



Mitral Valve Organ Culture

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Under direction of Dr. K. Jane Grande-Allen



Overview

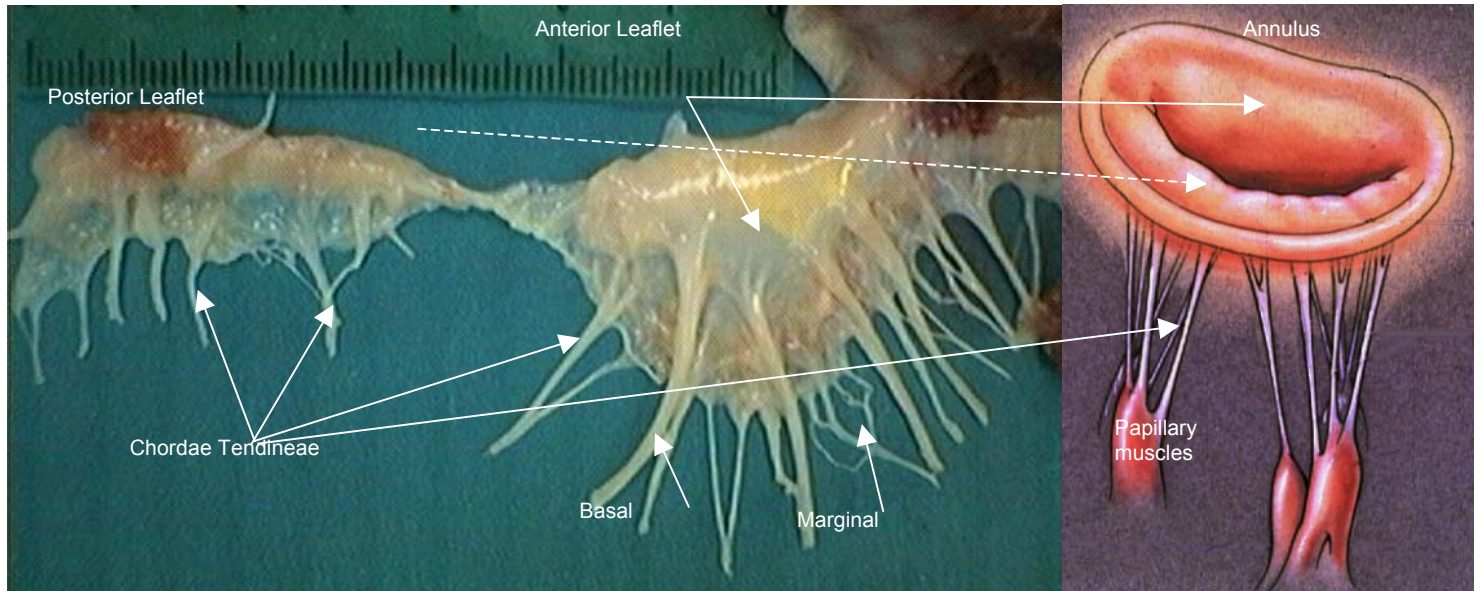
- Research Goal
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- Variables
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- Timeline
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Research Goal

- Establish an organ culture system for mitral valve tissue which will provide useful information on the physiological responses to various stimuli.

The Mitral Valve



Normal mitral valve (left) cut open to show ventricular aspect and (right) closed position (right figure courtesy of Cochran, Kunzelman). Basal chordae insert underneath the leaflet and marginal chordae insert at the edge of the leaflet.



Background

- Currently limited knowledge is available of the *in vivo* responses of the cells within mitral valve tissue.
- While Dr. Grande-Allen's lab is currently growing Mitral Valve Cells in culture
- A mono-layer of cells is not representative of the actual valve that exists in the human body.



