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Investigate the Effects of Yoda1 on Piezo1 Channels of Mouse Aorta through Myograph Techniques

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Ph.D. Rotation Report

Investigate the Effects of Yoda1 on Piezo1 Channels of Mouse Aorta through Myograph Techniques

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1 Introduction

Cardiovascular diseases, encompassing aortic abnormalities such as aortic aneurysms and stenosis, pose significant threats to the structural integrity and mechanical functionality of the aorta.[1] Conditions like supravalvular aortic stenosis (SVAS), occurring in approximately one out of every 20,000 new live births, result in focal stenosis of the ascending aorta.[2] While there are existing solutions to treat these cardiovascular diseases, they still have significant mortality, and early diagnosis is critical to avoid re-operation, especially in people with early diagnosis.[3,4] Therefore, a better understanding of the mechanical properties of the ascending aorta as well as the mechanisms at the molecule level may lead to novel therapeutic strategies that could reduce the need for multiple surgeries.

The myograph technique has become an important technique in the assessment of the mechanical properties of isolated vascular tissues.[5] It can be utilized to accurately measure the force in aortic rings in response to different molecular conditions. Understanding these force dynamics holds significant implications and offers precise insights into cardiovascular research, particularly in diseases affecting the aortic structure and function.

This project aims to investigate how Yoda1 would affect the force responses in mouse ascending aorta through the use of the myograph technique, indicating the role of Piezo1 activation in vascular mechanics.

2 Background

2.1 Piezo1 Channel and Yoda1

Piezo1 channels are expressed in endothelial cells lining the blood vessels.[6] They can sense shear stress by blood flow and induce the release of vasodilators. Mechanical signals sensed by Piezo1 can influence the proliferation and migration of endothelial cells, contributing to the formation of new vascular structures.

Yoda1 is a chemical activator of the Piezo1 channel.[7] Research into Yoda1 and Piezo channels reveals their critical role in mechanotransduction and their potential as therapeutic targets in cardiovascular diseases.

2.2 Myograph Technique

The myograph technique refers to the experimental tool for quantifying the isometric tension or force within muscle tissues or vascular rings.[5] It involves careful preparation of the sample, a cautious mounting procedure to fix the tissue in the myograph chamber, and bathing it in a physiological solution. The steps allow the myograph to stably control the dimensions so that it can accurately measure the contraction forces through time.

3 Experimental Design

3.1 Objectives

The major objective of this research project is to investigate how different concentrations of Yoda1 would affect the force responses in ascending aortic rings. To achieve this major objective, the following mini-objectives should be achieved:

(1) To extract the ascending aorta through mouse dissection and to cut it into rings;

(2) To successfully mount the aortic rings on the myograph; and

(3) To learn to use the myograph technique for stable, accurate, and normalized force measurements in aortic rings.

3.2 Sample Preparation

The Elnfl mice were used for this research. To prepare ascending aortic rings, euthanasia was performed and the aorta was carefully extracted from mice. Then, the ascending aorta was cleaned to get rid of fatty tissue and just kept the interested vessel. After that, the aorta was sectioned into ring segments with a length dimension ranging from 0.8mm to 1.2mm so that it could fit into the myograph.

3.3 Mounting Methods

Figure 1 shows the DMT 360CW Confocal Wire Myograph System, which was used for this research. The user guide and user manual (Volume 2)[8,10] show the details of the device setup and mounting procedures.

