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Mitral Valve Interstitial Cells and Respective Mechanobiological Response to Stress

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Introduction

Motivation

According to the American Heart Association, approximately five million people are diagnosed with heart valve disease in the United States each year^{1,2}.

Mitral Valve Regurgitation (MVR) is one of the most common valvular diseases, being increasingly prevalent^{3,4}. Approximately 41 000 hospitalizations per year are due to surgical corrections of MVR⁵. MVR is characterized by the leakage of blood from the left ventricle backwards into the left atrium during systole - where MV doesn't close completely^{3,5}. MVR can be caused by various mechanisms related to structural and functional abnormalities of the mitral apparatus or the left ventricle⁵.

Since the late 1970s and early 1980s have been developed different

MV and Interstitial Cells

MV has two leaflets, the anterior (also known as semicircular aortic) and posterior (also known as the mural) leaflets, that differ in structure, and thicknesses: the anterior leaflet is larger than the posterior which is narrower but has a longer attachment to the annulus^{4,6}. These leaflets are populated by cardiac interstitial cells (ICs) and it must be noted that both leaflets present distinct histological layers: atrialis, spongiosa, fibrosa and ventricularis. The respective non-cellular components of the cardiac valves presents a matrix of collagen, elastic fibres, proteoglycans (PGs) and glycoproteoglycans (GAGs)⁷.





Co Posterior Leafle

Papillary Muscles

Aims

For a better understanding about MVR, Agent-based models are developed (a novel approach to study but also simulate mechano-chemo-biological responses at the cellular level) 8 .

The long-term goal of this project is related to the development of an Agent-based modeling (ABM) framework on *NetLogo* or in **ComputceII3D** for MVIC mechanobiological response (dedicated to the Mitral Valve Anterior Leaflet, MVAL) to in vitro uniaxial stress/strain levels that allows rapid and focused hypothesis testing⁹. The validated ABM will be improved in the future, by incorporation into an organ-level model to be used as a predictive tool for different surgical repair scenarios, particularly, MVR.

This internship work, developed during only four weeks, was extremely exploratory, and presented specific goals:

techniques dedicated to MVR for surgical repair with subsequent progressive improvements⁶.

For a successful repair it is crucial to understand the anatomic and functional alterations that occur in MV.

> **Figure 1** - Components of Mitral Valve (MV). Adult heart specimen picture showing the mitral valve structure, where: Δ is the anterolateral commissure, the filled Δ is the posteromedial commissure, A1-A3 are divisions of the aortic mitral leaflet, P1-P3 are divisions of the mural leaflet of the mitral valve (left), and schematic illustration about mitral apparatus where can be appreciated the MV shape (right)⁴

1) Making an evaluation of *Compucell3D* as a tool for modelling mitral valve interstitial cell mechanobiological responses to stress overload with respect to the mitral valve stress/strain behavior;

2) The development of a preliminary modelling plan with the main aim of presenting the code using histology and immunohistochemistry data of MV previously obtained in experimental experiences.

Methods

First Part: Experiments

Step 1: Protocol and respective experimental Set-up

To reach the first step:

1. Acquire 10 porcine hearts (~10 months, ~250 pounds).

2. Isolate MVs into a Bell System isolation and place in petri dishes with regular phosphate buffered saline (PBS) to wash off the blood.

- 3. Separate anterior leaflet (MVAL) from posterior leaflet.
- 4. Trim each MVAL to measure 7.6 mm radially and 17.4 mm circumferentially.
- 5. Further trim MVAL tissue must provide three strips at the following dimensions (Figure 2):
- 11 mm by 7.6 mm for **bioreactor treatment**,
- 6.3 mm by 3.8 mm for mechanical characterization,
- 6.3 mm by 3.8 mm for **contro**l.

A uniaxial tissue fixation system was used to simulate physiological stress where the excised porcine MV anterior leaflet tissue specimen can be fixed under a range of stretches (10%, 20% and 30%) through of cyclic motion.

6. Each tissue strip is stored (Figure 3):

Bioreactor treatment – in a small plastic container with PBS.

biaxial testing device.

