Undergraduate Research Symposium
ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT
PROJECT 36

## Shweta Sapkota

**Caldwell University, Class of 2020**
Major: **Biological Science**

Faculty Advisor: **William Velhagen, Ph.D.,** Professor and Chair
Department of Natural Sciences

## *In Vitro Creation of Mouse Cardiac Tissue Using Mouse Embryonic Stem Cells*

A Report from American Heart Association 2017, says, every 40 seconds, someone in the United States has a heart attack. A heart attack is the irreversible death of cardiac muscle cells (cardiomyocytes) due to the lack of oxygen supply for a long time. The major problem of myocardial infarction in heart regeneration is that the dead cardiomyocytes are replaced by non-contracting fibrous scar tissue rather than new cardiac muscle cells. In the long term, this weakens the organ's ability to contract normally and increases the risk of a further heart attack. Currently, the only option for a severely failing heart is organ transplantation and recently developed heart-assist devices. The heart cannot regenerate the cardiomyocytes by itself, however, human stem cells are known to produce multiple cell lineages in the body and scientists hope that they will be able to generate new cardiomyocytes. Similarly, the goal of this research project was to apply a mouse stem cell culture technique to regenerate heart tissue which can be used for further research studies involving humans. Cell growth medium was prepared using 500ml of mouse ES cell Basal Medium supplemented with 10% FBS, 2.5µg/ml of the antibiotic (Amphotericin B) and 50µg/ml of antifungal (Gentamicin). The mouse cardiac embryonic stem cells from ATCC were thawed at 37oC and were transferred to sterile 15ml tube containing 10ml warm growth medium. The conical tubes were centrifuged at 1100 rpm for 2 minutes at room temperature. The supernatant was discarded, and the pelleted cells were resuspended into 2ml of ES growth medium. The recovered cells were placed in a tissue culture plate containing 10ml of ES growth medium. Several gelatin-coated plates were prepared using 200-300µl of type B 2% gelatin solution, in 25cm2 culture plates to subculture the cells for growth. The cell suspension was transferred into gelatin-coated plates for further growth. The number of live and dead cells were counted using a hemocytometer and trypan blue dye in a 60% confluency culture. 92% of the cells were alive while 8% were dead which represents a healthy culture. This proves that we were able to achieve our goal. The adapted successful protocol may help our future students to discover new ideas in related fields. These cultured cardiac tissues may be used in the biochemical and molecular investigation of cardiac physiology and their effects on the biomechanical properties of spontaneously beating cardiomyocytes.

**Independent Colleges Undergraduate Research Award Recipient 2020**

Acknowledgments: ***Dr. Darryl Aucoin and Dr. Agnes Berki***

# In-Vitro Creation Of Cardiac Tissue Using Neonatal Mouse Embryonic Stem Cells

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## INTRODUCTION

### Facts

- American Heart Association 2017, says, every 40 seconds an individual in the United States has a heart attack.

*Figure 1: Human Heart Model*

### Major Problems

- Heart cannot regenerate the dead cardiomyocytes by itself

### Current Prevention Methods

- Organ transplantation
- Recently developed heart assisting devices

### Research on Additional Prevention Measures

- Create artificial heart using stem cells
- Culture of pluripotent stem cells to generate new cardiomyocyte cells
- Replace the scar tissue with new tissues

## PURPOSE

- To use different protocols of mouse stem cell culture technique to regenerate heart tissue
- To adapt the culture technique to our lab
- To provide a base for further research studies involving human cardiac stem cells

## MATERIALS

- Mouse embryonic cardiac cells
- Mouse ES cell Basal Medium
- 10% FBS
- 2.5 µg/ml Amphotericin B
- 50 µg/ml of antifungal (Gentamicin)
- type B 2% gelatin solution
- Trypan Blue Dye
- Hemocytometer
- Sterile Hood
- Centrifuge machine
- $CO_2$ water jacketed incubator