Background

- Research conducted by Dr. W.M. Lester¹, in the late 1980's suggests that indeed an organ culture system is possible, while still leaving much to be determined.
- Dr. Lester studied the *in vitro* repair and responses (trauma etc.) of the mitral valve in organ culture, but not necessarily metabolism or cell viability



Background

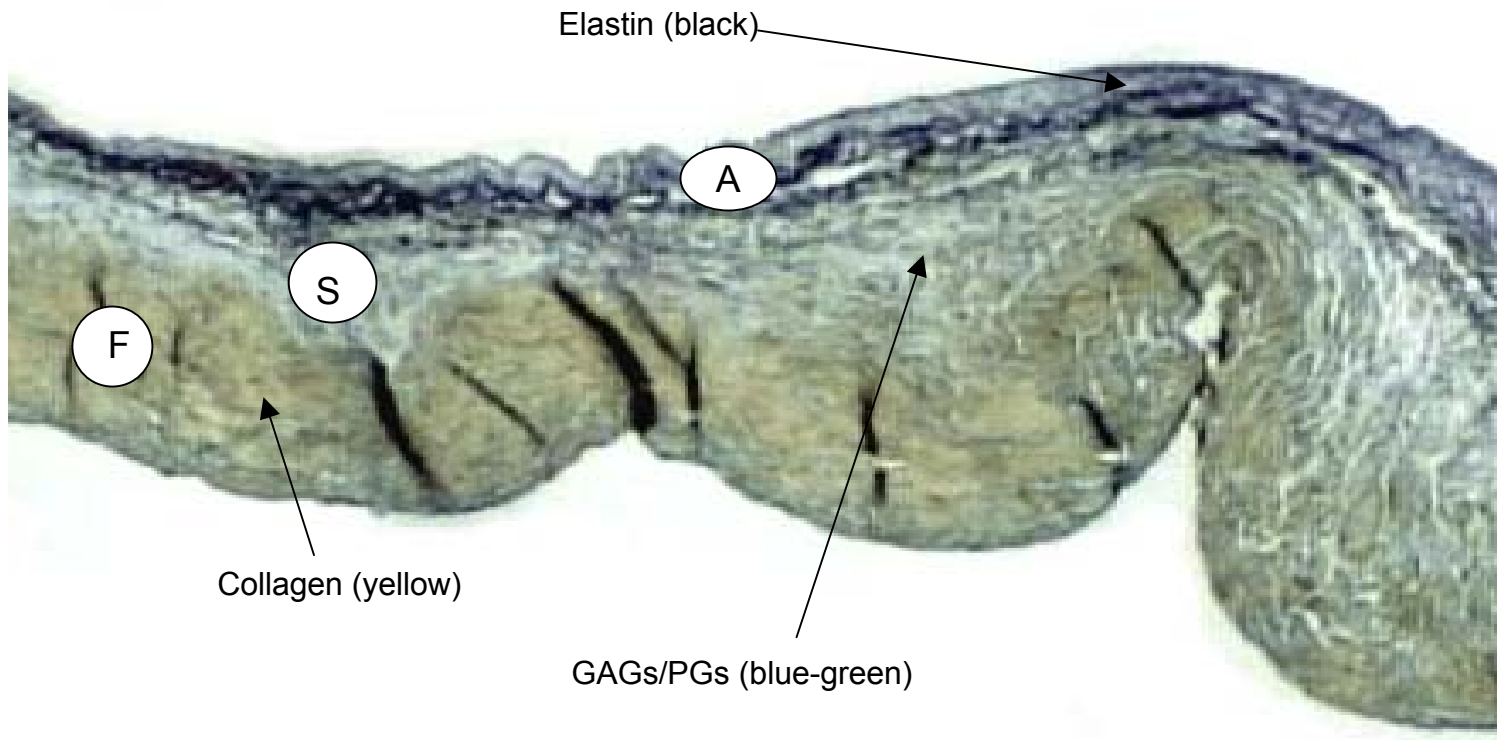
- There also appears to be information on corneal explant organ cultures that might serve as a basis for our research.
- Hippocampal slices have also been cultured and imaged using a process similar to what we intend to use



Defining Viability

- At the microscopic level, we can assess amount of living and non-living cells
- This has been done on valve tissue for years
 - Using flow cytometry
 - This fails to provide a location of the viable cells, but rather simply if they are alive
- This can also be done with Trypan Blue stain and a hemocytometer
- It is imperative that we know where the viable cells are in the tissue to better improve our organ culture. Also, the tissue is NOT homogenous, there are three distinct layers

The Structure of the Mitral Valve



Movat stain of the posterior leaflet. F = fibrosa, S = spongiosa, A = atrialis.