• Mechanical characterization – in small glass vials with PBS and place them in the a frigde. These samples must be tested within 24 hours.

• **Control** – 4 samples are used for biological assessment: 2 of them must use colorimetric assays and 2 for immunohistochemistry and histology.

After the tissue preparation experiment, the bioreactor must be in the incubator (Figure 3), which operates at 37°C (human temperature), being the duration of the experiment 48 hours.

Step 2: Histological Data

The Movat Pentachrome staining, the light microspopy imaging, the color deconvolution were methods used to do an efficient histological acquisition.

Determination of the relative thickness of Mitral Valve Anterior Leaflet (MVAL) layers:

Table 1 – MVAL layers (ventricularis, fibrosa, spongiosa and atrialis) thickness for 3 MVs¹⁰.

N=3	Average Thickness (μm)	Relative Thickness (%)
Ventricularis	53.1 ± 15.8	9.20 ± 2.70
Fibrosa	439 ± 77.0	76.0 ± 13.3
Spongiosa	41.0 ± 8.0	7.10 ± 1.39
Atrialis	44.4 ± 6.2	7.70 ± 1.07

Determination of the mass fractions of ECM components (collagen, elastin, PGs/GAGs) in layers of the anterior leaflet (Ventricularis, Fibrosa, Spongiosa, Atrialis):

Table 2 - ECM mass fraction for 3MVs, considering collagen, elastin and gags/pgs¹⁰.

N=3	Atrialis	Spongiosa	Fibrosa	Ventricularis
Collagen	0.208 ± 0.102	0.283 ± 0.078	0.662 ± 0.027	0.251 ± 0.076
Elastin	0.611 ± 0.144	0.226 ± 0.097	0.115 ± 0.55	0.612 ± 0.089
PGs/GAGs	0.180 ± 0.044	0.491 ± 0.111	0.223 ± 0.029	0.137 ± 0.018

Second Part: Computational Simulation

ComputCell3D (CC3D) is able to describe biological processes occurring at the cells, tissues and at the organism level using pixels¹¹. CC3D uses the Glazier-Graner-Hogeweg (GGH) approach which facilitates multiscale simulations by defining spatially-extended generalized cells, and represent clusters of cells¹¹.

Steps on CC3D:

Step 1

00

Step 1. Insertion of General Simulation Properties and upload of the PIFF file generated on CellDraw. Step 2. Regarding the PIFF file previously generated, the user must insert the necessary Cell Type Specifications.

Step 3. Insertion of Chemical Fields (diffusants). Step 4. Selection of Cell Properties and Behaviors. Step 5. Insertion of values for Secretion Plugin.

	Step 2			Step 3			
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Figure 2 - Mechanical tests, before the bioreactor treatment, using a planar biaxial testing device. (a) Observation of three strips (A,B,C) necessary for MV tissue mechanical characterization. (b) Tissue attachment.



Figure 3 – Experimental set-up: (a, b, c) Specifications of the bioreactor. (d) Controller of the bioreactor -Tissue Strip fixation system



Figure 4 - Histologic description of MVAL layers: Atrialis (A), Spongiosa (S), Fibrosa (F), Ventricularis (V), where black regions represent the elastin and the cell nuclei, the yellow region the collagen and the blue regions represent the Pgs and Gags.

Cell Properties and Behaviors			Secretion Plugin				
Cellular Behaviors	Constraints and Forces	Cellular Property Trackers	Field	CellType	Rate	On Contact With	Туре
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AdhesionFlex ContactLocalProduct	U VolumeLocalFlex	Moment Of Inertia	3 Collagen	darkBlueType	0.04		uniform
Compartments FocalPointPlasticity Elasticity	SurfaceFlex SurfaceLocalFlex <u>Ext.Force</u>	Cell Pixel Tracker					
ContactMultiCad (deprecated) Chemitasis	ExternalPotential	Aux. Modules					
Secretion Growth (Python)	Connectivity Clobal (2D/3D) Clobal (by cell id) Fast (2D square lattice)	BoxWatcher	Secretion Type	0	on contact) consta	ant concentration
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generation and analysis.