Figure 2. Hemocytometer

Figure 3. Centrifuge Machine

## RESULTS

When Cells Arrived

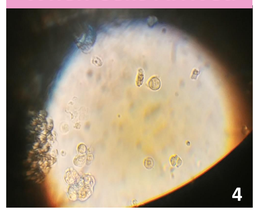


Figure 4

Cells Splitting

Figure 5

1st Generation Cells

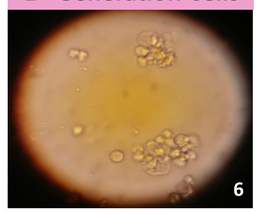


Figure 6

2nd Generation Cells

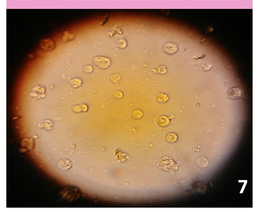


Figure 7

Live Cells

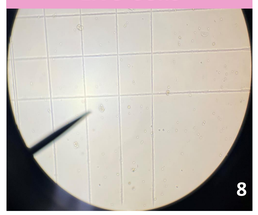


Figure 8

Live Cells Cluster

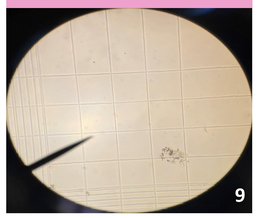


Figure 9

Tissue Formation

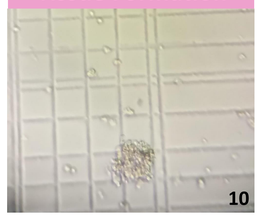


Figure 10

Dyed Dead Cells

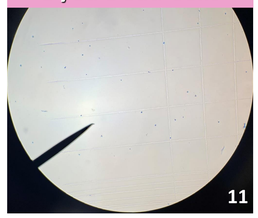


Figure 11

**Figure 4:** Microscopic image of the stem cells when arrived (before thawing). **Figure 5:** Medium used to grow cells and cells being split in gelatin coated flasks, **Figure 6:** 1st generation of cells growing (sub culture from original cells**), Figure 7:** 2nd generation of cells growing (sub culture from 1st generation), **Figure 8:** Living cells growing in 0.1µl of cell suspension viewed in hemocytometer grid, **Figure 9:** The living cells are clustering beginning to form a tissue, **Figure 10:** Magnified image of tissue formation (Figure 9), **Figure 11:** The dead cells dyed using trypan blue solution and being viewed in hemocytometer grid.

### Hemocytometer Readings

| Live Cells | | |
|---|---|---|
| 198 | | 91 |
| | 157 | |
| 216 | | 79 |

| Dead Cells | | |
|---|---|---|
| 21 | | 15 |
| | 13 | |
| 6 | | 17 |

### Cell Viability

**Total Cells = Avg of Live Cells + Avg of Dead Cells**
**= 148 + 14 = 162**

**% Viability =** $100\% - \left(\frac{14}{162} \times 100\%\right)$

$= 91.36\%$

## METHODS

**Cell-Splitting and Medium Change and Gelatin-coating** were performed under a sterile hood

| | |
|---|---|
| • **Preparation of Cell Growth Medium** | 500ml of mouse ES cell Basal Medium supplemented with 10% FBS, 2.5µg/ml Amphotericin B and 50µg/ml Gentamicin |
| • **Thawing and Centrifuging** | 2000rpm for 2minutes |
| • **Gelatin coating** | 200-300µl of type B 2% gelatin solution, in 25 $cm^2$ culture plates |
| •**Cell Counting** | Using trypan blue dye and Hemocytometer |
| • **Incubation** | place plates in a 37°C incubator for at least 48 hours |

Figure 4. Sterile hood

## CONCLUSIONS

- 91.36% of cultured cells were viable
- Cardiac cells can be successfully cultured in our Biology Laboratory in Caldwell University

## FURTHER RESEARCH

- Discover new ideas to attach the cells to the surface of the culture
- Investigate biochemical and molecular properties and cardiac physiology of the tissue and their effects on spontaneously beating cardiomyocytes.
- Apply similar protocols using human cell lines and create usable beating heart tissues

## REFERENCES

**Mouse Embryonic Stem Cells Culturing Protocols by Coriell Institute for Medical Research**
https://www.coriell.org/0/Sections/Collections/NIA/nia_protocol_mesc.pdf

**ATCC**
https://www.atcc.org/~/media/7E031EF950594BC3B85A411AE1DC9684.ashx

## ACKNOWLEDGEMENTS

**Independent College Fund of New Jersey**

**Sagar Lamichhane**

**Darryl Aucoin, Ph.D.**

**Caldwell University**

# INTRODUCTION

**Problem**

Heart cannot regenerate the dead  cardiomyocytes by itself

**Current Solution**

Organ transplantation or heart assisting devices

**Ongoing Research**

- Culture of pluripotent stem cells to generate new cardiomyocyte cells
  - Replace the scar tissue with new tissues

**My Research using Mouse Stem Cells**

Cell Growth Medium was prepared

The cells from ATCC were thawed and resuspended in the medium

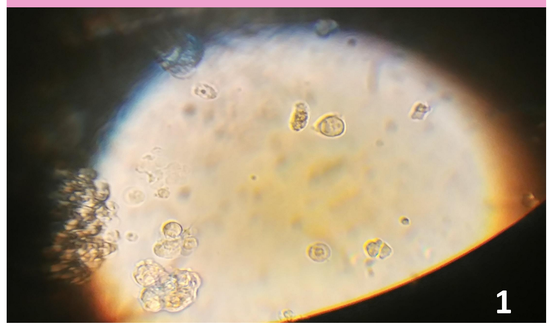
Cell suspension was transferred into gelatin coated cell culture plates

