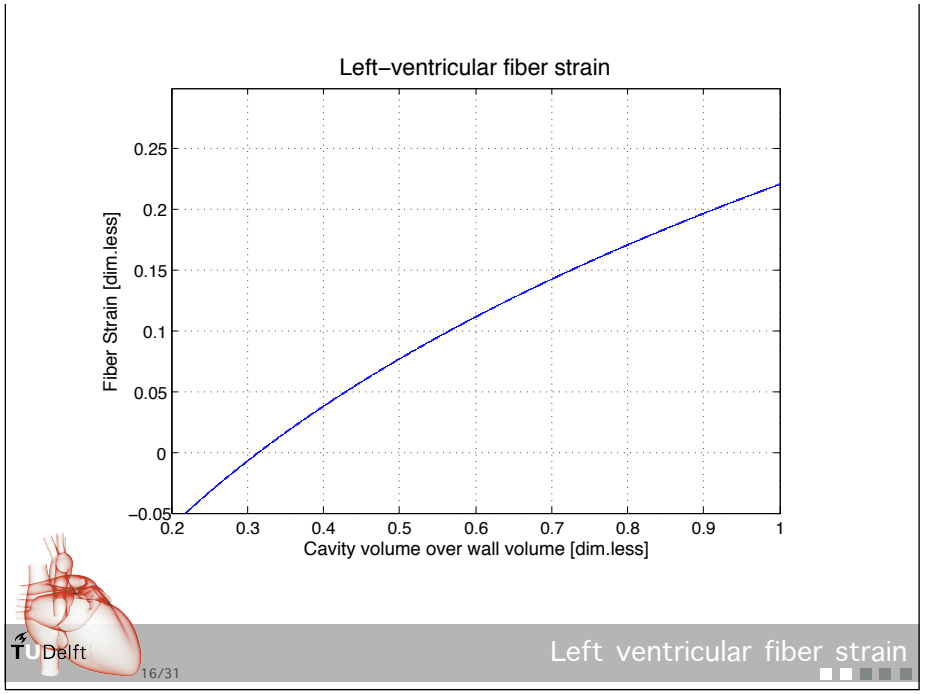

Chosen Arts, vd Bovendeerd. fiber orientation, close to anatomical findings. FLUID-FIBER CONTINUUM. Homogeneous nice->single value



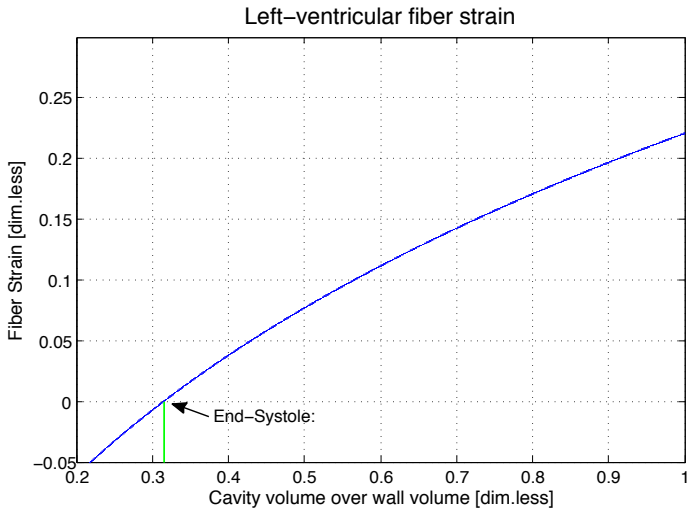

Rotationally symmetric (excluding geometry effects). Thick walled structure build up from various thin walled shells. Energy: mechanical work by myocardial fibers is equal to pumping work of the chamber.

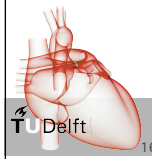


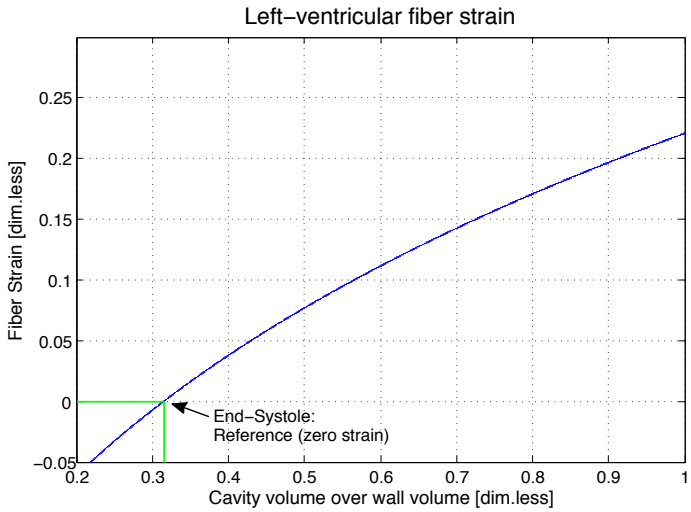

mainly depends on cavity volume – wall volume ratio. Bovendeerd approximation one third wall thickness elongation sphere. Basal boundary true left ventricle is open without derivative dr/dz being zero.

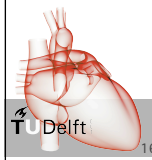


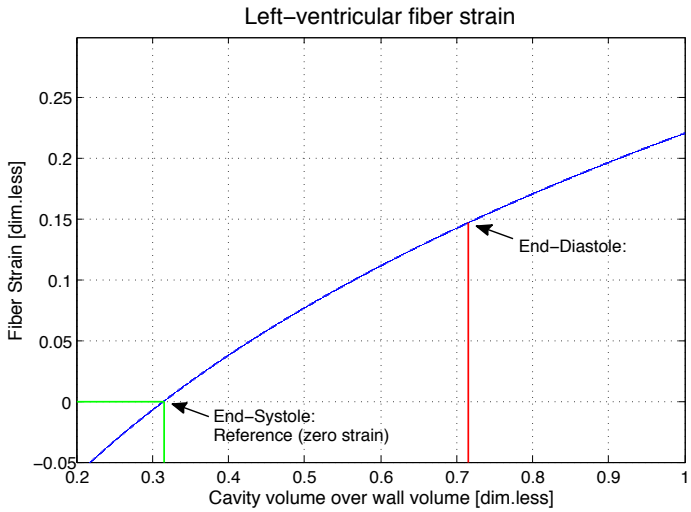

end syst: 0.315 (range 0.2-0.4) end diast: 0.715 (range 0.6-0.8) (LVESV=63ml,LVWV=200ml,LVEDV=143)

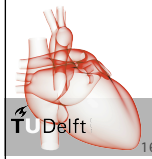

Left−ventricular fiber strain
Fiber Strain [dim.less]
Cavity volume over wall volume [dim.less]
End−Systole:
0.25
0.2
0.15
0.1
0.05
0
−0.05
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

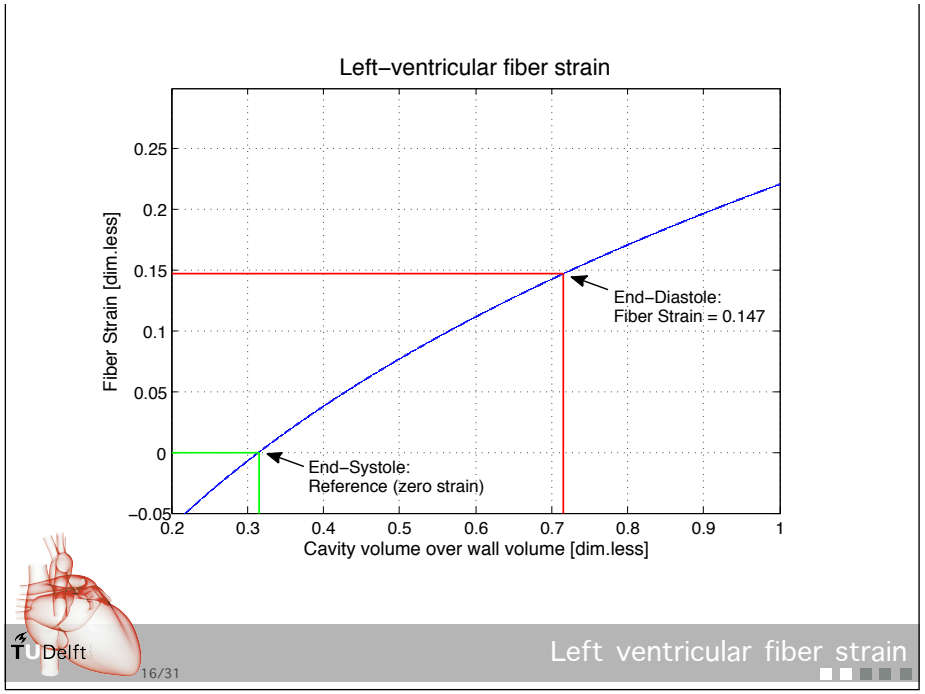



Left–ventricular fiber strain
Fiber Strain [dim.less]
Cavity volume over wall volume [dim.less]
End–Systole:
Reference (zero strain)
0.25
0.2
0.15
0.1
0.05
0
−0.05
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

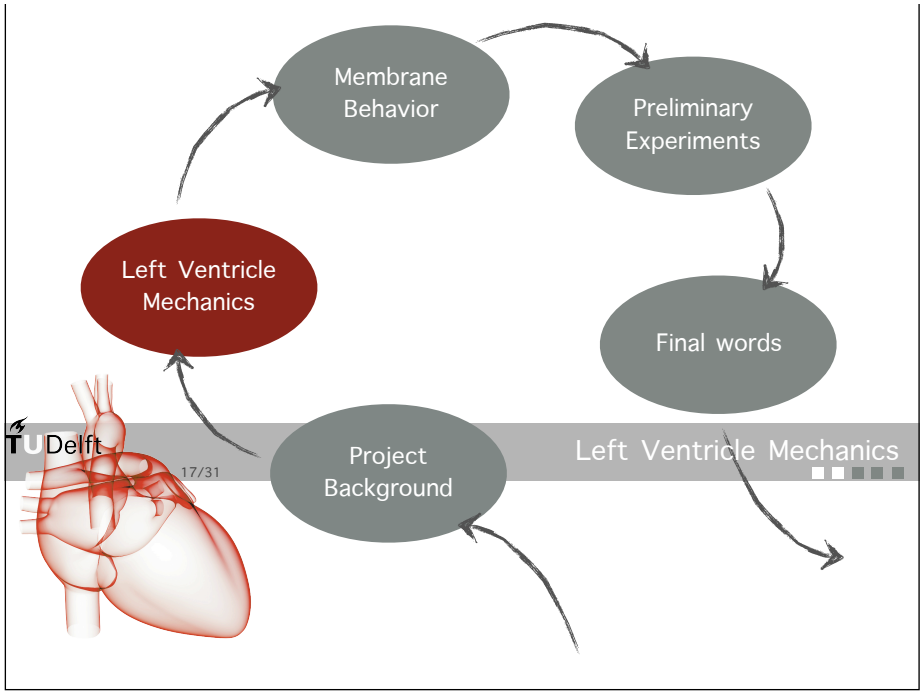



Left−ventricular fiber strain
Fiber Strain [dim.less]
Cavity volume over wall volume [dim.less]
End−Diastole:
End−Systole:
Reference (zero strain)
0.25
0.2
0.15
0.1
0.05
0
−0.05
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1






14.7 % strain in normal cardiac cycle. MRI data adult human left ventricle. Specific range cavity volume over wall volume during human development. Strain value seems reasonable, Salameh et al. 10 and 20% stretch significantly more elongated cells than 5% stretch.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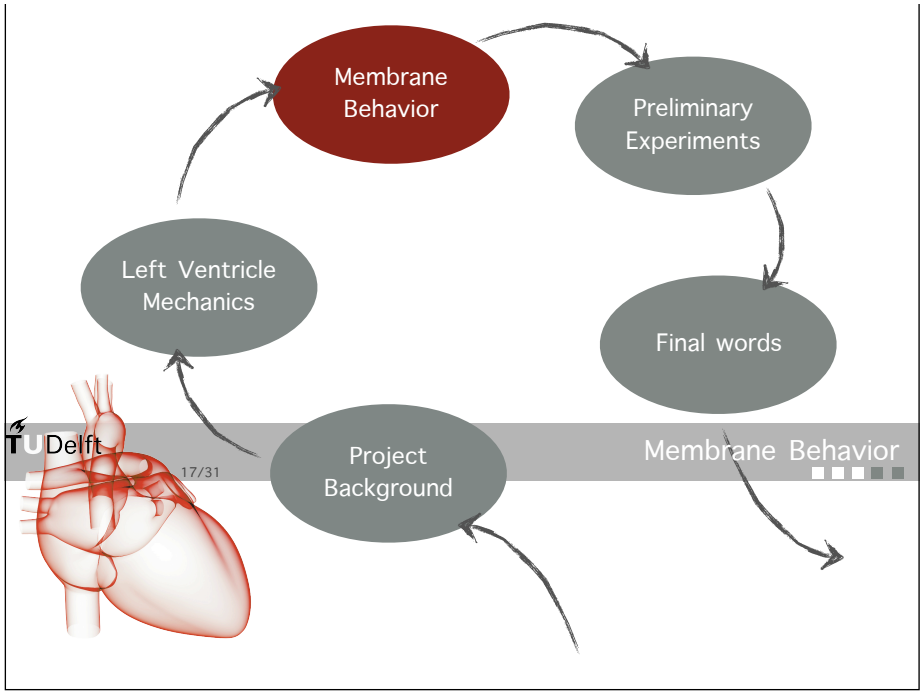
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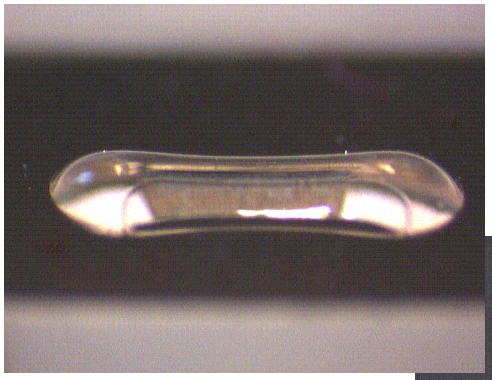
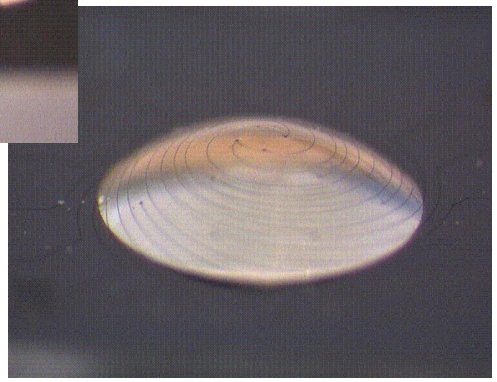
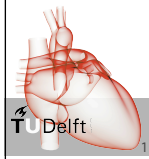
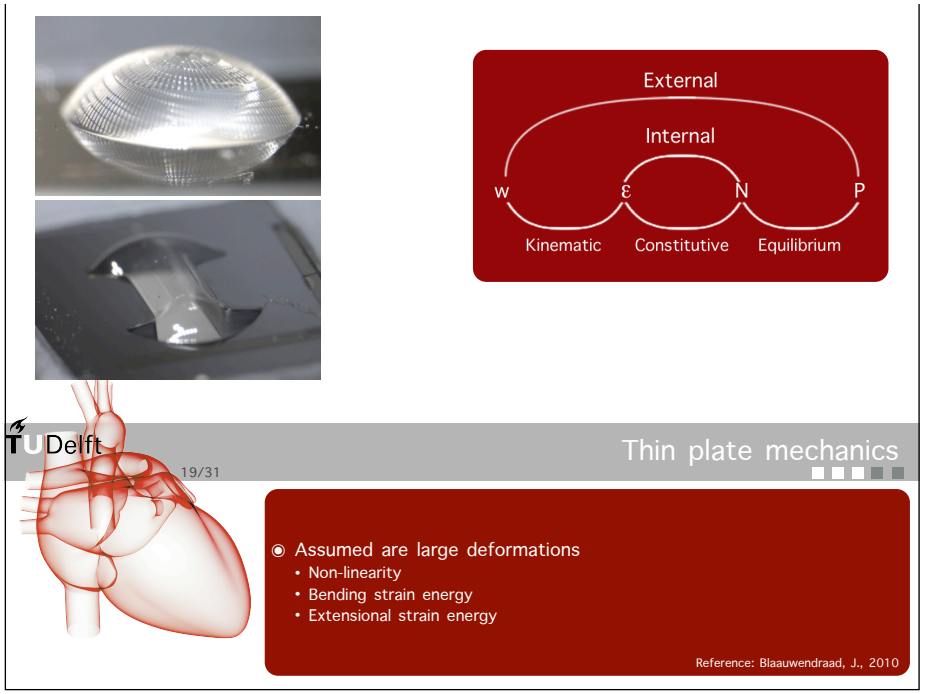