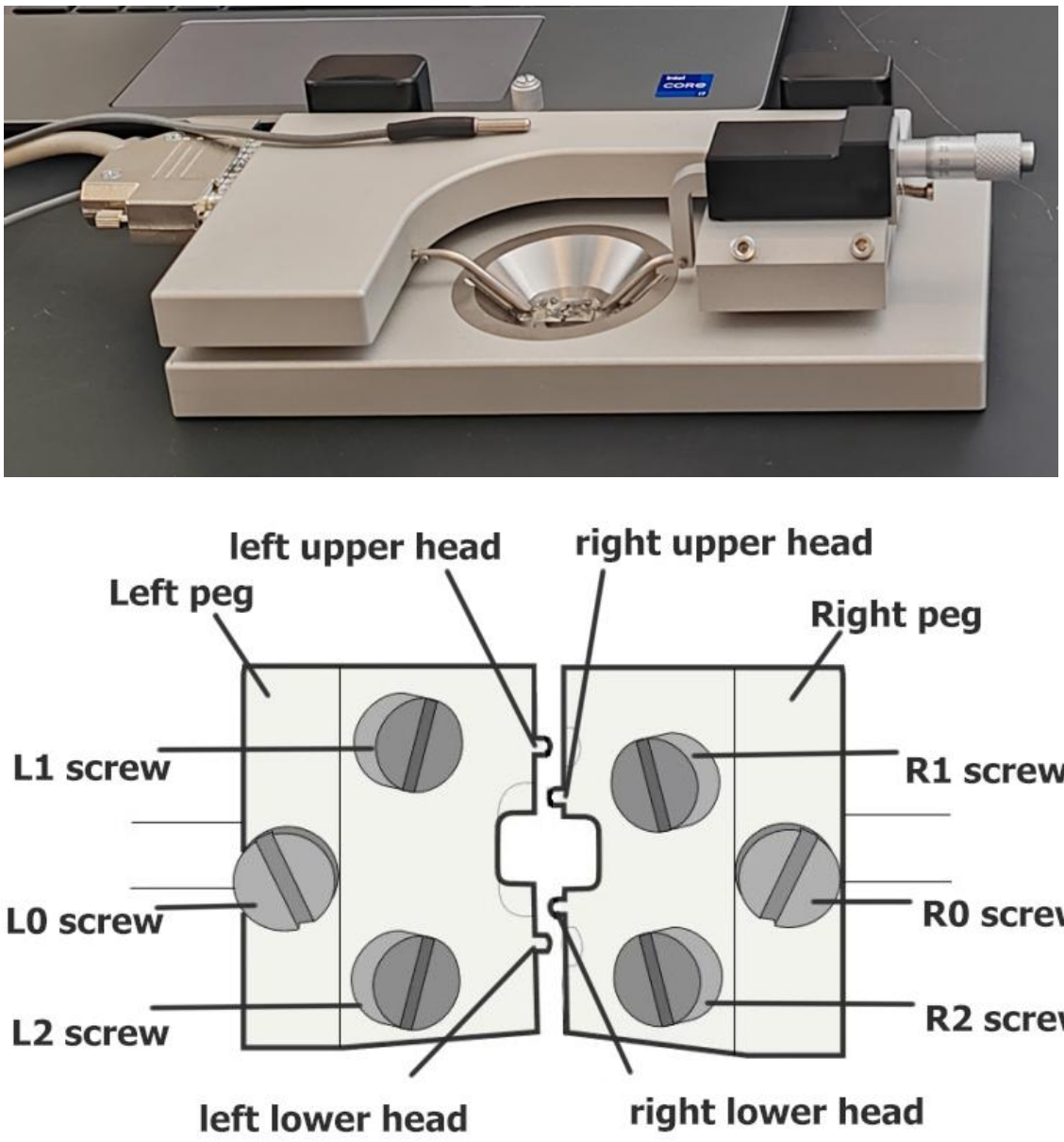

Figure 1. *The DMT 360CW Confocal Wire Myograph System. The upper part of the figure shows the actual device. The bottom part of the figure shows the detailed parts inside the chamber, including 6 screws, 4 heads, as well as the left peg and the right peg.*

While the user guide and user manual provide detailed mounting procedures, the actual steps used in practice are slightly different:

[1] Before the mounting process, make sure both the left and right pegs are flat. If not,

screw L0 and screw R0 can be adjusted to make them flat. Besides, it is always good to add some buffer such as PBS to prepare to mount the tissue.