Objective

- Phase I: Establish viability throughout the mitral valve organ cultures
 - Specifically, to have an acceptable level of viability after a three week culture period
 - To optimize culture medium
 - To have curbed the swelling in my cultures to closer represent the physiological valve state



Objective

- Phase II: Determine the cell/tissue phenotypes expressed in organ culture
 - Immunohistochemistry for proliferating cell nuclear antigen (PCNA) (desmin, actin, vimentin, and prolyl-4-hydroxylase)
 - Tells us if tissue maintains normal phenotype as well as viability in culture
 - Determine how the ECM is maintained in culture
 - Measure the responses to various drugs
 - Preliminary attempts at cardiovascular disease models



Approach for Phase I

- The method I have chosen to determine viability is confocal microscopy
 - This allows to look within the layers and establish viability
 - We can see where the cells are alive/dead in relation to the rest of the tissue
- Why confocal?
 - Most surface cells will be dead...



Approach for Phase I

- In order to use confocal microscopy, a fluorescent stain was tailored for our specific needs.
- I needed a stain that would work on live tissue, but would not wash off during fixation.
- Also, one that is able to penetrate the dense collagenous matrix



Methods for Stain

- Trypan Blue- Membrane stain
- XTT- Metabolic activity marker
- JC-1-Mitochondria membrane potential marker
- TUNEL-Indicated broken DNA strands
- Oversimplified----not specific enough
- Dense collagen is a problem
- Not correlated with viability
- Too complex, expensive, but comprehensive

Chosen methods

- Propidium Iodide and Fluorescein Diacetate combination
 - PI marks the dead cell nuclei (RED)
 - FDA marks the live cell membranes (Green)
- Molecular Probes Live/dead kit
 - Uses Ethidium Bromide to stain dead and Calcein AM to stain live cells