- Analytical compared with Numerical and Experimental








Will not pay attention to the first two sets of equations. Shortly discuss the equilibrium of the system to show where the load-deflection relation comes from.

constitutive results in stiffness matrix

Energy method:

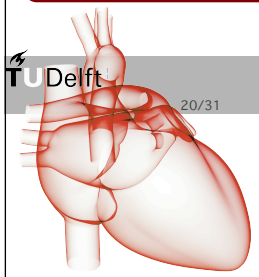
$$U = W_{membrane} + W_{load}$$

$$W_{membrane} = W_{bending} + W_{extension}$$

- Minimum potential energy

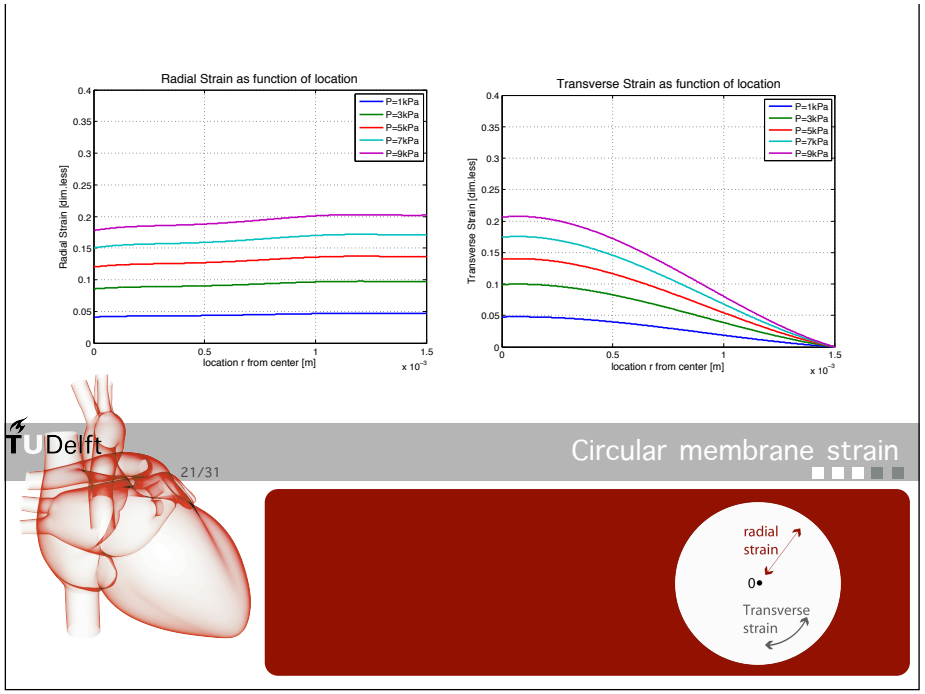
Equilibrium:

$$\frac{\partial U}{\partial c_1} = 0 \ldots \frac{\partial U}{\partial c_j} = 0 \ldots \frac{\partial U}{\partial c_n} = 0$$

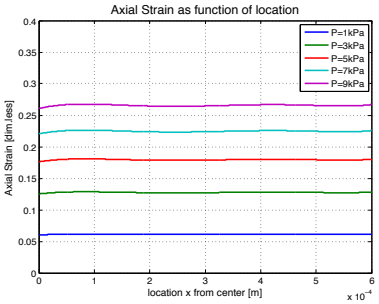




A structure will deform or displace to a position (stationary point), that minimizes its potential energy.

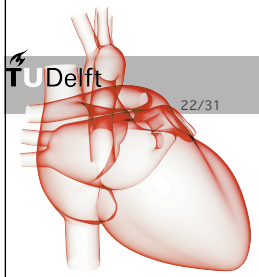


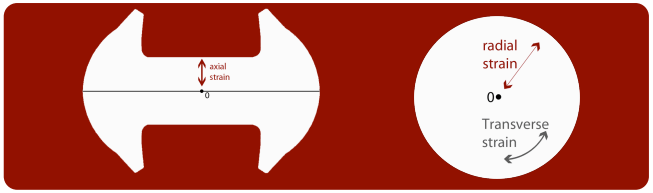

Homogeneous, isotropic material (PDMS), Linear elastic material model, Simply supported boundary condition, In plane trial displacement function *'u'* contains five terms

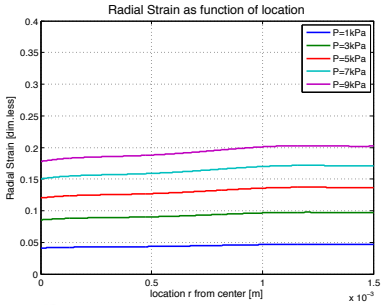


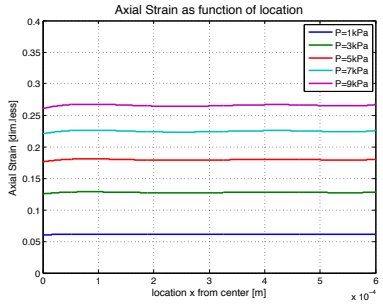



# Dogbone membrane strain



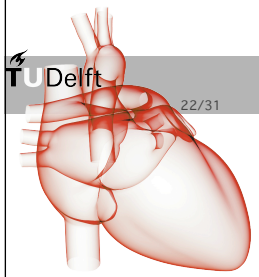


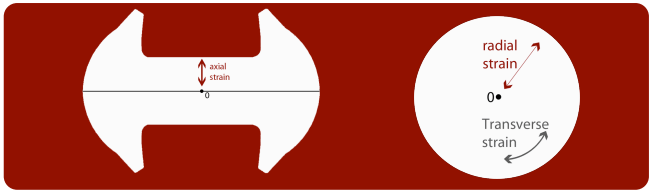





# Dogbone membrane strain

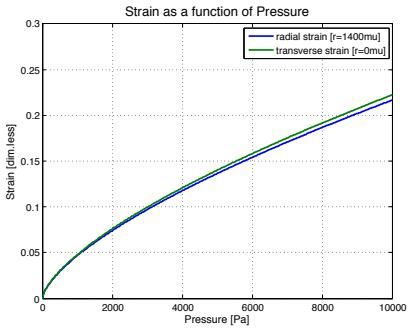


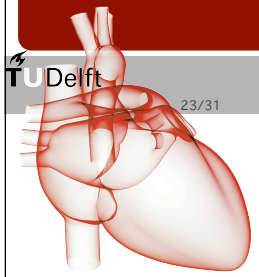


- For pressures >3kPa bending strain energy can be neglected

  Bending: $h^3$ Extension: $h$

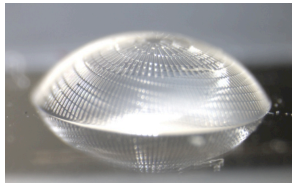
- 14.7% strain when applying 5.375 kPa of pressure





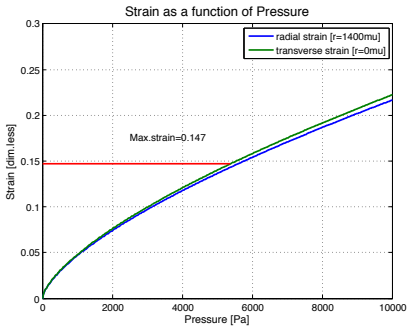
## Circular membrane applied pressure

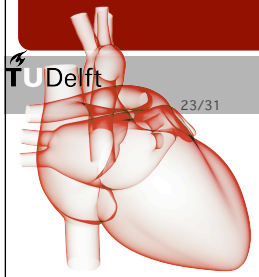


- For pressures >3kPa bending strain energy can be neglected

  Bending: $h^3$ Extension: $h$

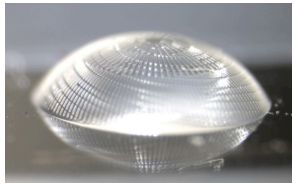
- 14.7% strain when applying 5.375 kPa of pressure





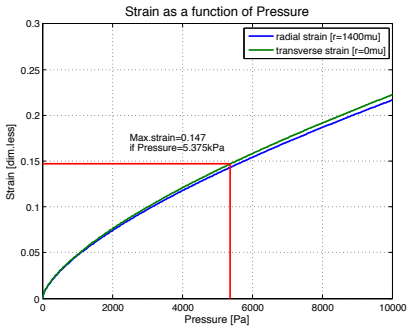
## Circular membrane applied pressure

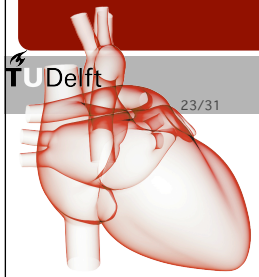


- For pressures >3kPa bending strain energy can be neglected

  Bending: $h^3$ Extension: $h$

- 14.7% strain when applying 5.375 kPa of pressure





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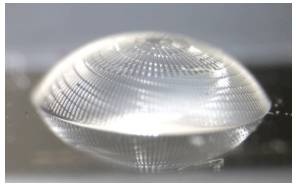

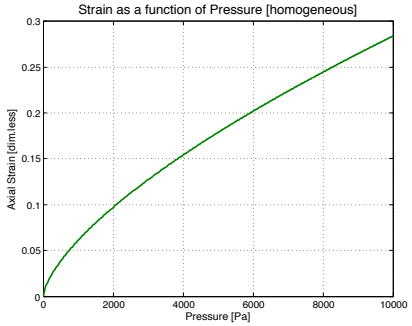


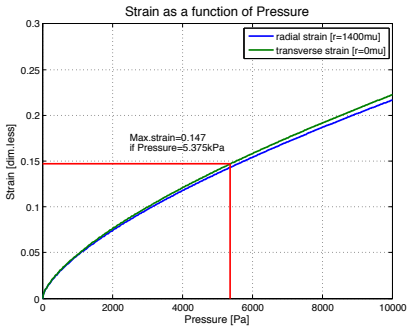


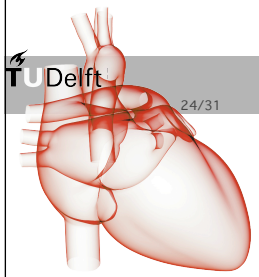

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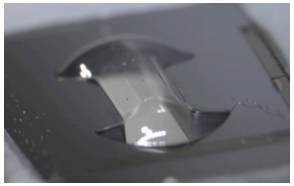
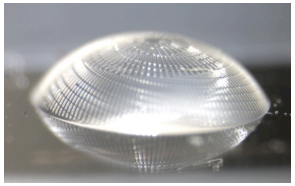
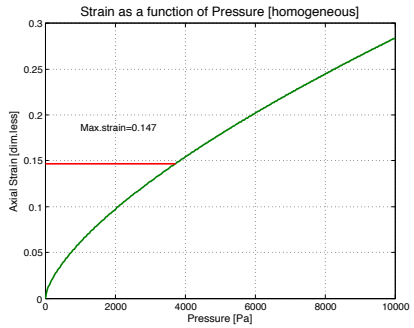
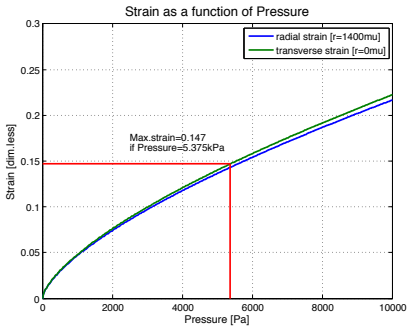
## Dogbone membrane applied pressure