[2] After adding some PBS into the chamber, hold a wire with the forceps to place it near the left upper head. Then, bend the wire around the left upper head, and fix and tighten it with the L1 screw. Remember to keep long enough wire for the left lower head and the L2 screw. Excess wire above the L1 screw can be cut off.

[3] Then, transfer the aortic ring into the chamber. Use the forceps to place the wire passing through the aortic ring, and place the ring between the left heads.

[4] After setting the ring into the right place, place the wire under the left head, and then hold the wire close to the L2 screw while keeping the wire under the heads.

[5] Fix the wire around the L2 screw while keeping the wire tighten. After tightening the L2 screw, excess wire can be cut off. Now the left peg is successfully mounted.

[6] To mount the right peg, the procedures are similar but it should be really careful not to break the aortic ring. Besides, the wire used to mount the right peg should be able to pass the ring without physical contact with the left wire. It is either fine to pass the wire first, followed by fixing the R1 and R2 screws while keeping the wire under the right heads, or to fix the R1 screw first, followed by passing the wire through the ring and fixing the R2 screw while keeping the wire under the right heads.

[7] After successful mounting, remember to measure the length of the ring. Furthermore, it is always good to replace the fluids in the chamber with the buffer that will be used in the experiment in the correct amount after mounting. This is because

replacing fluids after the normalization step may cause a change of situation that needs another normalization process.

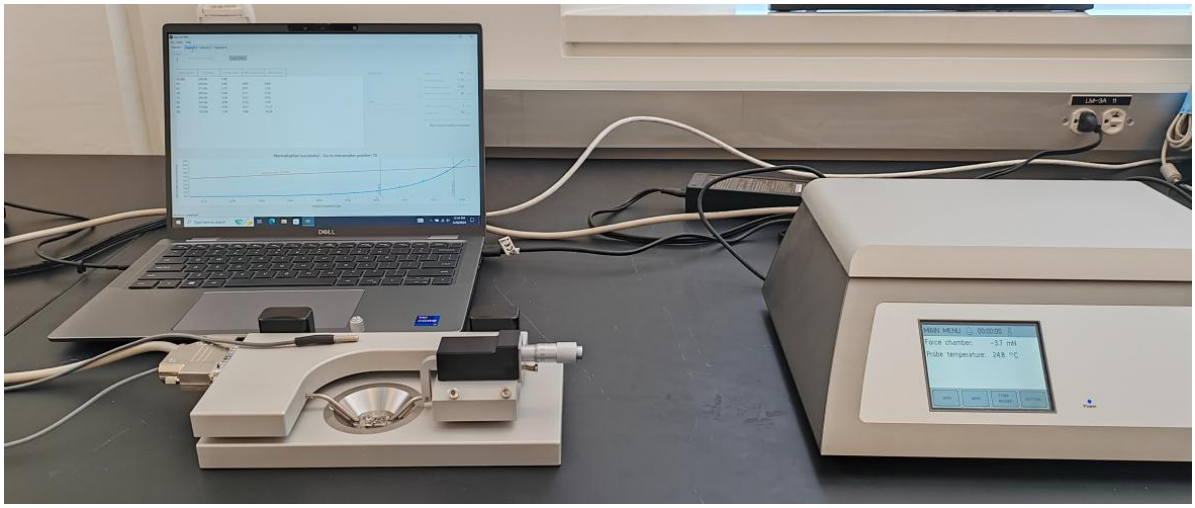

Figure 2. *The myograph connected to the interface (right) and the laptop.*

[8] When setting up everything about the myograph, connect the myograph to the wire interface and the laptop as shown in **Figure 2**. The wire interface is able to show readings of force and temperature. The force value can be adjusted by the larger screw on the myograph, and the temperature is always set to 25°C in the lab.

3.4 Normalization

In order to normalize the internal circumference of the mounted ring in the myograph to be the same as the condition in a given transmural pressure *in vivo* (in this case, 100 mmHg = 13.3 kPa), the MyoNORM platform is used to simply the normalization procedures. The user manuals (Volume 1 and Volume 2)[9,10] show the details of the

MyoNORM Normalization.