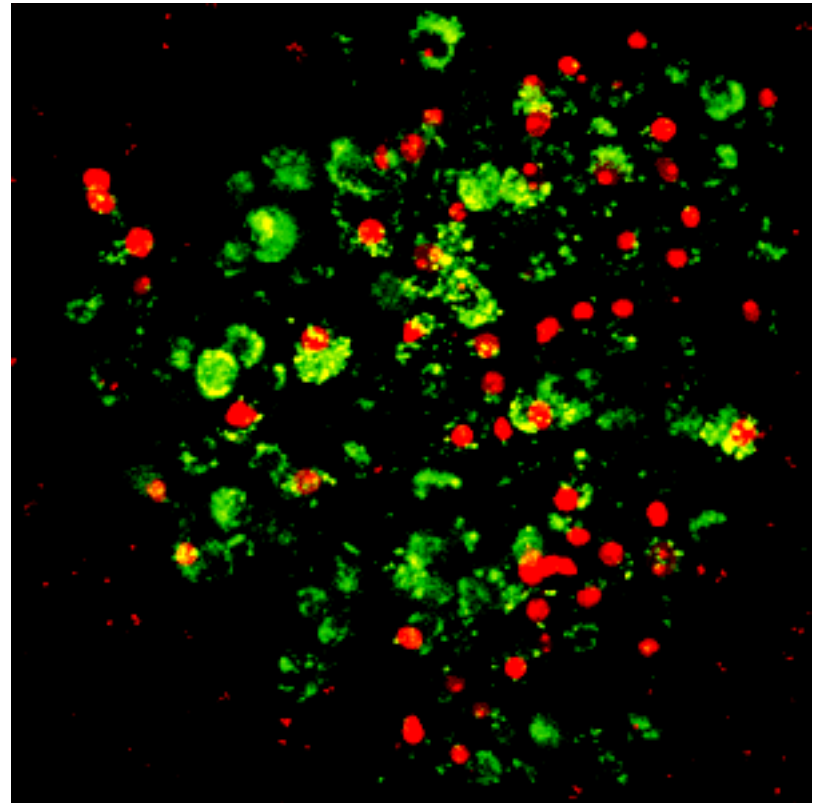
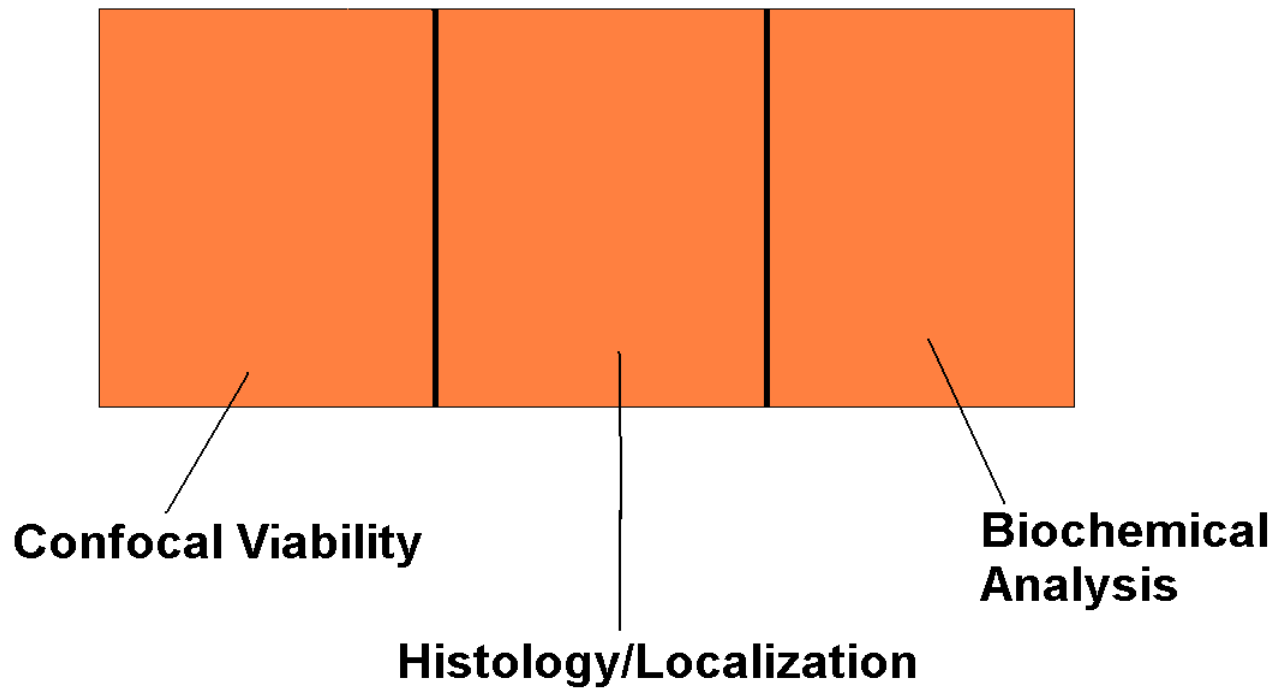


Diagram of Mitral Valve Organ Culture Preparation





Methods

- Culture
- Slice as thin as possible
- Stain
- Fix
- Prepare on slides—
 - Difficult*
- Image

- Culture
- Slice Pseudo-Thin*
- Stain
- Freeze → Cryostat
- Prepare on slides
- Image



Mounting the Samples

- Method I

- This requires an amount of vacuum grease and a wet mount with DAPI
- This is a time consuming method that is VERY messy

- Method II

- Here the sections are thin enough to be able to mount with a normal cover slip



Variables

- Swelling
- Time in culture
- Optimizing the dye concentrations
- Contamination
- Images and Quantification
- Negative Control for auto fluorescence
- Controlling the medium



Progress

- Dissection/anatomy training
- Research different methods available for viability
- Swelling study...
- Cell culture fine tuning
 - Making medium
 - Freezing/thawing cells...etc
 - Passaging
- Imaging



Progress

- Splitting into thirds
- How to normalize data
 - Biochemical DNA count
 - Field of view size
 - Total DAPI cells versus the stained cells
- How to localize? Histology? Movat?



Progress for Phase I

- Thus far, I have made great progress with the confocal microscopy. With the assistance of Judy Drazba, and several hours of confocal training, I can now image my own samples.
- I am also exploring the use of de-swelling medium for the cultures.
 - Plasma-Lyte or a Dextran solution?