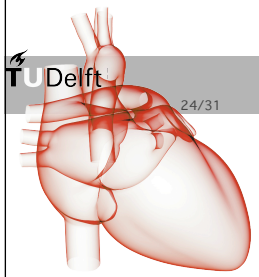


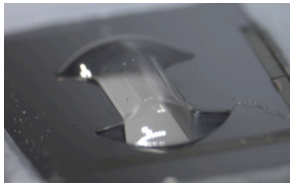


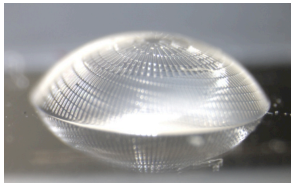



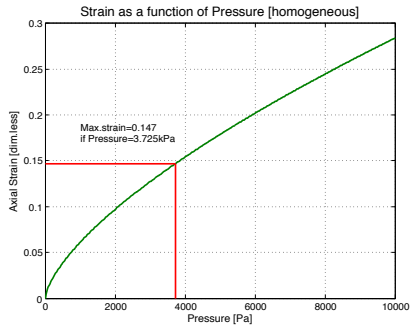


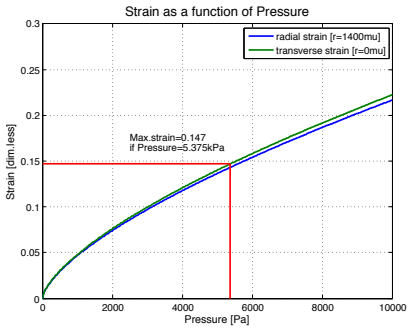

## Dogbone membrane applied pressure





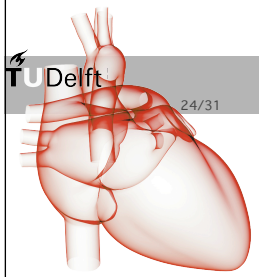


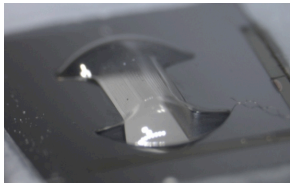


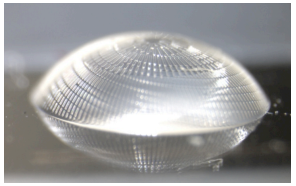



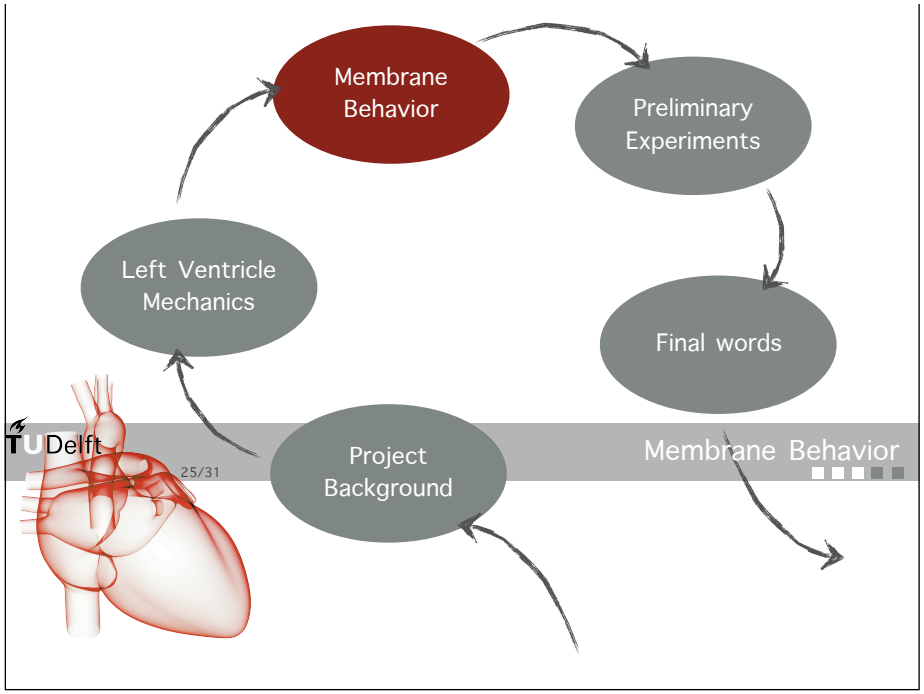



## Dogbone membrane applied pressure





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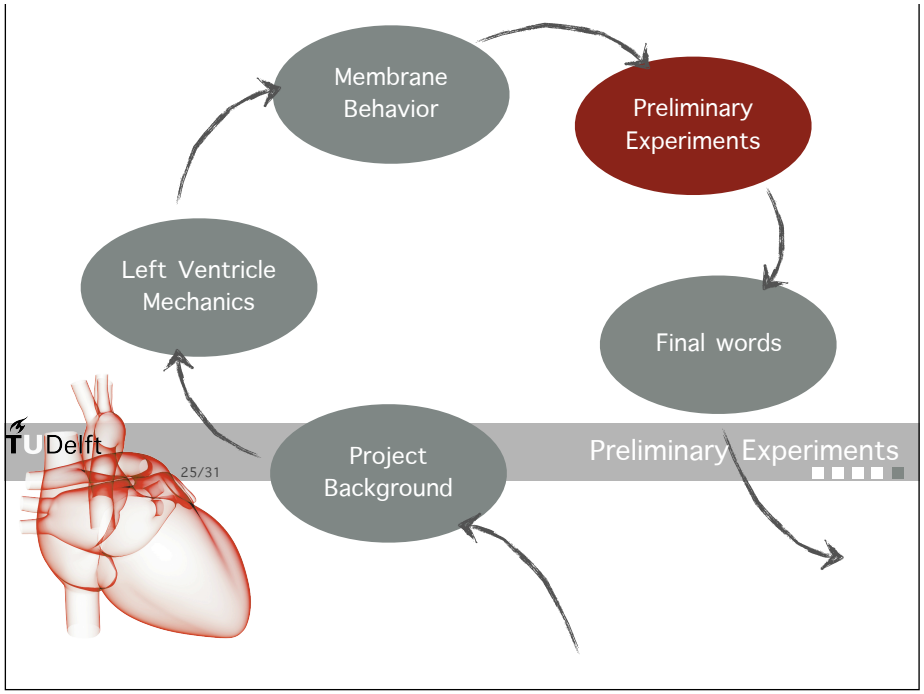
Membrane Behavior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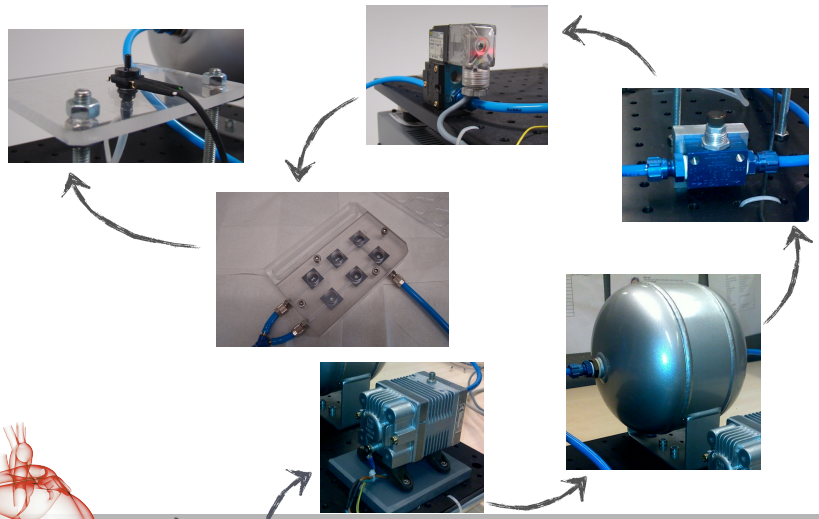
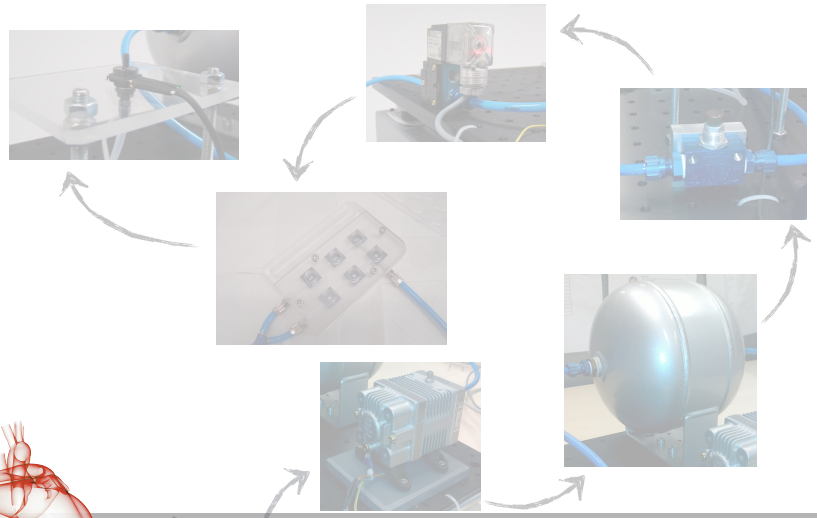




Experimental setup





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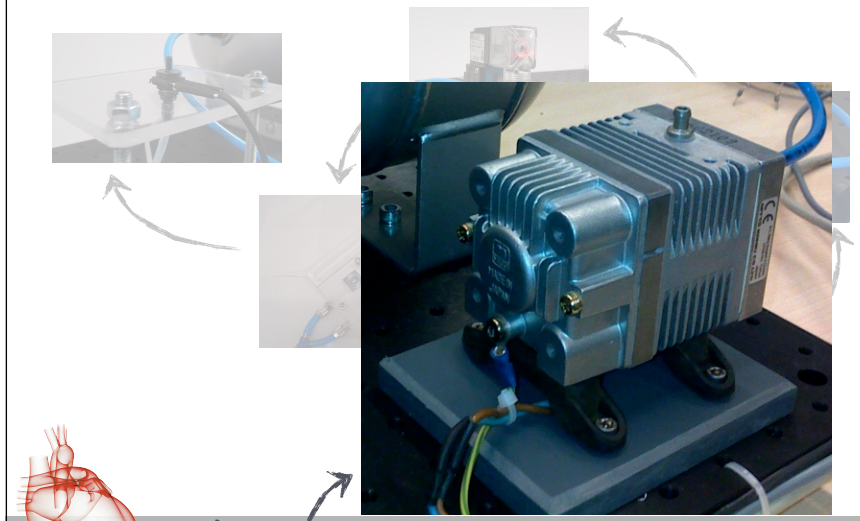
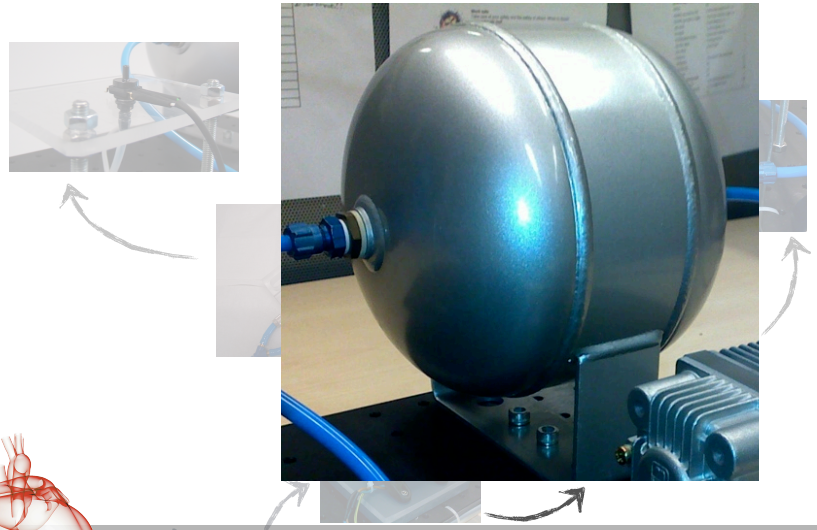






## Experimental setup





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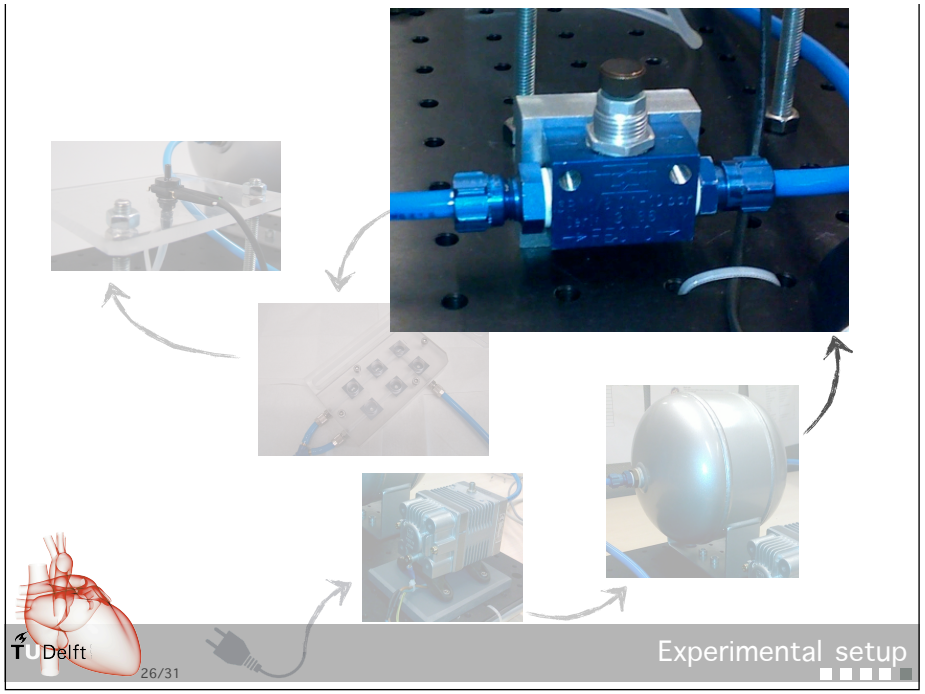



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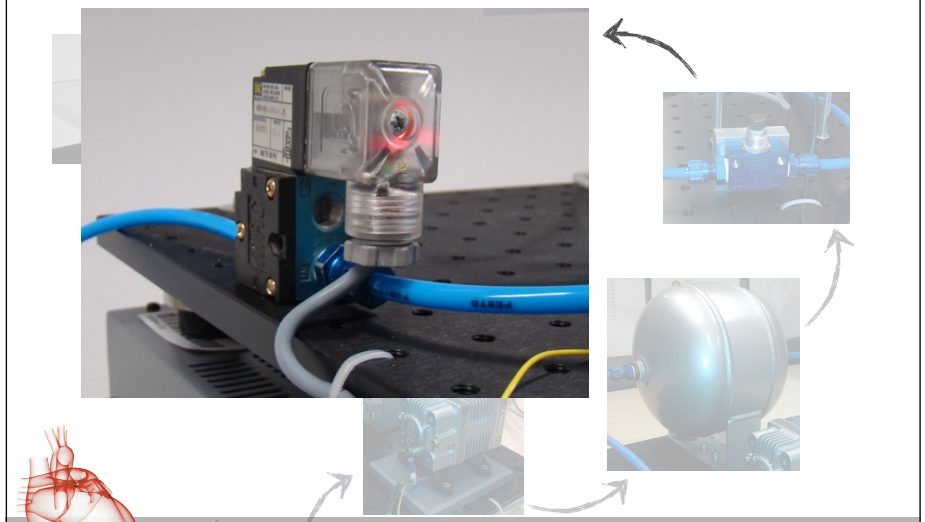



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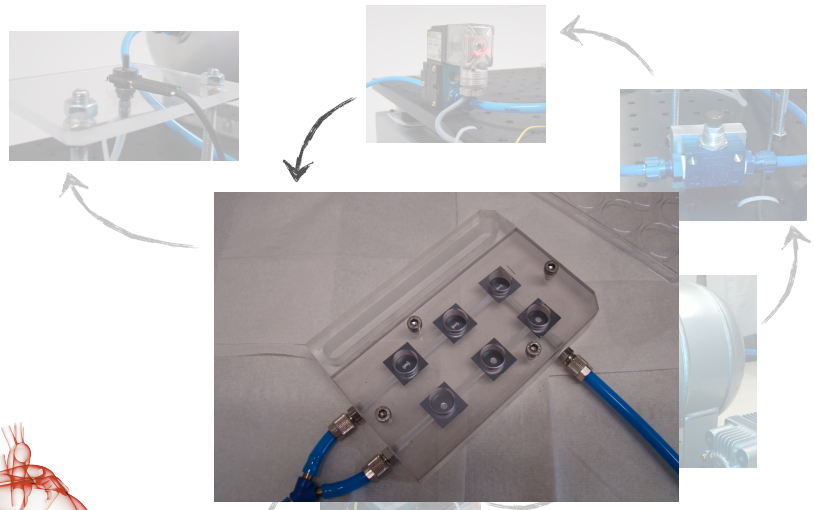



