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
Embryonic stem cells have the ability to differentiate into any cell type.
Earlier studies have showed that cardiac constructs exposed to cyclic stretch show enhanced maturation.
References:

Salameh, A., et al., 2010

Zimmermann, W.-H., et al., 2002
Two Cytostretch configurations have been developed. The circular configuration ensures a multi-directional cell-stretch. The dogbone configuration ensures a uni-directional cell-stretch.
Two Cytostretch configurations have been developed. The circular configuration ensures a multi-directional cell-stretch. The dogbone configuration ensures a uni-directional cell-stretch.
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 fulfilled
• Gain insight in the strain cardiac myocytes endure in vivo
• Determine membrane behavior of both Cytostretch configurations
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Two main objectives have to be fulfilled
- Gain insight in the strain cardiac myocytes endure in vivo
- Determine membrane behavior of both Cytostretch configurations
Each simplification is a compromise between accuracy and calculation time.

In vivo difficult: mathematically.
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,LWWV=200ml,LVEDV=143)
Left ventricular fiber strain

End-Systole:

Fiber Strain [dim.less] vs. Cavity volume over wall volume [dim.less]

16/31
Left ventricular fiber strain

Cavity volume over wall volume [dim.less]

Fiber Strain [dim.less]

End-Systole: Reference (zero strain)

16/31
Left ventricular fiber strain

Fiber Strain [dim.less]

Cavity volume over wall volume [dim.less]

End-Systole:
Reference (zero strain)

End-Diastole:
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.
Membrane Behavior

Project Background

Preliminary Experiments

Left Ventricle Mechanics

Final words
Membrane behavior

- 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
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
Axial Strain as a function of location

Axial Strain [dim.less]

P=1kPa
P=3kPa
P=5kPa
P=7kPa
P=9kPa

Dogbone membrane strain
Axial Strain as function of location

Radial Strain as function of location

Axial Strain 

Radial Strain 

P=1kPa 
P=3kPa 
P=5kPa 
P=7kPa 
P=9kPa

Dogbone membrane strain

location x from center [m]

location r from center [m]
• For pressures >3kPa bending strain energy can be neglected

Bending: \( \frac{h^3}{h} \)

• 14.7% strain when applying 5.375 kPa of pressure

Circular membrane applied pressure

Strain as a function of Pressure

radial strain \( r=1400 \mu m \)
transverse strain \( r=0 \mu m \)
● For pressures >3kPa bending strain energy can be neglected

Bending: \[ h^3 \]
Extension: \[ h \]

● 14.7% strain when applying 5.375 kPa of pressure

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

Bending: \[ h^3 \]
Extension: \[ h \]

Circular membrane applied pressure

Strain as a function of Pressure

- Max strain = 0.147
- Radial strain: \[ r = 1400 \mu \]
- Transverse strain: \[ r = 0 \mu \]

14.7% strain when applying 5.375 kPa of pressure.
- For pressures >3 kPa bending strain energy can be neglected.

Bending: \[ h^3 \]

Extension: \[ h \]

- 14.7% strain when applying 5.375 kPa of pressure.

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

14.7% strain when applying 5.375 kPa of pressure.
Dogbone membrane applied pressure

Strain as a function of Pressure (homogeneous)

Pressure [Pa]

Axial Strain [dim.less]

Max. strain = 0.147 if Pressure = 5.375 kPa

radial strain [r=1400 μm]
transverse strain [r=0 μm]
Dogbone membrane applied pressure

Pressure [Pa] vs. Strain as a function of Pressure (homogeneous)

Max. strain = 0.147 if Pressure = 5.375 kPa
Dogbone membrane applied pressure

Strain as a function of Pressure [homogeneous]

Maximum strain = 0.147 if Pressure = 5.375 kPa

Strain as a function of Pressure

Maximum strain = 0.147 if Pressure = 3.725 kPa

Axial strain [dim.less]

Radial strain [r=1400 μm]

Transverse strain [r=0 μm]
Membrane Behavior

Project Background

Left Ventricle Mechanics

Preliminary Experiments

Final words
Experimental setup
Experimental setup
Experimental setup
Experimental setup
Experimental setup
Experimental setup
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.
Preliminary Experiments

Project Background

Membrane Behavior

Left Ventricle Mechanics

Preliminary Experiments

Final words
Project

Background

Preliminary Experiments

Membrane Behavior

Left Ventricle Mechanics

Final words

Project Background

Final words
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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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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Transverse strain of cells on the circular membrane is location dependent.
Future area of research

- Adaptation of Cytostretch configuration
- Improving left ventricular strain model
- Improving accuracy of membrane 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
Future area of research

- Adaptation of Cytostretch configuration
- Improving left ventricular strain model
- Improving accuracy of membrane model
- Experimental testing

![Graph showing Transverse Strain as function of location](image)

- Fluid-Fiber-Collagen Continuum

![Material Model](image)
• Variation in material model
• Shear deformations due to transverse forces
• Plating the cardiac myocytes in mono-layer
- Experimenting with various applied pressures
Membrane load-deflection