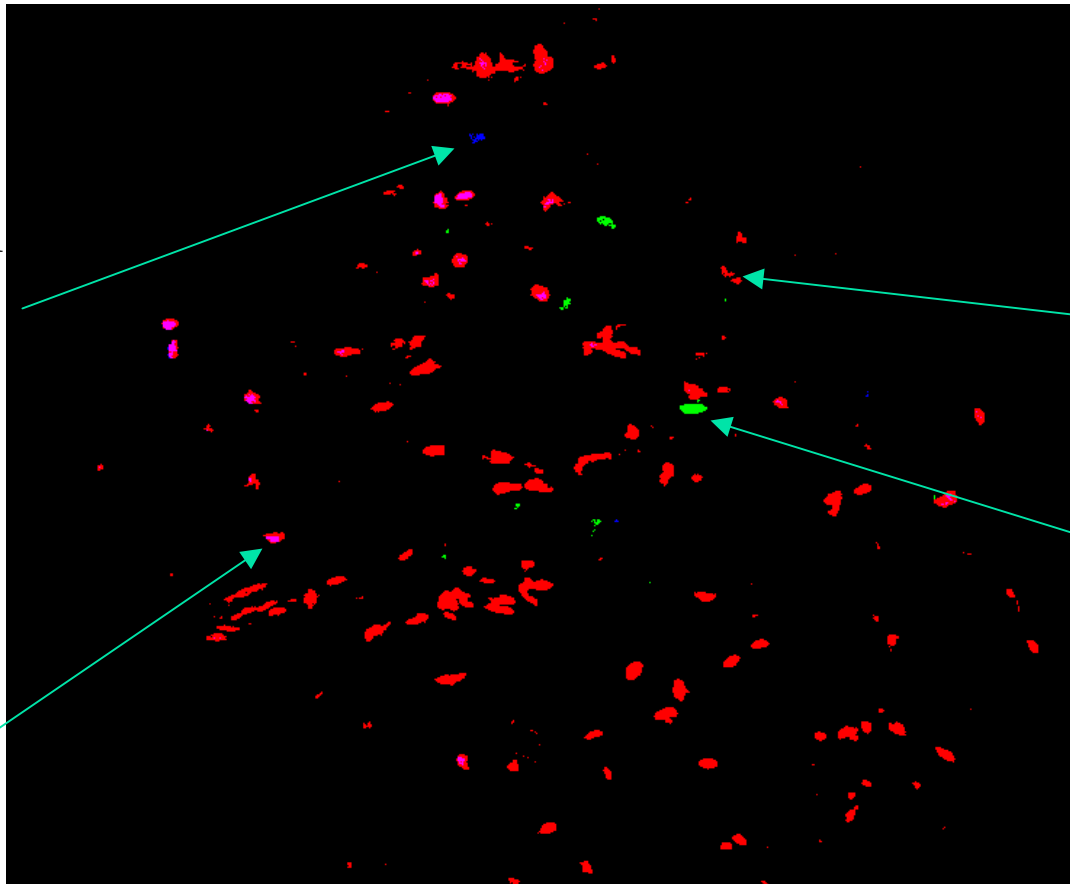
Confocal Images

DAPI Stain
Cell Nuclei

PI Stain
Dead Cells

FDA Stain
Live Cells

DAPI/PI
Overlay





Phase II Design

- Once the tissue is in culture and is deemed to be viable, then the Phase II process can begin
- Most of the tests conducted in phase II of this project can take place while the tissue is in culture



Applications

- Through the establishment of viability in the mitral valve cultures, we will be able to conduct several tests on the tissue trauma, proliferation and cell cycle.
- We will also be able to test the *in vivo* response to agents like neurohormones, cytokines, and drugs.



Timeline

- Ideally, Phase I will be complete by mid September, and Phase II will begin shortly thereafter.
- By the October mid-term presentation, I would like to have definitive data on the viability phase of this project, and also have a start on the phenotype of the tissues.



Conclusion

- We see that this project can yield valuable results for many applications
 - The establishment of viability will allow scientists to study the in vivo responses of tissue while in culture
 - The studies of the phenotypic responses of this tissue in culture will allow us to closely study the actual responses of the body without having to study these valves through an invasive procedure.
 - Also, Dr. Vesely is interested in other tissue engineering applications based on this same project



Acknowledgments

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Questions?