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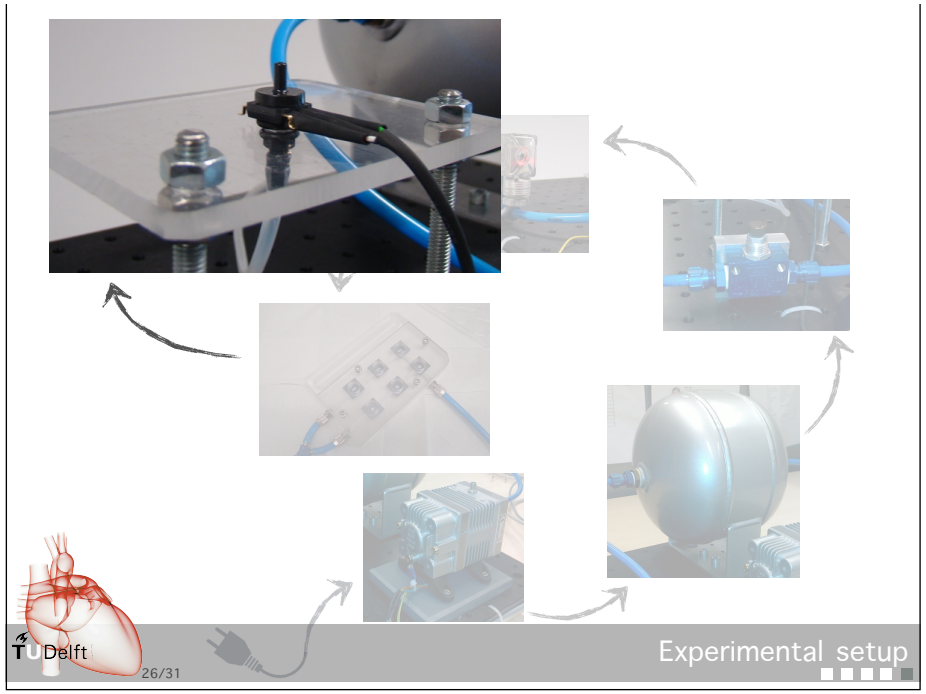



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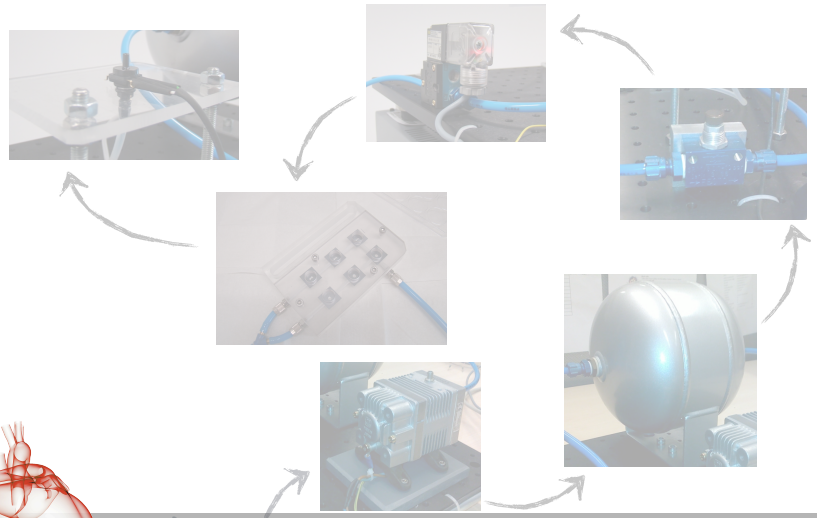



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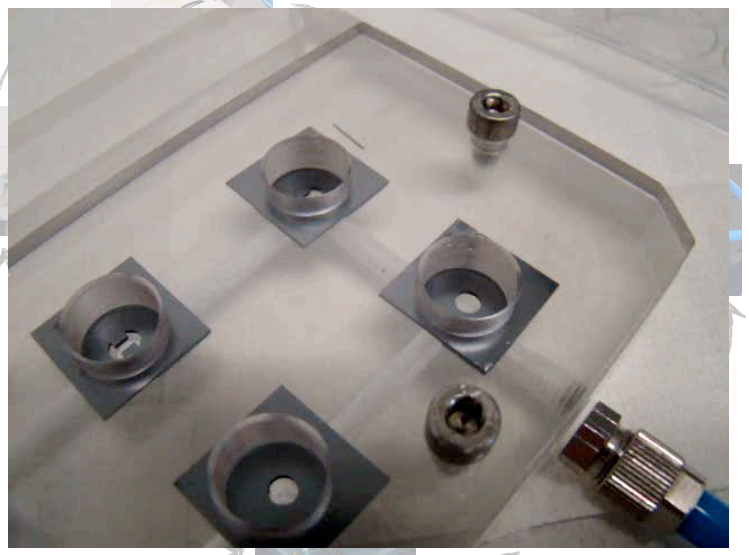



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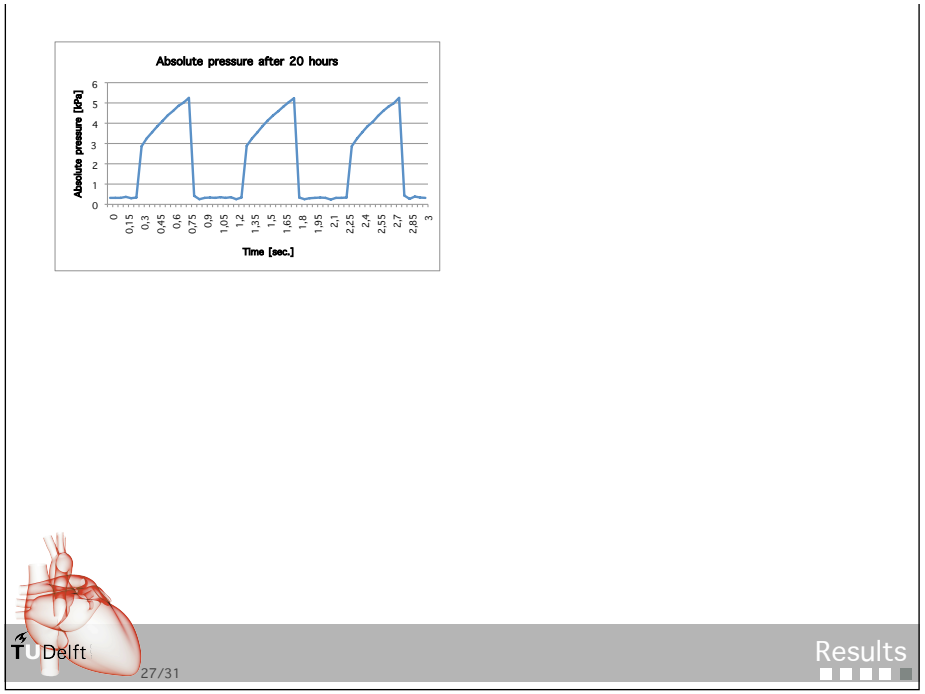
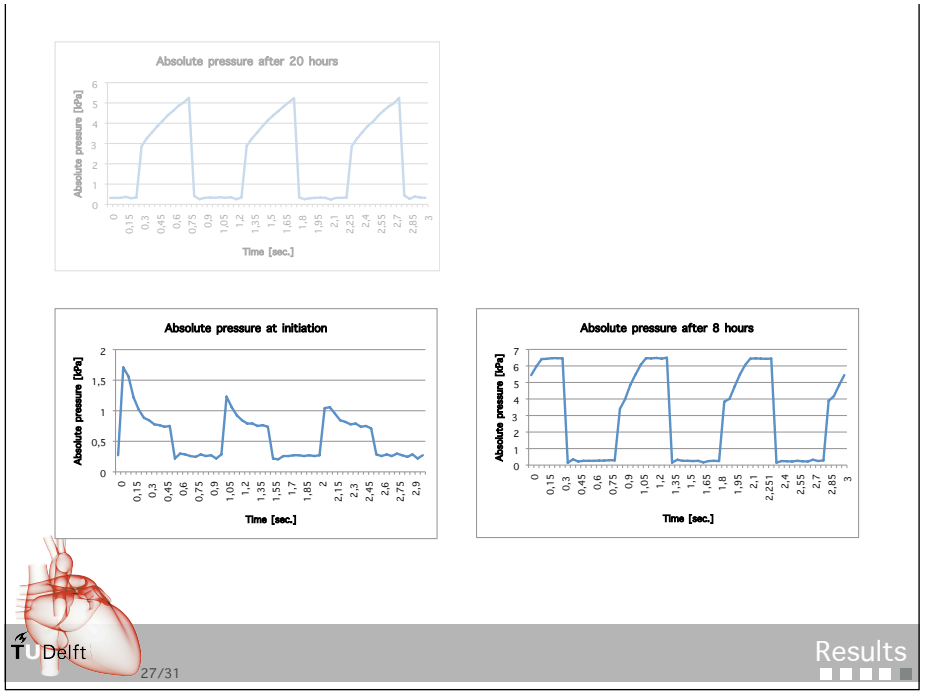
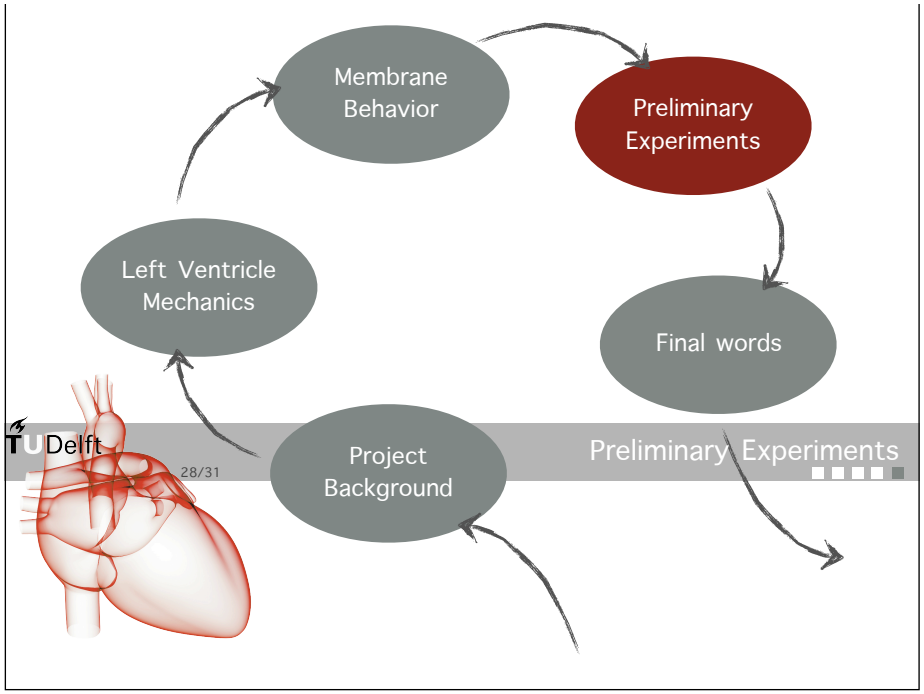



First set of experiments: Pressure remains constant! The first set of experiments were successful in that we have seen that the cells remain attached when subjected to load. Over the weekend however the cells died due to a lack in nutritious fluid, the chip holder has been modified to prevent this for further experiments.




Pressure rise due to leakage, parafilm. Third set of experiments now running.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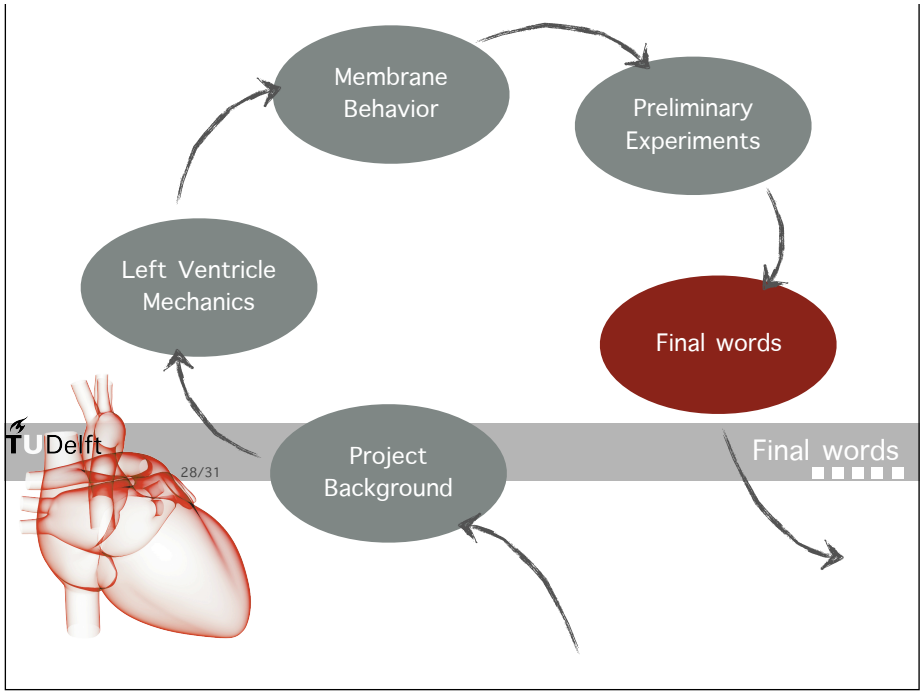
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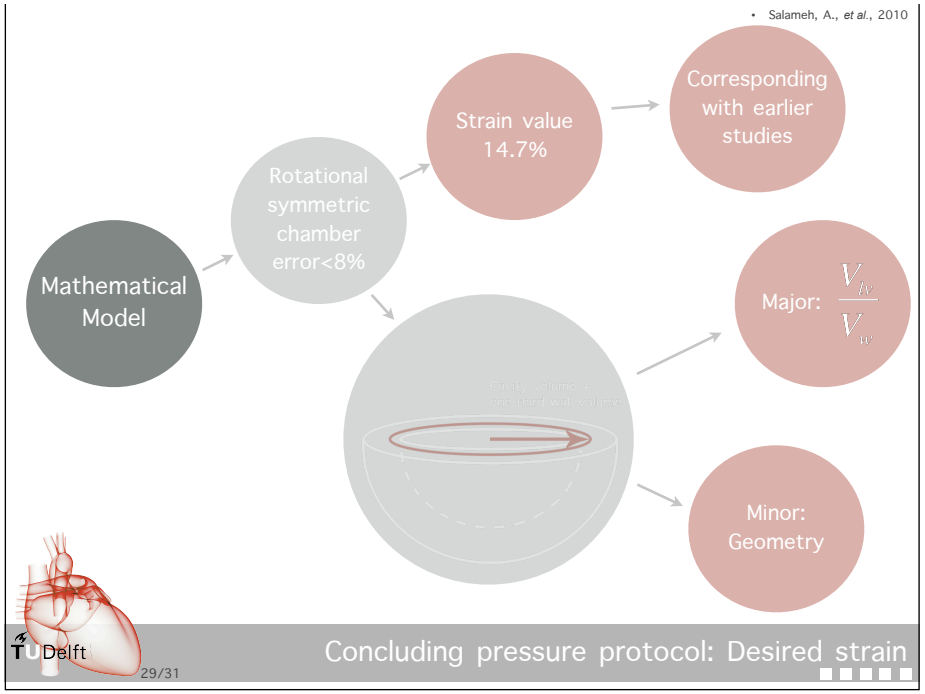
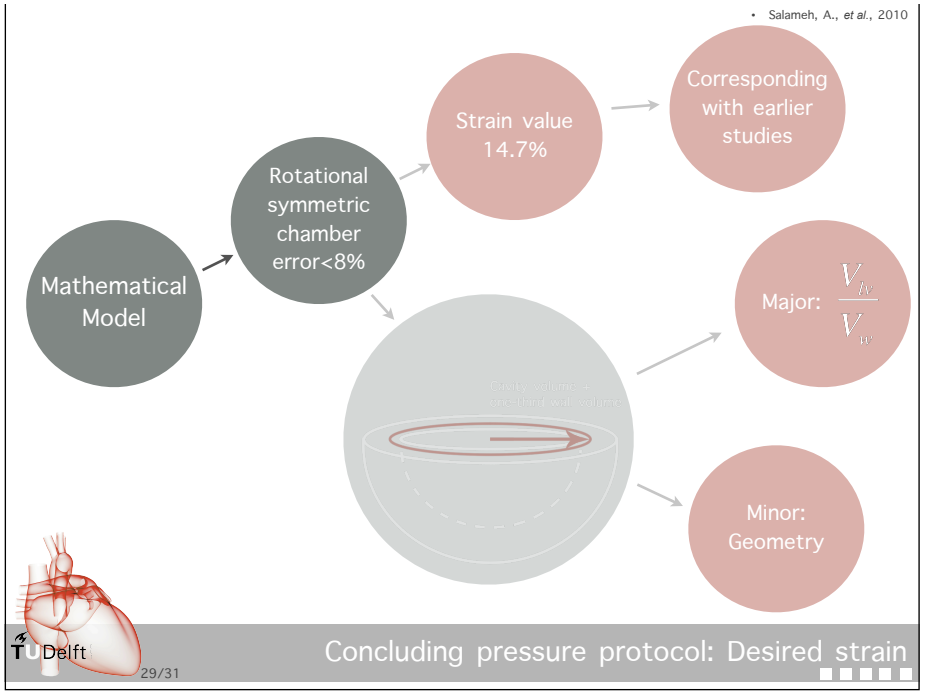