- Dogbone modelled two-dimensional
- Circular modelled by polar coordinates
Nodal displacement
Cardiomyocytes cultured using conventional methods do not align and remain poorly differentiated.
Configurations including interconnects
Replica moulding

a) Microcontact Printing

b) Replica Moulding
\[
\frac{\sigma_f}{P_{lv}} = 1 + 3 \frac{V_{lv}}{V_w} \\
\Delta \varepsilon_f = \frac{1}{3} \Delta \ln \left(1 + 3 \frac{V_{lv}}{V_w}\right)
\]
The displacement field is described by:
- Out of plane deflection 'w'
- In plane displacement 'u'

Displacement field:
\[
\begin{bmatrix}
\frac{\partial \varphi}{\partial x_1} & \ldots & \frac{\partial \varphi}{\partial x_n}
\end{bmatrix}
\begin{bmatrix}
C_1, C_2, \ldots, C_n
\end{bmatrix}
\]

Equilibrium:
\[
\frac{\partial E}{\partial x_1} = 0, \quad \frac{\partial E}{\partial x_2} = 0, \quad \frac{\partial E}{\partial x_3} = 0
\]
In plane displacement ‘u’
\[ \hat{w} = w_0 \left(1 - \frac{r^2}{a^2}\right) \]
\[ \hat{u} = \frac{(a - r)}{r} \left(c_1 + c_r r + c_r r^2 + c_r r^3 + c_r r^4 + \ldots \right) \]
\[ \hat{u} = r(a - r) \left(c_1 + c_r r + c_r r^2 + c_r r^3 + c_r r^4 + \ldots \right) \]
\[ \hat{u} = r(a - r)(c_1 + c_r) \]

\[ \hat{w} = \frac{w_0}{2} \left(1 + \cos \frac{2\pi x}{L}\right) \]
\[ \hat{u} = x \left( \frac{L}{2} - x \right) \left(c_1 + c_r x + c_r x^2 + c_r x^3 + c_r x^4 + \ldots \right) \]
\[ \hat{u} = x \left( \frac{L}{2} - x \right)(c_1 + c_r x) \]

Displacement field clamped
\[
\begin{align*}
\tilde{w} &= w_r \left(1 - \frac{2(3 + v) r^2}{5 + v} \frac{1}{a^2} + \frac{1 + v r}{5 + v a^2}\right) \\
\tilde{u} &= (a - r) \left(c_i + c_r r + c_r r^3 + c_r r^5 + c_r r^7 + \ldots\right) \\
\hat{u} &= r(a - r) \left(c_i + c_r r + c_r r^3 + c_r r^5 + c_r r^7 + \ldots\right) \\
\hat{u} &= r(a - r) (c_i + c_r) \\
\tilde{w} &= w_r \left(1 - \frac{2(3 + v) x^2}{5 + v} \frac{1}{(L/2)^2} + \frac{1 + v x}{5 + v (L/2)^2}\right) \\
\tilde{u} &= x \left(\frac{L}{2} - x\right) \left(c_i + c_r x + c_r x^3 + c_r x^5 + c_r x^7 + \ldots\right) \\
\hat{u} &= x \left(\frac{L}{2} - x\right) (c_i + c_r x)
\end{align*}
\]
Circular membrane deflection at prescribed pressure of 5kPa

Clamped \[ u = r(a - r)(c_1 + c_2r) \]

Deflection \[ w \]

Supported \[ u = r(a - r)(c_1 + c_2r + c_3r^2 + c_4r^3 + c_5r^4) \]

Deflection \[ w \]
Circular membrane in plane displacement

Circular displacement ($u$) at prescribed pressure of 5kPa

Clamped

Radial displacement $u$ [m]

Location $r$ from center [m]

$u = r(a - r)(c_1 + c_2r)$

Supported

Displacement $u$ [m]

Location $r$ from center [m]

$u = r(a - r)(c_1 + c_2r + c_3r^2 + c_4r^3 + c_5r^4)$
\[ U = \frac{32\pi}{3} \frac{w_0^3}{a^2} \frac{Eh^3}{12(1-v^2)} + \frac{\pi Eh}{1-v^2} \left( \frac{425v^2w_0^3}{3969a^2} - \frac{222v^3w_0^3}{3157a^2} + \frac{1501w_0^3}{7938a^2} \right) - \frac{1}{3} \pi Pa^2 w_0 \]

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

\[ \frac{\partial U}{\partial w_0} = 0 \]
\[
U = \frac{Eh^3}{12(1 - v^2)} \frac{\pi^4 w_0^2}{L^4} + \frac{Eh}{(1 - v^2)} \left\{ \frac{3w_0^2 (\pi^4 - 30)}{64L^4} \right\} - \frac{LPw_0}{2} \\

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

\[
\frac{\partial U}{\partial w_0} = 0
\]
Circular membrane radial strain

Clamped: $u = r(a - r)(c_1 + c_2r)$

Supported: $u = r(a - r)(c_1 + c_2r + c_3r^2 + c_4r^3 + c_5r^4)$

P = 1kPa, 3kPa, 5kPa, 7kPa, 9kPa
Experimental comparison

Centre-deflection as a function of Pressure

Pressure [Pa]

Centre-deflection $w_0$ [m]

Experimental deflection
Finite Element approximation
Analytical approximation clamped
Analytical approximation supported
Left ventricular volume
Labview fluctuations

Sensor readout

Absolute pressure [kPa]

Time [sec.]

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
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.