The MyoNORM platform first asks the length of the ring. After filling up all the parameters, enter the stretch vs force data points while gradually screwing up the force readings; The MyoNORM platform will find a place of normalization after enough data points. Then, set the large screw of the myograph to the told position as suggested by the MyoNORM platform. The position is always a little bit of stretching the ring.

3.5 Experimental Design

To investigate the effects of different concentrations of Yoda1, several different amounts of PBS and 1mM Yoda1 stock solutions made in dimethyl sulfoxide (DMSO) were prepared to make different concentrations as shown in **Table 1**.

Table 1. Amount of PBS and Yoda1 Stock Solution for Different Concentrations

Yoda1 Concentration	Yoda1 Stock Volume	PBS Volume	Solution Volume
10μM	25μL	2475μL	2500μL
20μM	50μL	2450μL	2500μL
50μM	125μL	2375μL	2500μL
250μM	625μL	1875μL	2500μL

In each experiment, the corresponding amount of PBS shown in **Table 1** was added before the normalization procedure. After that, the force recording was turned on with the

normalized position placed throughout the entire recording period.

After the recording was started, the normalized position was recorded without any addition for 60 seconds; the first dose of Yoda1 stock solution was added right at the 0s and this situation was kept for 600 seconds. In order to investigate the “refractory period effect” of the Piezo1 channels, the second dose of the same amount as the first dose of Yoda1 stock solution was added at 600s, and this situation was kept for another 600 seconds before ending the recording. As a result, the concentration of the chamber solution was doubled after adding the second dose. **Table 2** shows the amount of doses in each of the two-dose experiments in 10μM, 20μM, and 50μM Yoda1 conditions; An illustration of the timeline of this two-dose experiment is also shown in **Figure 3** on the next page.

Table 2. Amount of Doses in Different Two-Dose Experiments

Experiment Type	PBS Volume at -60s	Yoda1 Stock Volume Added		Total Volume after Dose 2
		Dose 1 at 0s	Dose 2 at 600s	
10μM Yoda1	2475μL	25μL	25μL	2525μL
20μM Yoda1	2450μL	50μL	50μL	2550μL
50μM Yoda1	2375μL	125μL	125μL	2625μL

The control groups were using the same amount of volume of DMSO or PBS to replace the Yoda1 stock doses while keeping all other factors the same.

Besides, while the action of adding doses may influence the force readings, the no-dose experiment that could record the force readings of PBS in the chamber alone without any dose additions was also conducted.

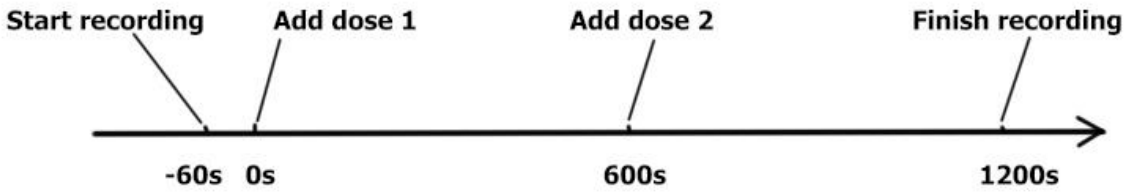

Figure 3. *The timeline of the two-dose experiment.*

4 Results

4.1 Two-Dose Experiments

Two-dose experiments were conducted with 10μM, 20μM, and 50μM Yoda1 solutions. The control group used 125μL of DMSO to replace the Yoda1 stock solution in the 50μM Yoda1 two-dose experiments. That said, there were four conditions for the two-dose experiments: 10μM, 20μM, 50μM, and DMSO (as 50μM Yoda1 volumes). Three experiments (meaning 3 rings) were conducted in each condition. The results of each condition are shown in **Figures 4 to 7.**