Left ventricle can be modelled mathematically by: Fibrous structure embedded in soft-incompressible material. Rotationally symmetric (maximum error <8%). Mainly depending on cavity volume over wall volume ratio. Shape of the left ventricular representation is of minor importance Strain due to volume difference between end-systolic and end-diastolic circumference elongation of one-third of the wall thickness of sphere Normal human cardiac cycle results in an absolute strain on the cardiac myocytes of approximately 14.7%. Reasonable when looking at previous experiments (Salameh, A., *et al.*, 2010)

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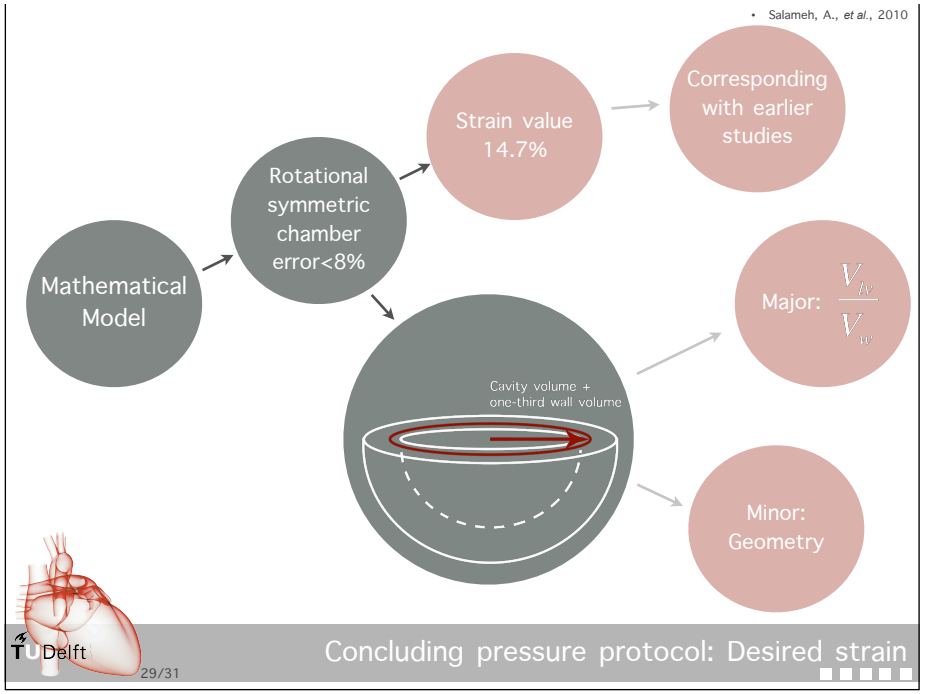


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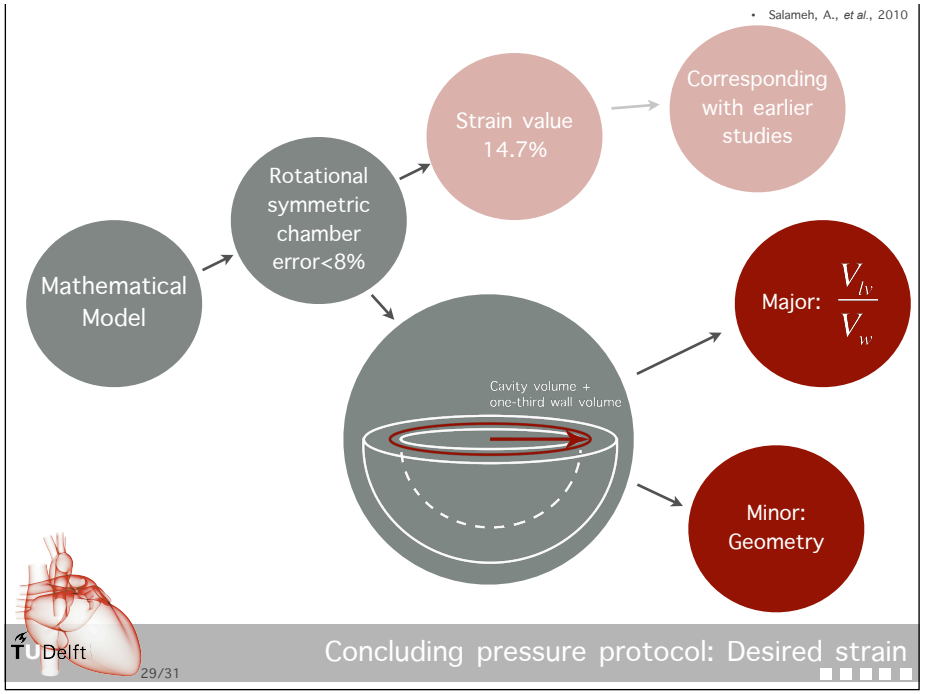


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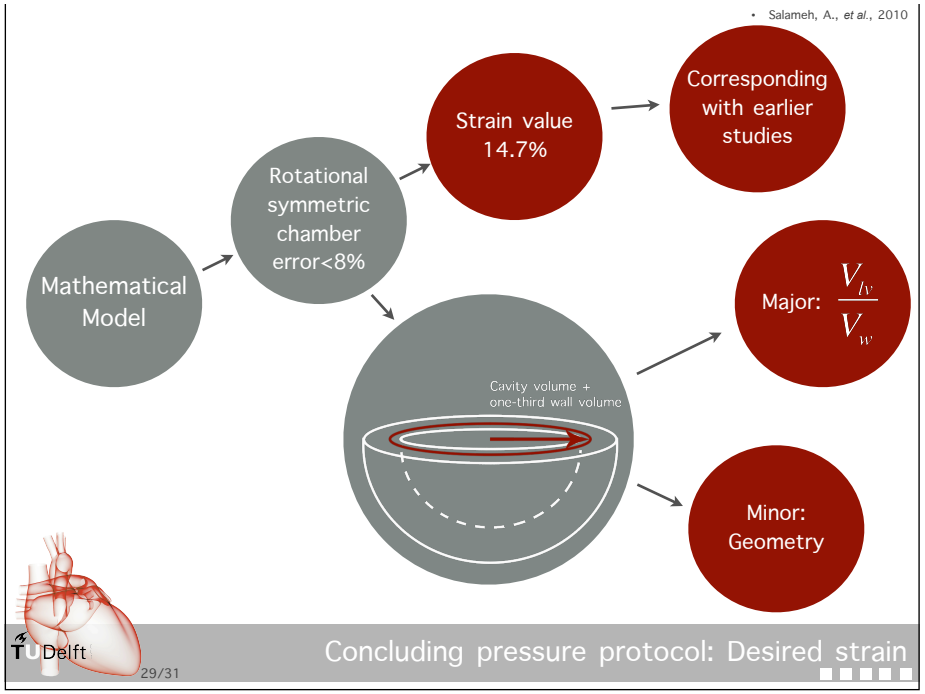


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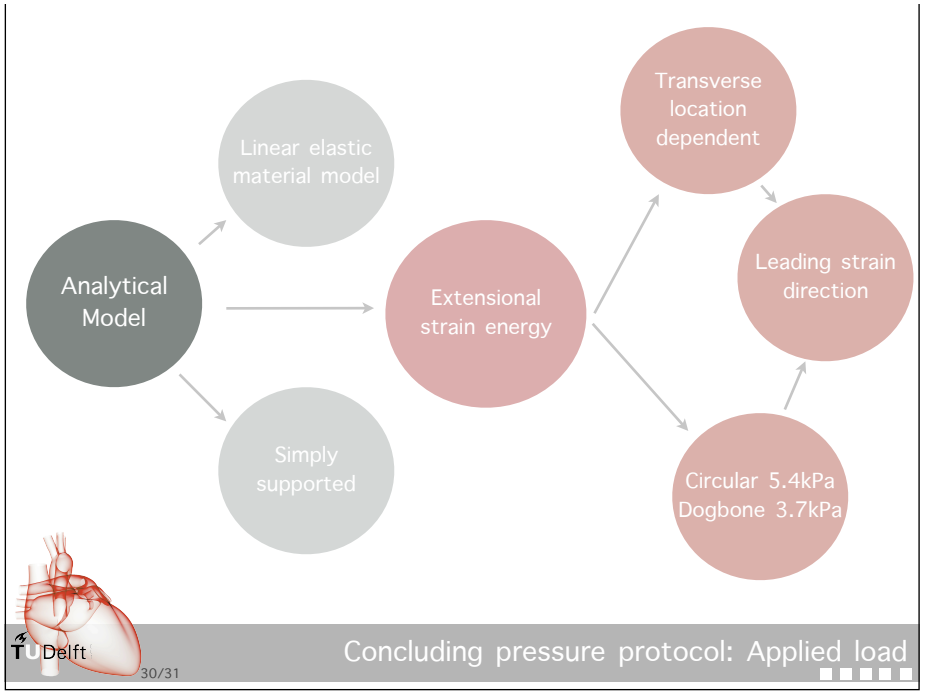
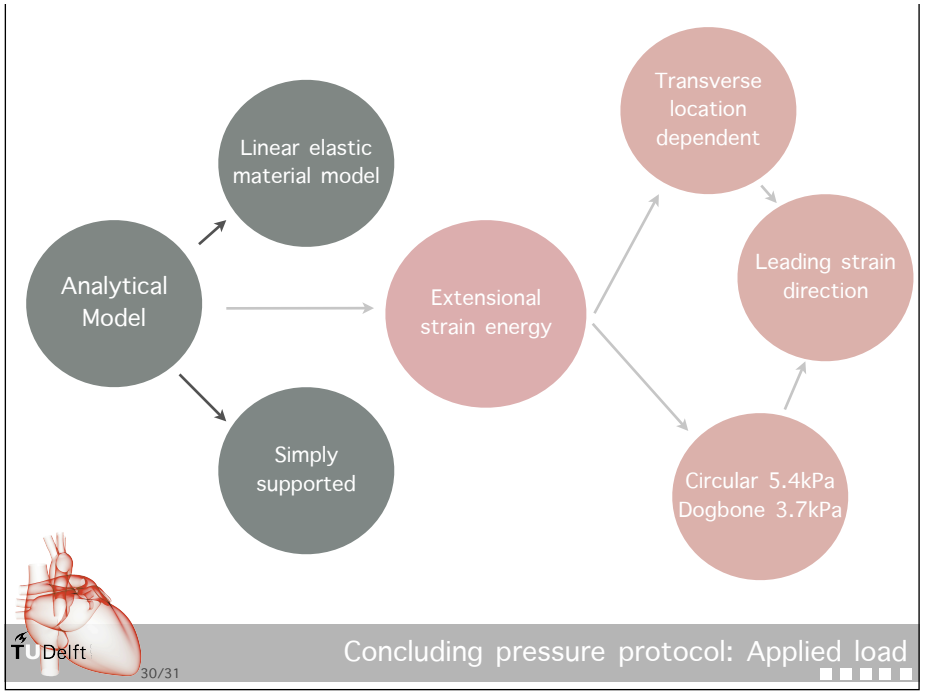



- Both Cytostretch membranes can be modelled most accurately analytically by a simply supported analytical model assuming a linear elastic isotropic material model
- Trail displacement field has big influence on analytical strain outcome
- Membrane behavior mainly depends on extensional strain energy
- 14.7% strain on the cells during the cardiac cycle
  - Circular membrane: 5.375 kPa applied pressure
  - Dogbone membrane: 3.725 kPa applied pressure
- Comparison between uni-directional and multi-directional strained cardiomyocytes cannot accurately be made
- Transverse strain of cells on the circular membrane is location dependent

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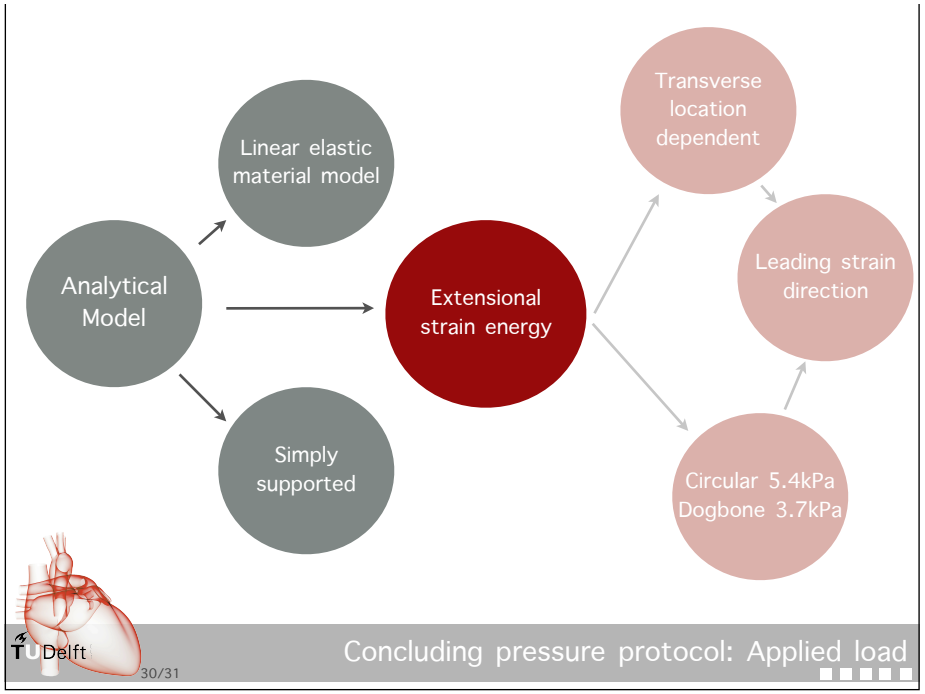
- Comparison between uni-directional and multi-directional strained cardiomyocytes cannot accurately be made
- Transverse strain of cells on the circular membrane is location dependent




