# Mitral Valve Interstitial Cells and Respective Mechanobiological Response to Stress

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***Advanced Computing Research***

# 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 MVR[5]. 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 ventricle[5].

Since the late 1970s and early 1980s have been developed different techniques dedicated to MVR for **surgical repair** with subsequent progressive improvements[6].

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

## 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].

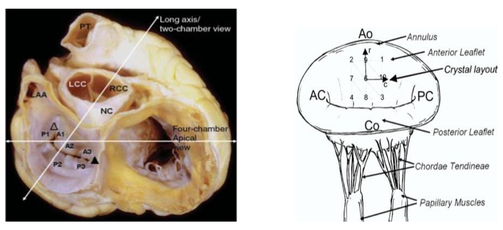



**Figure 1** - Components of Mitral Valve (MV). Adult heart specimen picture showing the mitral valve structure, where: Δ is the anterolateral commissure, the filled Δ is the posteromedial commissure, A1-A3 are divisions of the aortic mitral leaflet, P1-P3 are divisions of the mural leaflet of the mitral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

## 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 ***Computcell3D*** 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 testing[9]. 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:

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.
• **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 37ºC (human temperature), being the duration of the experiment 48 hours.

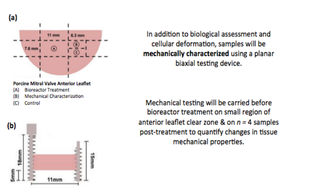



**Figure 2** - Mechanical tests, before the bioreactor treatment, using a planar biaxial testing device. (a) Observation of three strips (A,B,C) necessary for MV tissue mechanical characterization. (b) Tissue attachment.

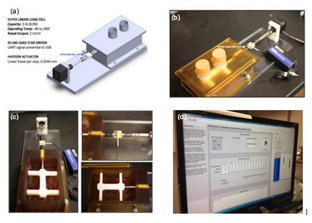



**Figure 3 –** Experimental set-up: (a, b, c) Specifications of the bioreactor. (d) Controller of the bioreactor - *Tissue Strip fixation system*

### 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 MVs[10].

| N=3 | Average Thickness ($\mu m$) | 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/pgs[10].

| 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 |

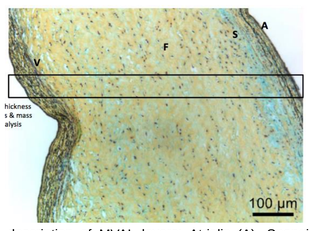


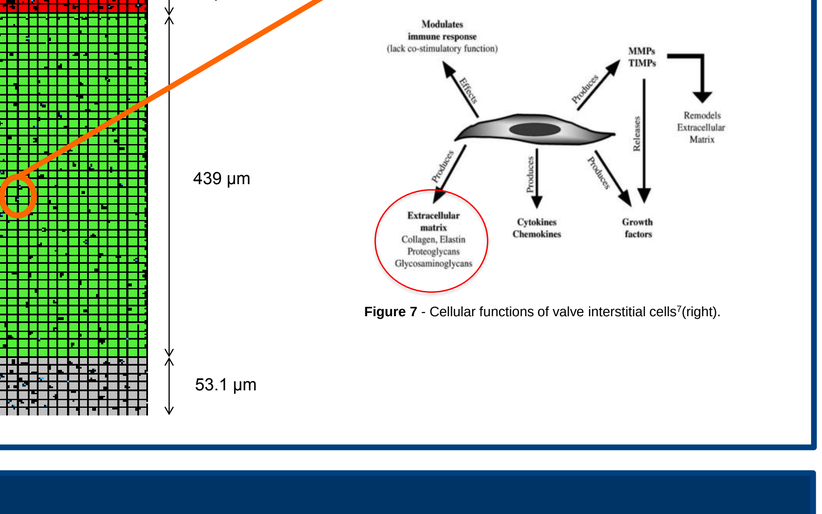

**Figure 4** - Histologic description of MVAL layers: Atrialis (A), Spongiosa (S), Fibrosa (F), Ventricularis (V), where black regions represent the elastin and the cell nuclei, the yellow region the collagen and the blue regions represent the Pgs and Gags.

## Second Part: Computational Simulation

***ComputCell3D (CC3D)*** is able to describe biological processes occurring at the cells, tissues and at the organism level using pixels[11]. CC3D uses the ***Glazier-Graner-Hogeweg (GGH)*** approach which facilitates multiscale simulations by defining spatially-extended generalized cells, and represent clusters of cells[11].

### Steps on CC3D:

**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.**




***Twedit ++: Xml* code and Python Code generation and analysis.**

# Results

## Histological Data Analysis

**Table 3** - Analysis of histologic data in *Image J*: average area and number of IC per layer[10].

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

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

| MV Layer | Percentages (%) | Total area for environment simulation ($\mu m^2$) | Necessary pixels number (= Ics) |
|---|---|---|---|
| **Atrialis** | 7.69 | 22200 | ≈108 |
| **Spongiosa** | 6.84 | 20500 | ≈110 |
| **Fibrosa** | 76.92 | 219500 | ≈420 |
| **Ventricularis** | 8.55 | 26550 | ≈167 |

## PIFF file

**obtained from *CellDraw***

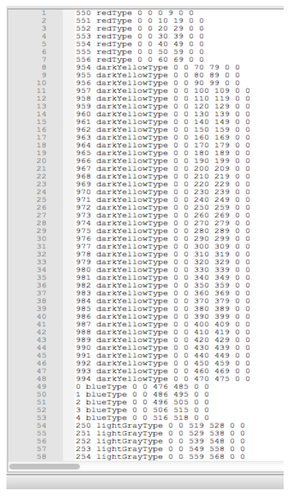


**Figure 5** – PIFF file.

## *ComputCell 3D*

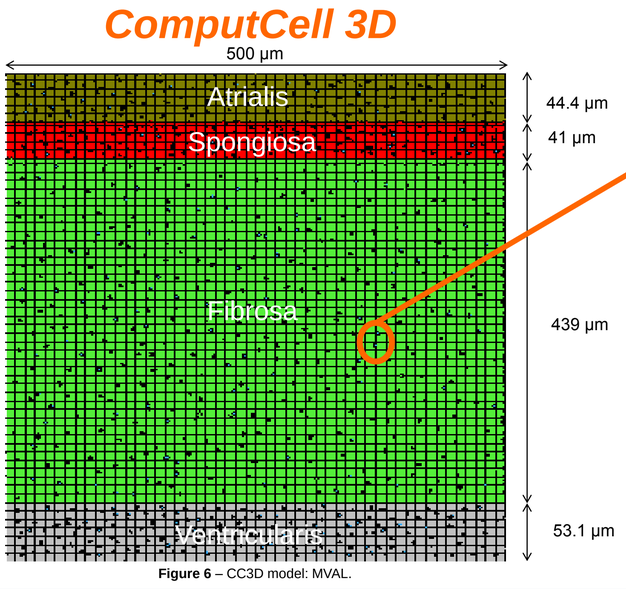



**Figure 6** – CC3D model: MVAL.

## Interstitial Cells




**Figure 7** - Cellular functions of valve interstitial cells[7](right).

# 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.

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