Number of live and dead cells were counted using trypan blue dye and hemocytometer

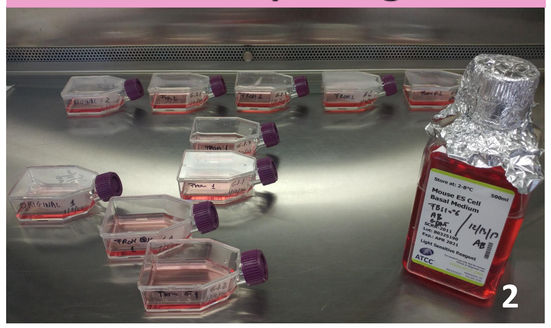
92% viability of cells

# RESULTS

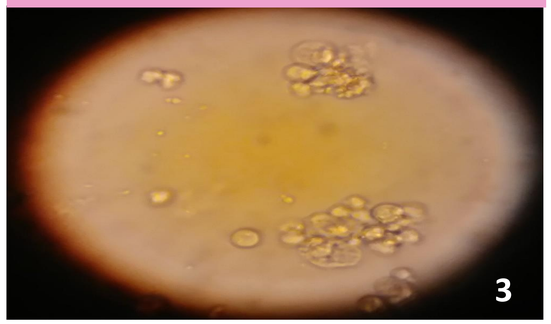
## When Cells Arrived



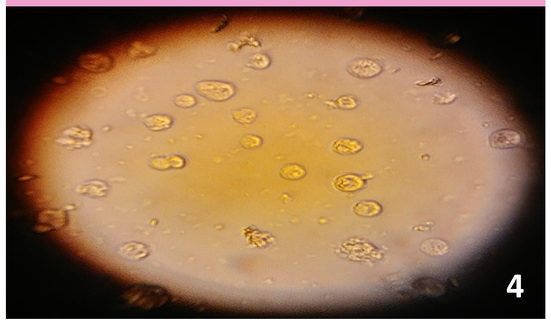

## Cells Splitting



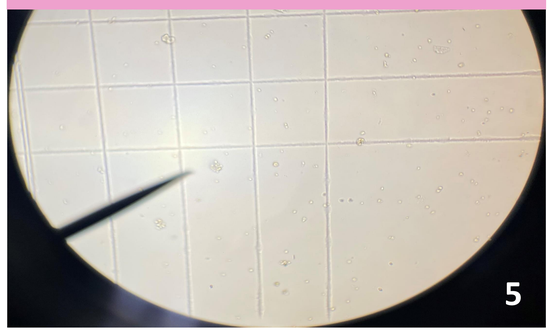

## 1st Generation Cells



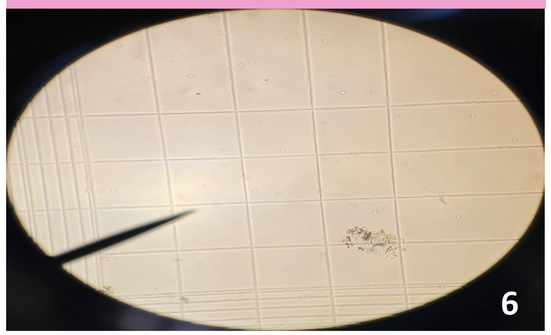

## 2nd Generation Cells



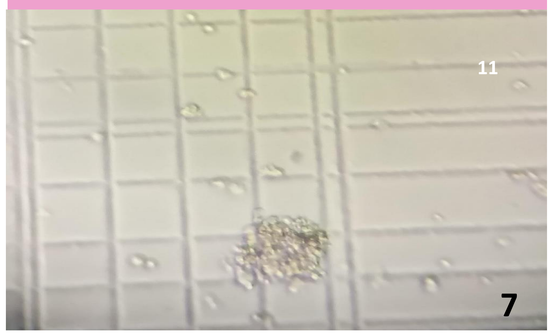

## Live Cells



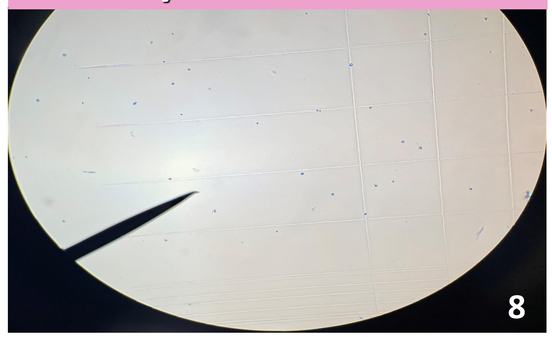

## Live Cells Cluster




## Tissue Formation




## Dyed Dead Cells

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