- Both Cytostretch membranes can be modelled most accurately analytically by a simply supported analytical model assuming a linear elastic isotropic material model
- Trail displacement field has big influence on analytical strain outcome
- Membrane behavior mainly depends on extensional strain energy
- 14.7% strain on the cells during the cardiac cycle
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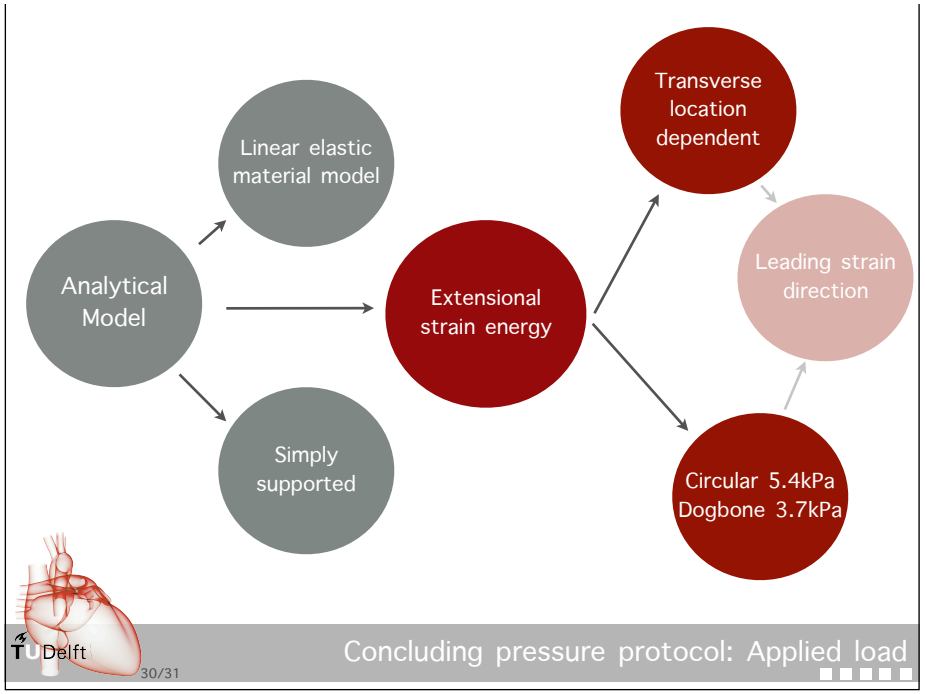


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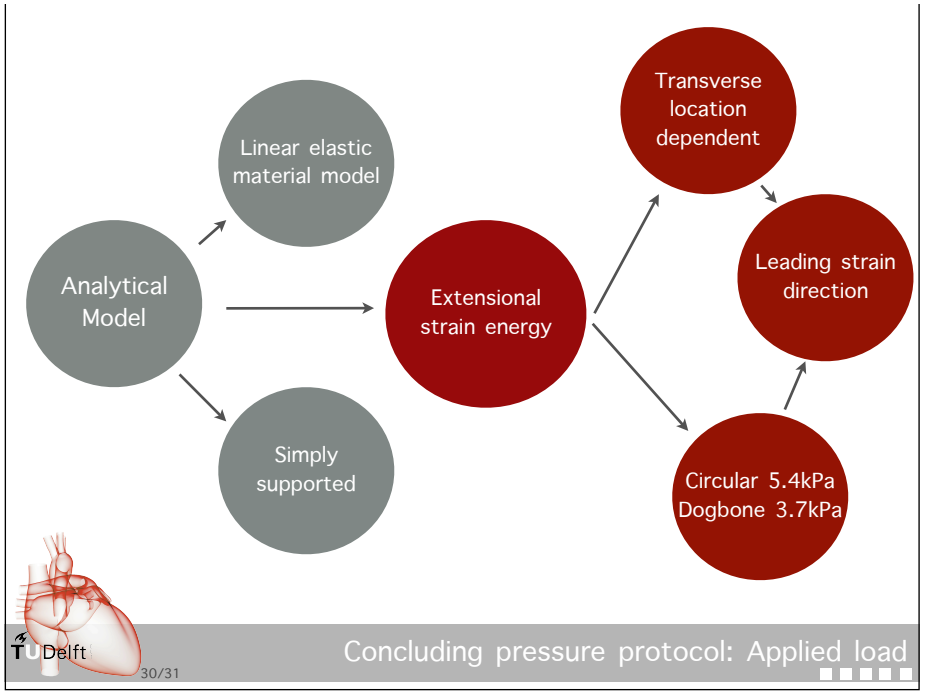


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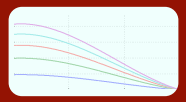
- Adaptation of Cytostretch configuration



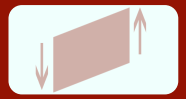
- Improving left ventricular strain model

$V_{left\cdot ventricle} / V_{wall}$

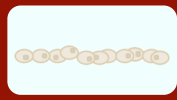
Fluid-Fiber-Collagen Continuum

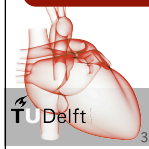
- Improving accuracy of membrane model

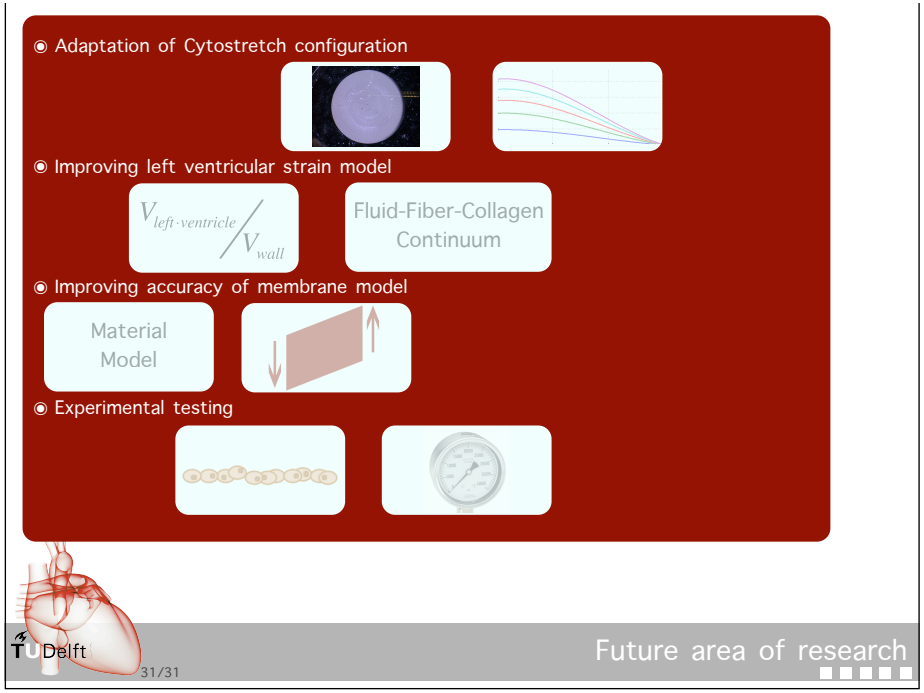
Material Model



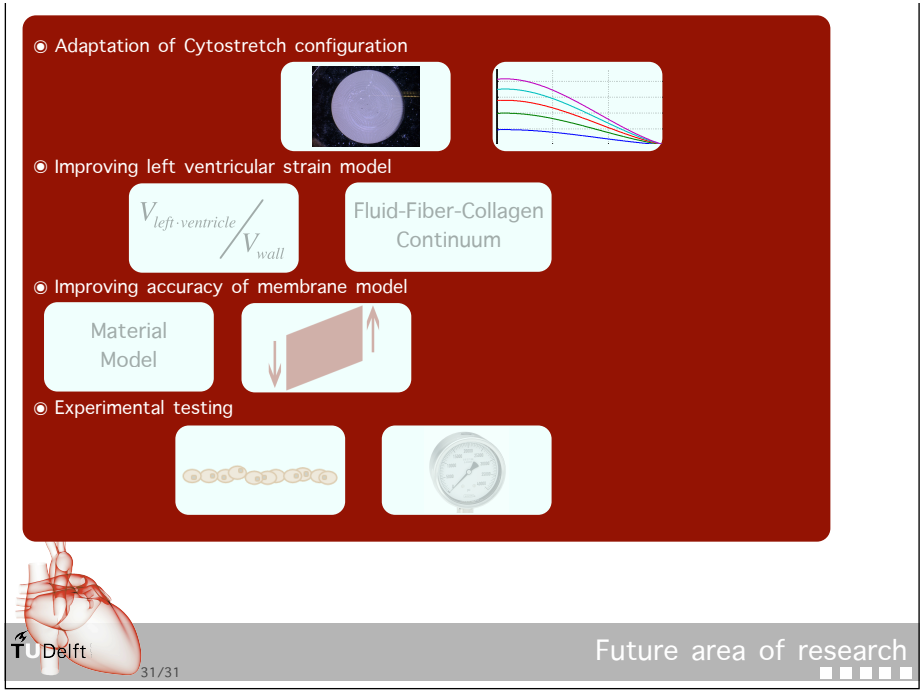
- Experimental testing



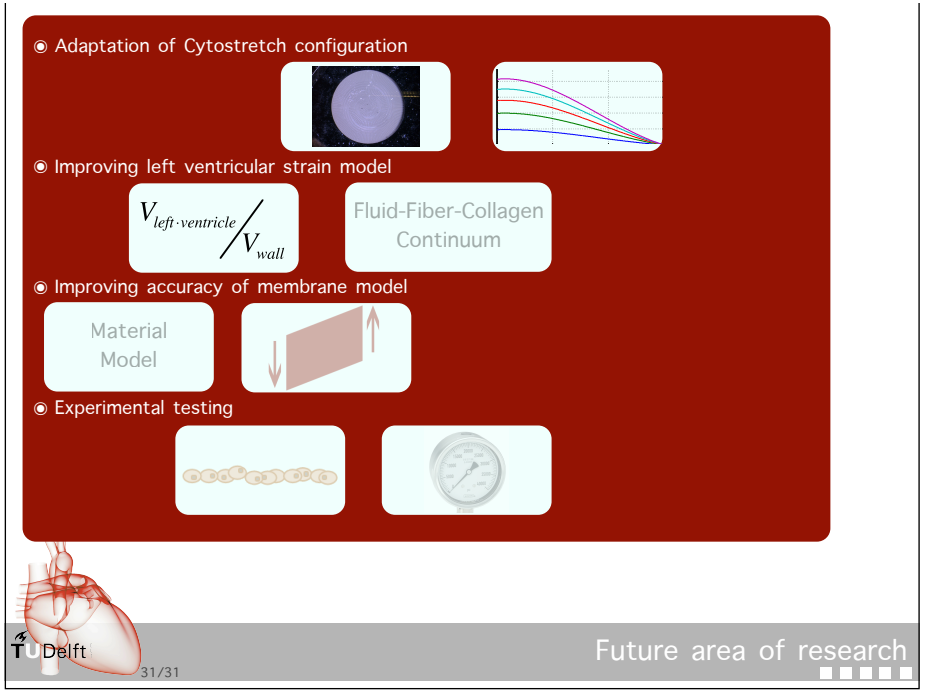





- A circular membrane more than twice as large

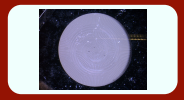


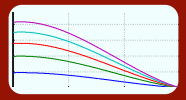

- Ensure only attachment in centre section




- Implementation MRI data LV cavity volume over wall volume during development

- Adaptation of Cytostretch configuration





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Fluid-Fiber-Collagen Continuum

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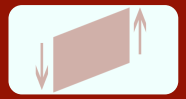
Material Model



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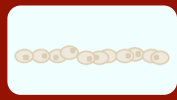
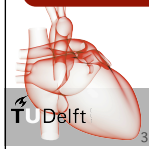
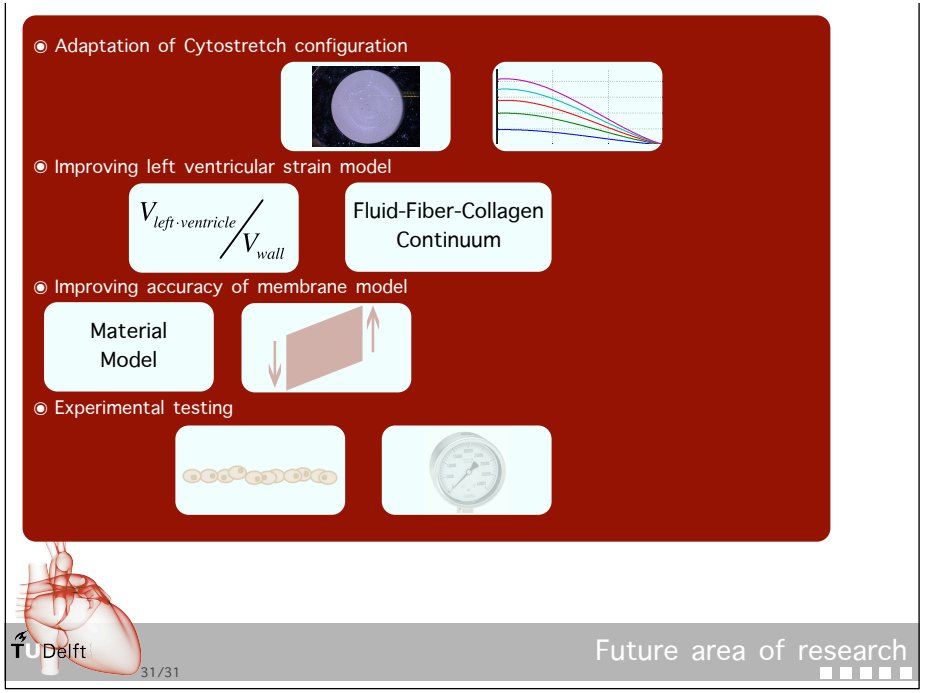
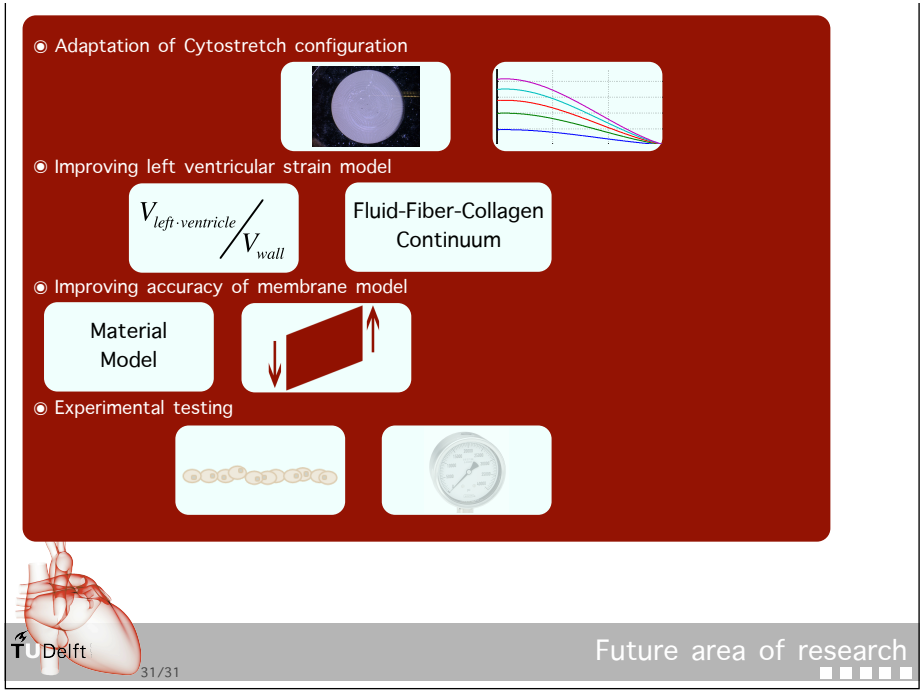