Results

Histological Data Analysis

Table 3 - Analysis of histologic data in *Image J*: average area and number of IC per layer¹⁰.

MV Layer	Average area	Number of IC per layer
Atrialis	0.00693 mm ² = 6930 μm ²	≈ 34
Spongiosa	0.01562 mm ² = 15620 μm ²	84
Fibrosa	$0.02615 \text{ mm}^2 = 26150 \ \mu \text{m}^2$	50
Ventricularis	$0.00238 \text{ mm}^2 = 2380 \ \mu \text{m}^2$	≈ 15

Table 4 –MV layers: respective percentages (from the analysis in *Image J*) and respective necessary calculations for environment development on CC3D.

	(%)	environment simulation (μm^2)	pixels number (= lcs)
Atrialis	7.69	22200	≈108
Spongiosa	6.84	20500	≈110
Fibrosa	76.92	219500	≈420
Ventricularis	8.55	26550	≈167

PIFF file

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3	552 redType 0 0 20 29 0 0	
4	553 redType 0 0 30 39 0 0	
5	554 redType 0 0 40 49 0 0	
6	555 redType 0 0 50 59 0 0	
7	556 redType 0 0 60 69 0 0	
8	954 darkYellowType 0 0 70 79 0 0	1
9	955 darkYellowType 0 0 80 89 0 0	1
10	956 darkYellowType 0 0 90 99 0 0	1
11	957 darkYellowType 0 0 100 109 0	
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13	959 darkYellowType 0 0 120 129 0	i
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18	964 darkVellowType 0 0 170 179 0	
19	965 darkVellovType 0 0 180 189 0	
20	966 darkVellovType 0 0 100 109 0	
21	967 darkVellovType 0 0 200 209 0	
22	968 darkVellovType 0 0 210 219 0	
23	969 darkVellowType 0 0 220 229 0	
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25	971 darkVellovType 0 0 240 249 0	
25	972 darkVellovType 0 0 250 259 0	
22	972 darkVellowType 0 0 250 259 0	
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36	982 darkVellovType 0 0 350 359 0	
37	983 darkVellovType 0 0 360 369 0	1
38	984 darkVellovType 0 0 370 379 0	
39	985 darkVellovType 0 0 380 389 0	
40	986 darkVellovType 0 0 390 399 0	
41	987 darkVellovType 0 0 400 409 0	
42	988 darkVellovType 0 0 410 419 0	
43	989 darkVellovType 0 0 420 429 0	
44	990 darkVellovType 0 0 430 439 0	
45	991 darkYellowType 0 0 440 449 0	
46	992 darkVellovType 0 0 450 459 0	
47	993 darkVellovType 0 0 460 469 0	
48	994 darkVellovTupe 0 0 470 475 0	
49	0 bluerupe 0 0 476 485 0 0	
50	1 blueTupe 0 0 486 495 0 0	
51	2 blueTupe 0 0 496 505 0 0	
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52	A blueType 0 0 506 515 0 0	
53	250 lightCrowTupe 0 0 510 529 0	0
55	250 lightGrauTupe 0 0 529 528 0	0
55	251 lightGrauTupe 0 0 529 538 0	0
26	252 lightGrayType 0 0 539 548 0	U



Conclusions

The final goal of this project is the development of an ABM model to incorporate it into an organ-level model, to be used as a predictive tool for different surgical repair scenarios (related to MV) regurgitation). CC3D was evaluated and it can be a good choice to develop an ABM model, because it is extremely specific for biological processes description. CC3D is an intuitive tool, allowing quick results when very specific cell parameters (for example, dimensions, behavior), according the user preferences, are given. In a few words, CC3D has appellative characteristics: it is simple, physics based, uses an energy formalism to describe cell properties, cell-cell interactions, as well as cell's behaviors. Regarding the next steps, for example: (i) to extend this work to 3D dimensions; (ii) there are yet needed more experiments, namely, an understanding about the secretion rates of GAGs/PGs, elastin and collagen.

References

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