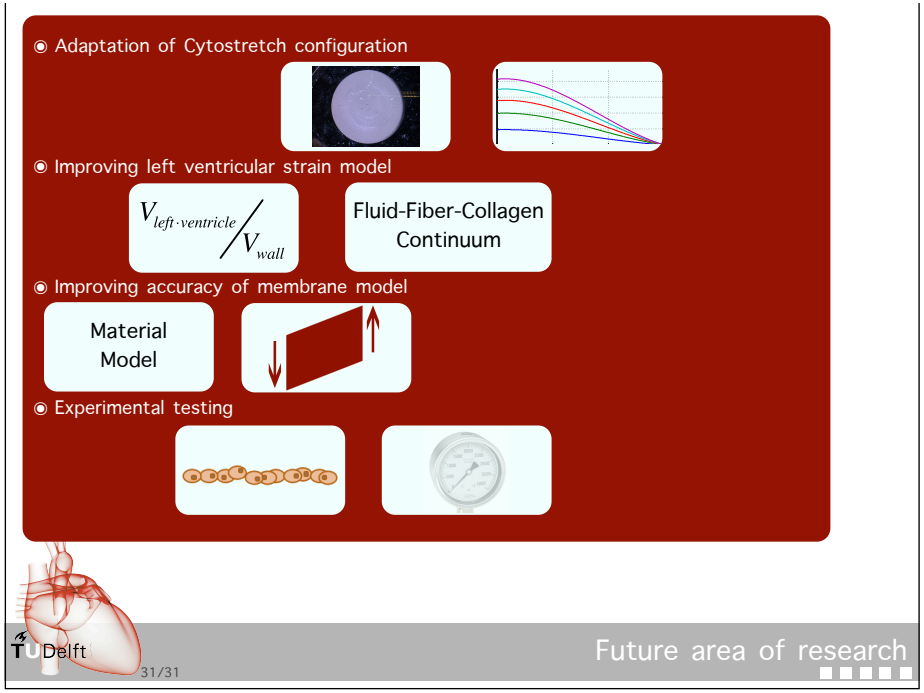




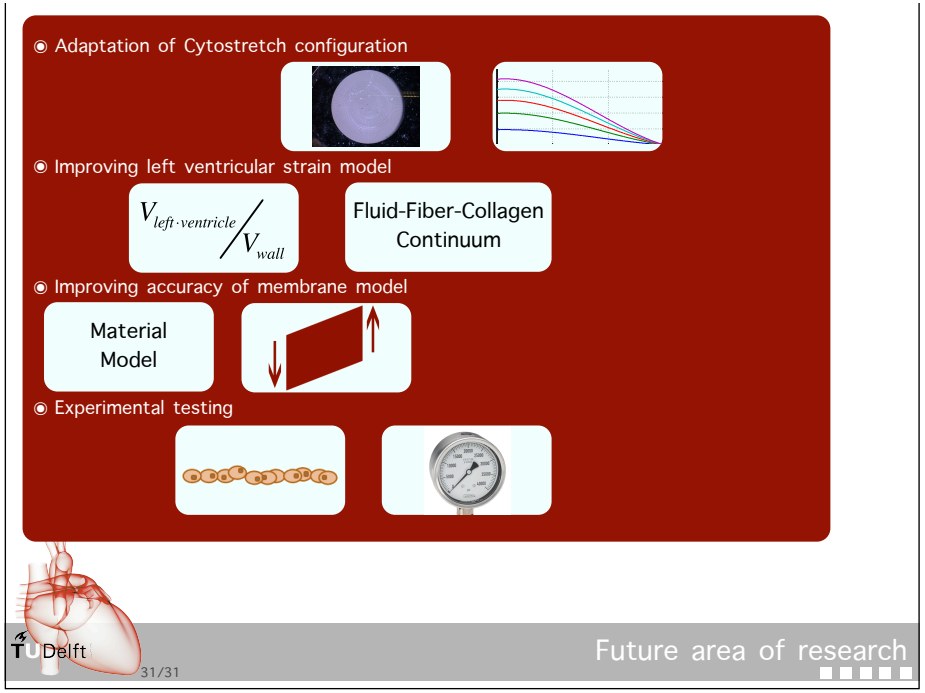

- Variation in material model



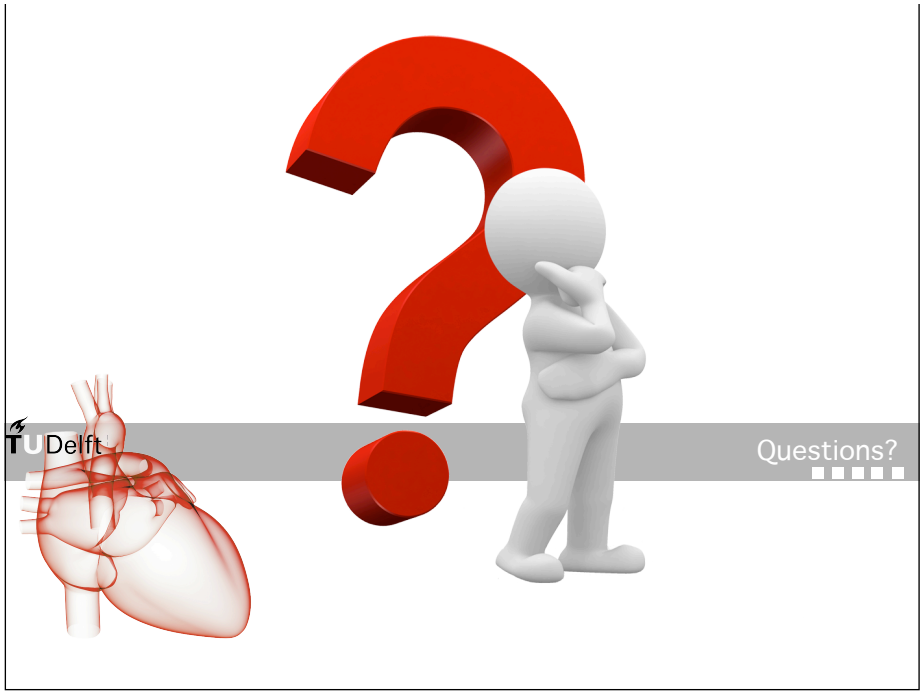

- Shear deformations due to transverse forces




- Plating the cardiac myocytes in mono-layer




- Experimenting with various applied pressures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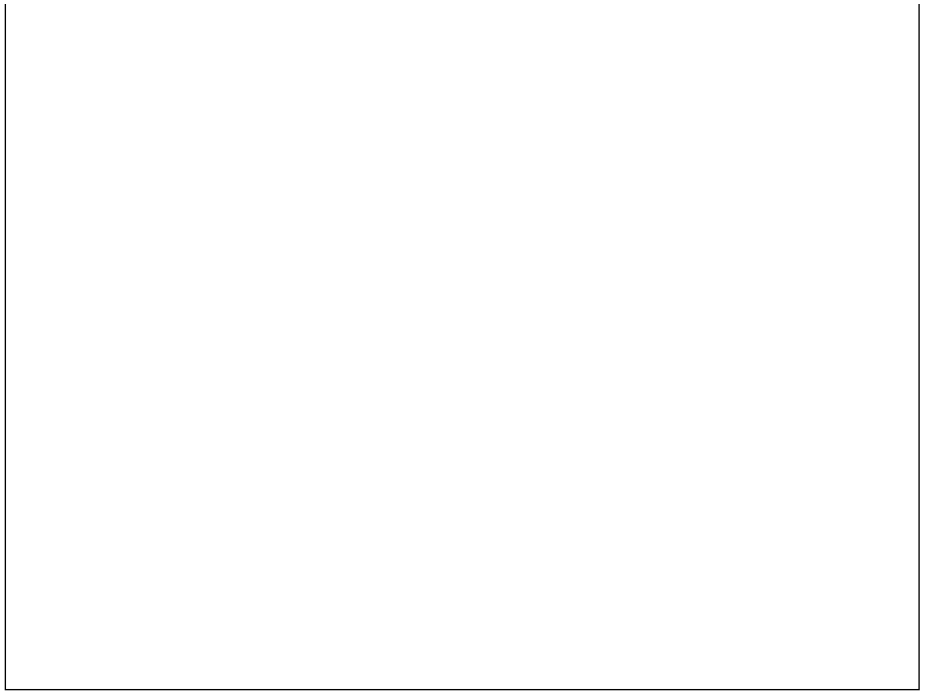
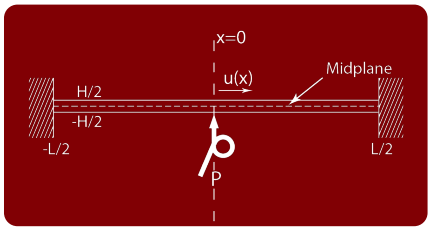
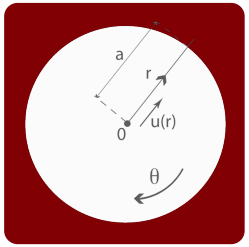
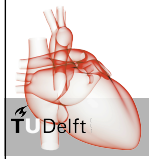
Questions?

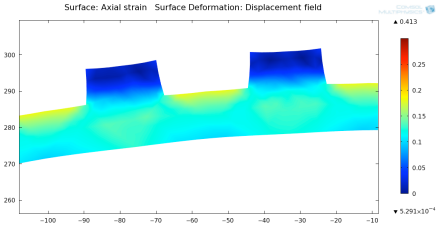


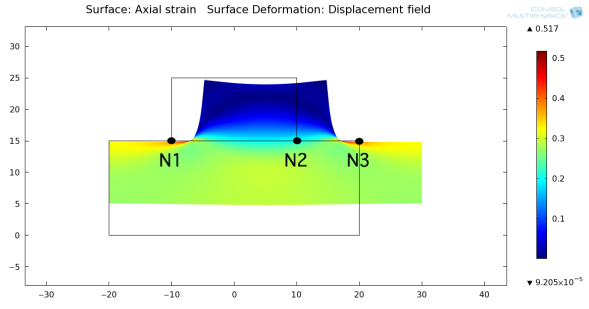


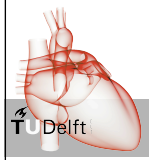

- Dogbone modelled two-dimensional
- Circular modelled by polar coordinates



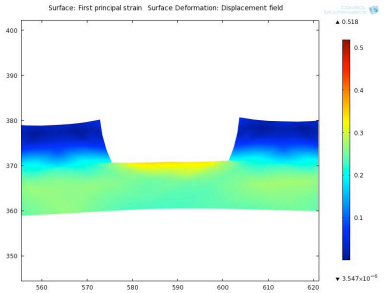


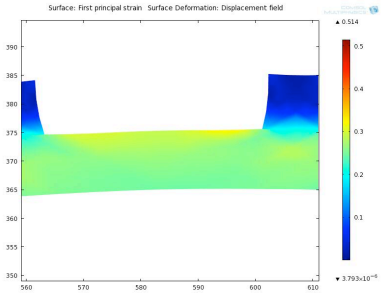



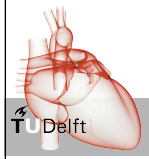





# Nodal displacement



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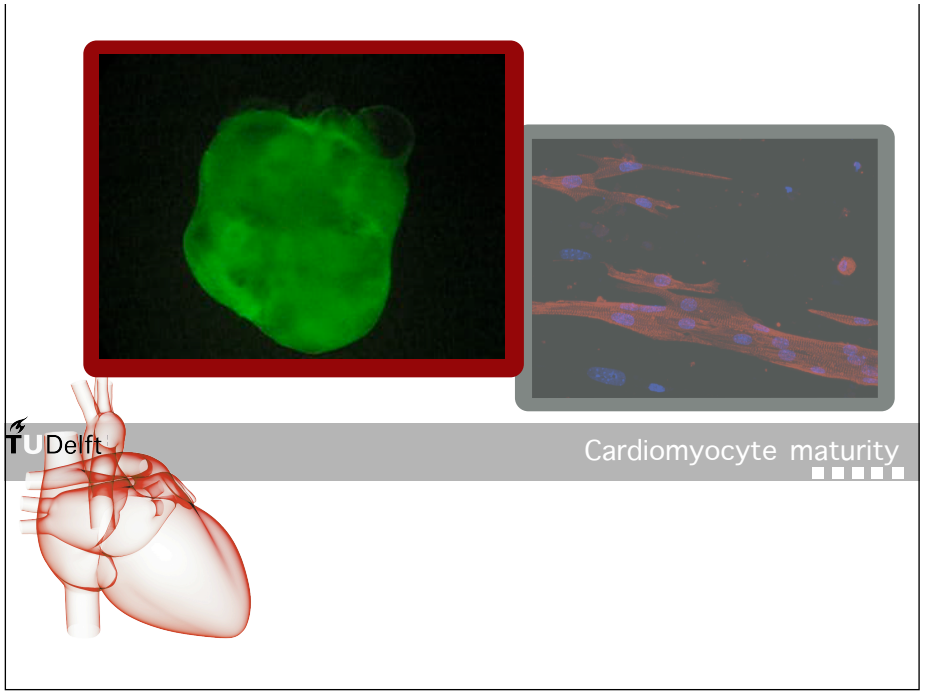
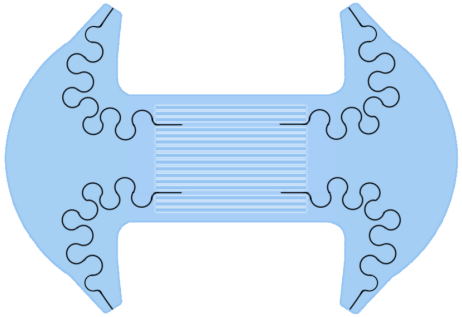
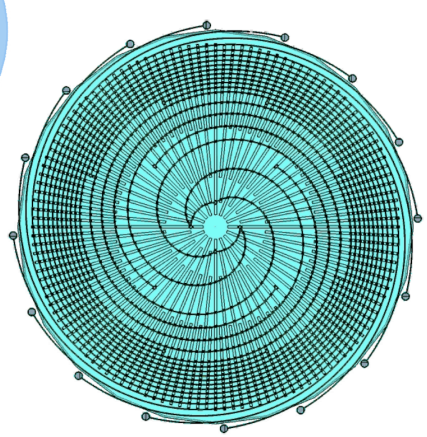
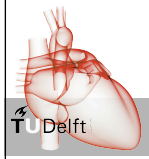
Groove width

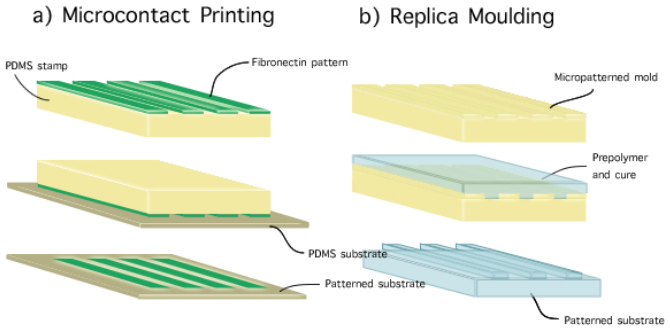



Cardiomyocytes cultured using conventional methods do not align and remain poorly differentiated.



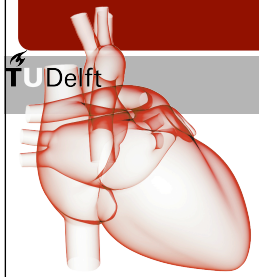





a) Microcontact Printing
b) Replica Moulding
PDMS stamp
Fibronectin pattern
PDMS substrate
Patterned substrate
Micropatterned mold
Prepolymer
and cure
Patterned substrate

$$\frac{\sigma_f}{P_{lv}} = 1 + 3\frac{V_{lv}}{V_w}$$

$$\Delta\varepsilon_f = \frac{1}{3}\Delta \ln\left(1 + 3\frac{V_{lv}}{V_w}\right)$$



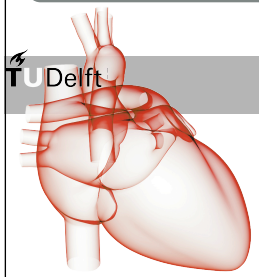
- The displacement field is described by:
  - Out of plane deflection *'w'*
  - In plane displacement *'u'*

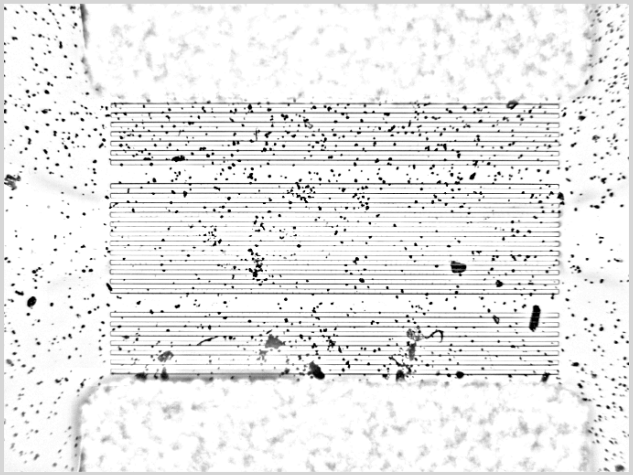
Displacement field:

$$\hat{u}(x, y, z : c_1, c_2, ..., c_n)$$
$$\hat{w}(x, y, z : c_1, c_2, ..., c_n)$$

Equilibrium:

$$\frac{\partial U}{\partial c_1} = 0 ... \frac{\partial U}{\partial c_j} = 0 ... \frac{\partial U}{\partial c_n} = 0$$





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In plane displacement *'u'*

$$\hat{w} = w_0 \left(1 - \frac{r^2}{a^2}\right)^2$$

$$\frac{\hat{u}}{r} = (a-r)\left(c_1 + c_2 r + c_3 r^2 + c_4 r^3 + c_5 r^4 + \ldots\right)$$

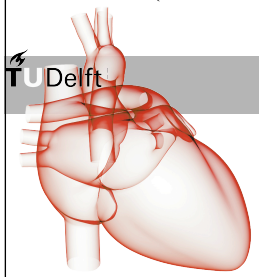
$$\hat{u} = r(a-r)\left(c_1 + c_2 r + c_3 r^2 + c_4 r^3 + c_5 r^4 + \ldots\right)$$

$$\hat{u} = r(a-r)(c_1 + c_2 r)$$

$$\hat{w} = \frac{w_0}{2}\left(1 + \cos\frac{2\pi x}{L}\right)$$

$$\hat{u} = x\left(\frac{L}{2} - x\right)\left(c_1 + c_2 x + c_3 x^2 + c_4 x^3 + c_5 x^4 + \ldots\right)$$

$$\hat{u} = x\left(\frac{L}{2} - x\right)(c_1 + c_2 x)$$



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Displacement field clamped

$$\hat{w} = w_0\left(1 - \frac{2(3+\nu)}{5+\nu}\frac{r^2}{a^2} + \frac{1+\nu}{5+\nu}\frac{r^4}{a^4}\right)$$

$$\frac{\hat{u}}{r} = (a-r)\left(c_1 + c_2 r + c_3 r^2 + c_4 r^3 + c_5 r^4 + \ldots\right)$$

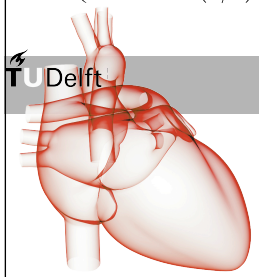
$$\hat{u} = r(a-r)\left(c_1 + c_2 r + c_3 r^2 + c_4 r^3 + c_5 r^4 + \ldots\right)$$

$$\hat{u} = r(a-r)(c_1 + c_2 r)$$

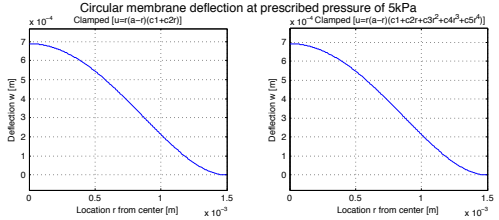
$$\hat{w} = w_0\left(1 - \frac{2(3+\nu)}{5+\nu}\frac{x^2}{(L/2)^2} + \frac{1+\nu}{5+\nu}\frac{x^4}{(L/2)^4}\right)$$

$$\hat{u} = x\left(\frac{L}{2} - x\right)\left(c_1 + c_2 x + c_3 x^2 + c_4 x^3 + c_5 x^4 + \ldots\right)$$

$$\hat{u} = x\left(\frac{L}{2} - x\right)(c_1 + c_2 x)$$

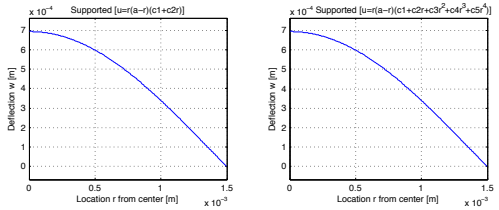


Circular membrane deflection at prescribed pressure of 5kPa



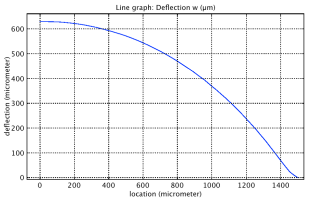


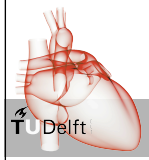










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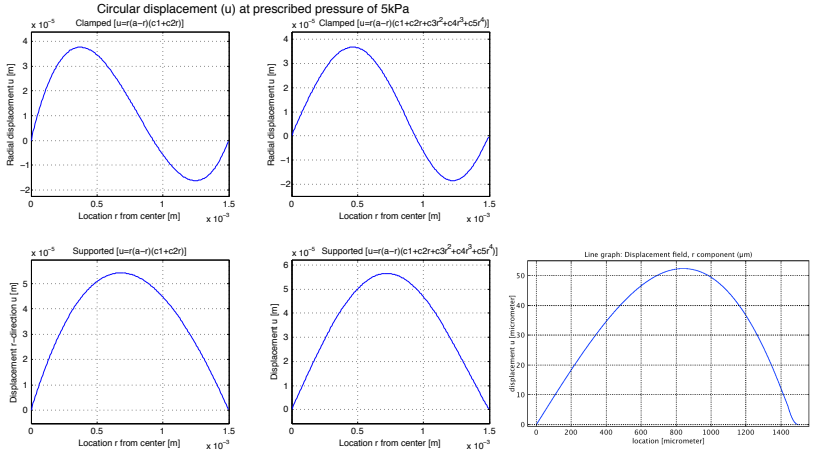
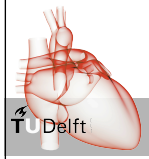
Circular membrane deflection



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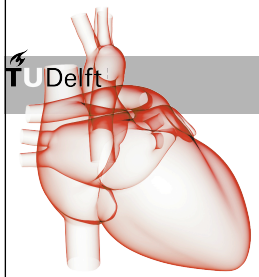
Circular membrane in plane displacement

$$U = \frac{32\pi}{3}\frac{w_0^2}{a^2}\frac{Eh^3}{12(1-\nu^2)} + \frac{\pi Eh}{1-\nu^2}\left(\frac{425\nu w_0^4}{3969a^2} - \frac{222\nu^2 w_0^4}{3157a^2} + \frac{1501 w_0^4}{7938a^2}\right) - \frac{1}{3}\pi P a^2 w_0$$

$$P = \frac{Eh^3}{12(1-\nu^2)}\frac{64 w_0}{a^4} + \frac{Eh}{1-\nu^2}\frac{w_0^3}{a^4}\left\{\frac{1700\nu}{1323} - \frac{589\nu^2}{698} + \frac{1813}{799}\right\}$$



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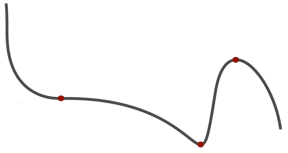
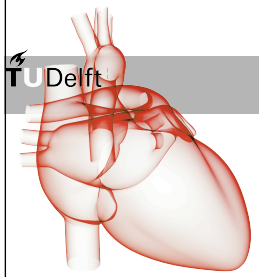
## Load deflection circular membrane

$$\frac{\partial U}{\partial w_0} = 0$$



$$U = \frac{Eh^3}{12(1-\nu^2)}\frac{\pi^4 w_0^2}{L^3} + \frac{Eh}{(1-\nu^2)}\left\{\frac{3w_0^4(\pi^4-30)}{64L^3}\right\} - \frac{LPw_0}{2}$$

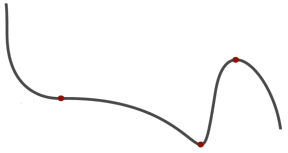
$$P = \frac{Eh^3}{12(1-\nu^2)}\frac{4\pi^4 w_0}{L^4} + \frac{Eh}{(1-\nu^2)}\left\{\frac{3w_0^3(\pi^4-30)}{8L^3}\right\}$$

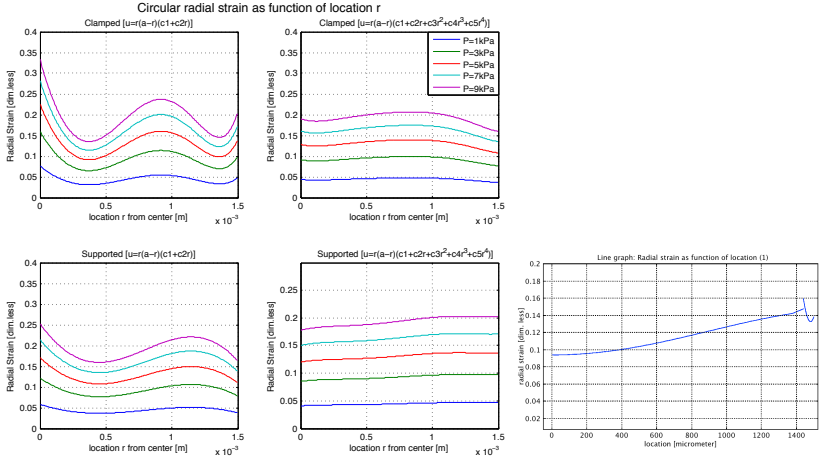


TU Delft

## Load deflection dogbone membrane

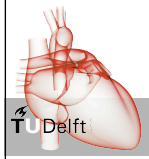
$$\frac{\partial U}{\partial w_0} = 0$$





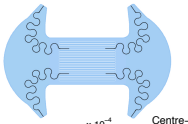


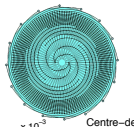


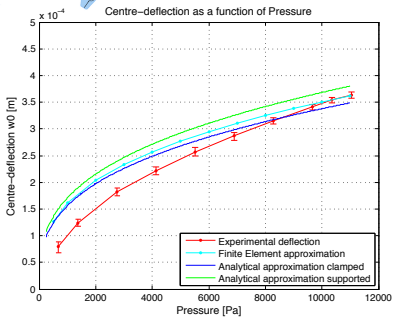


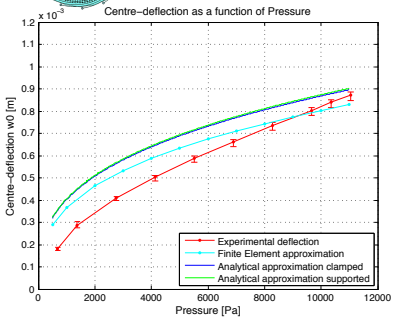
TU Delft

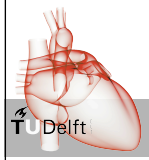
# Circular membrane radial strain





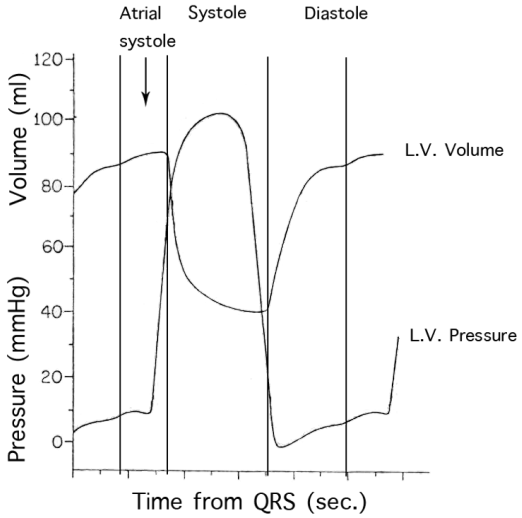


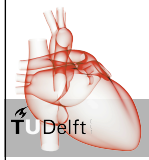



TU Delft

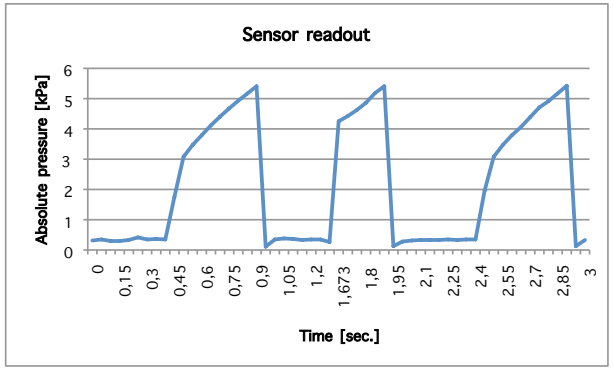
## Experimental comparison

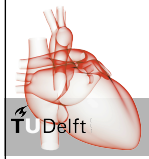


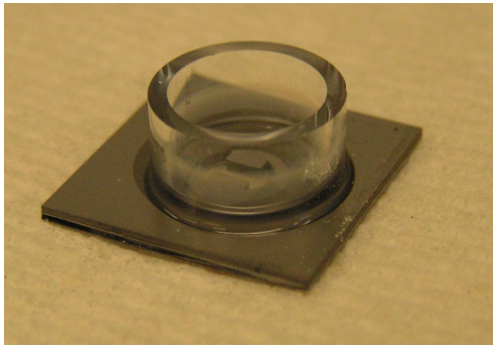


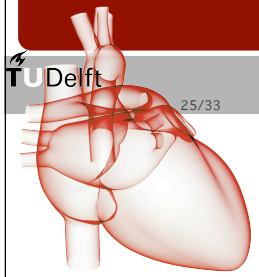
Left ventricular volume


Sensor readout
Absolute pressure [kPa]
6
5
4
3
2
1
0
0
0,15
0,3
0,45
0,6
0,75
0,9
1,05
1,2
1,673
1,8
1,95
2,1
2,25
2,4
2,55
2,7
2,85
3
Time [sec.]



- Plasma treatment
  - Hydrophilic membrane surface
- Fibronectin coated (20% sol.)
  - Provides attachment
- Cocultured with endoderm cells
- Beating areas on >12 day cocultures dissected and plated on chip
- Incubated without stretching for at least 48 hours







Plasma treatment: electric glow discharge. Cocultured with endoderm cells to induce differentiation into cardiomyocytes. In also in plane dissection to ensure little thickness beating areas on the chips