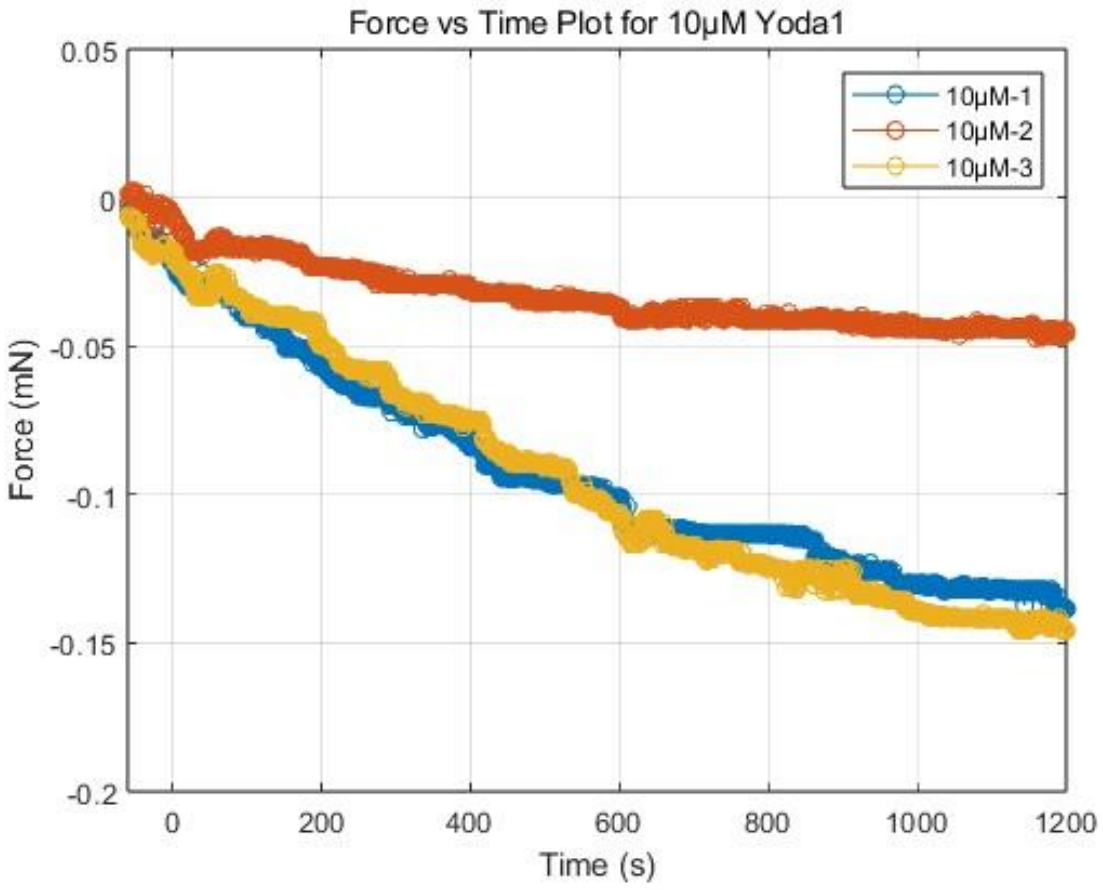

Figure 4. *The result of the two-dose experiments for 10μM Yoda1.*

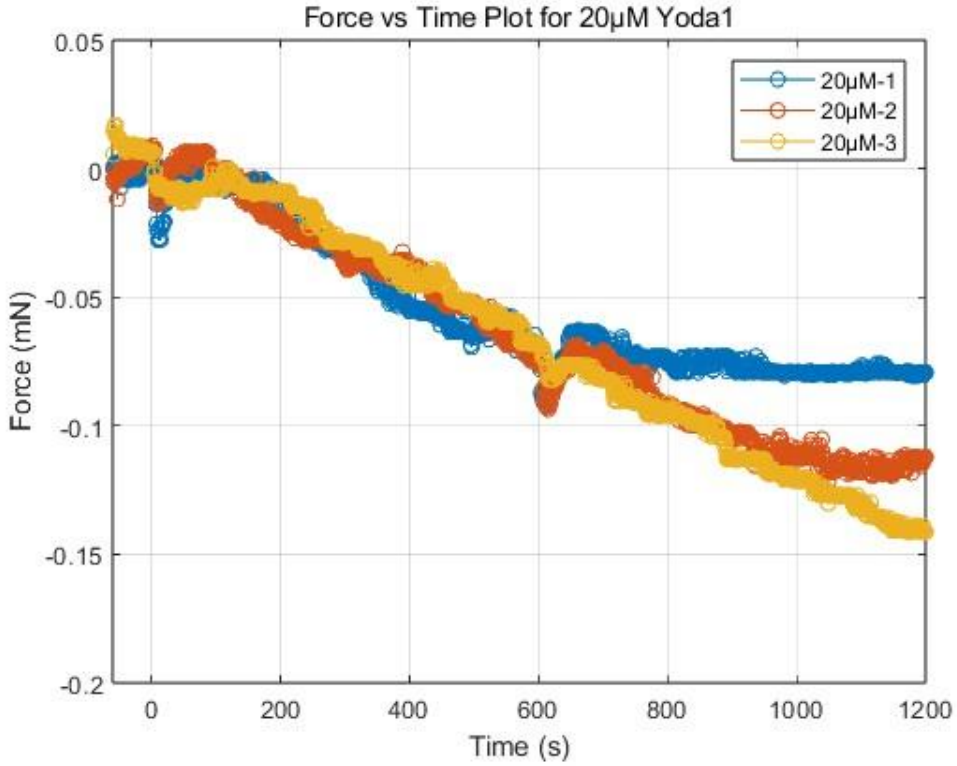

Figure 5. *The result of the two-dose experiments for 20μM Yoda1.*

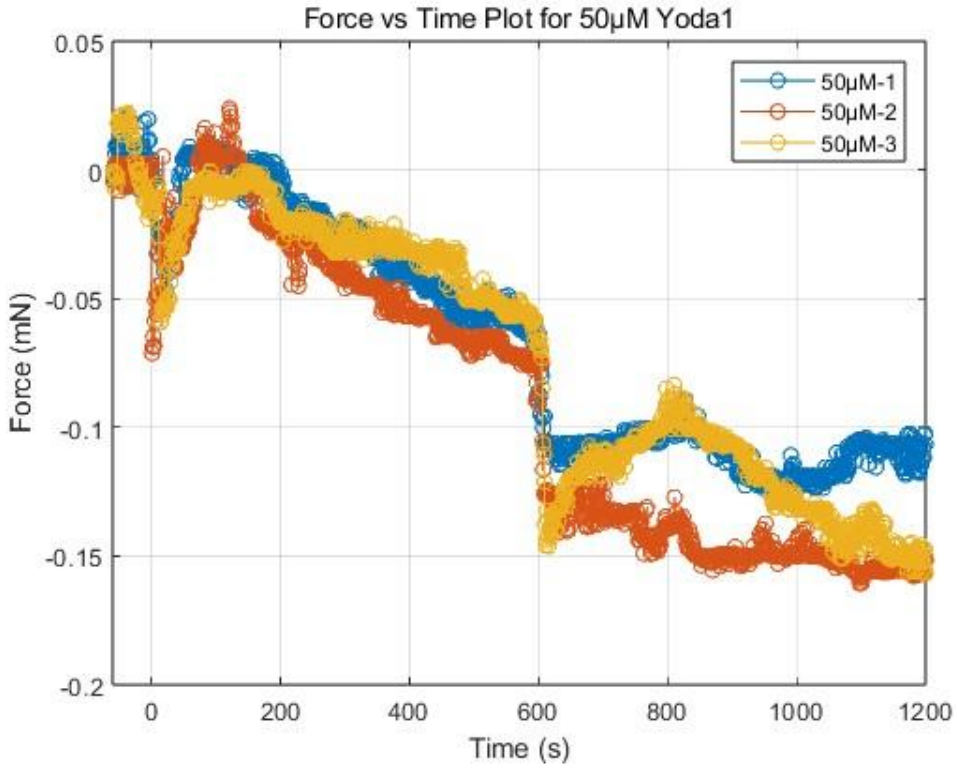

Figure 6. *The result of the two-dose experiments for 50μM Yoda1.*

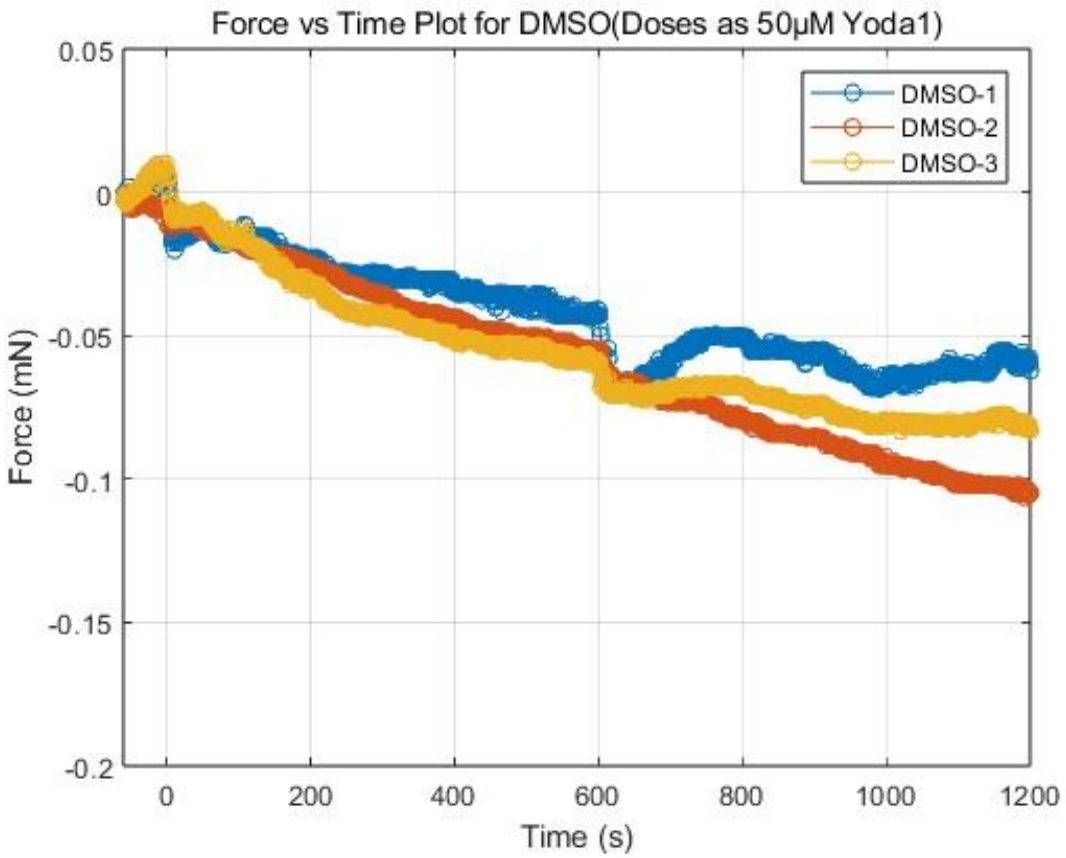

Figure 7. *The result of the two-dose experiments for DMSO (as 50μM Yoda1 volumes).*

4.2 One-Dose & No-Dose Experiments

Besides the two-dose experiments, another set of experiments regarding 250μM Yoda1 were conducted. Since the high concentration would use too many stock solutions, only the first dose (first 660 seconds) was conducted in these experiments.

These conditions were: 250μM Yoda1 dose, DMSO dose (625μL as 250μM Yoda1 volumes), and PBS dose (625μL as 250μM Yoda1 volumes).

Besides, another experiment with PBS alone was conducted without any dose additions.

Two experiments (meaning 2 rings) were conducted in each condition mentioned above. The results of each condition are shown in **Figure 8.**

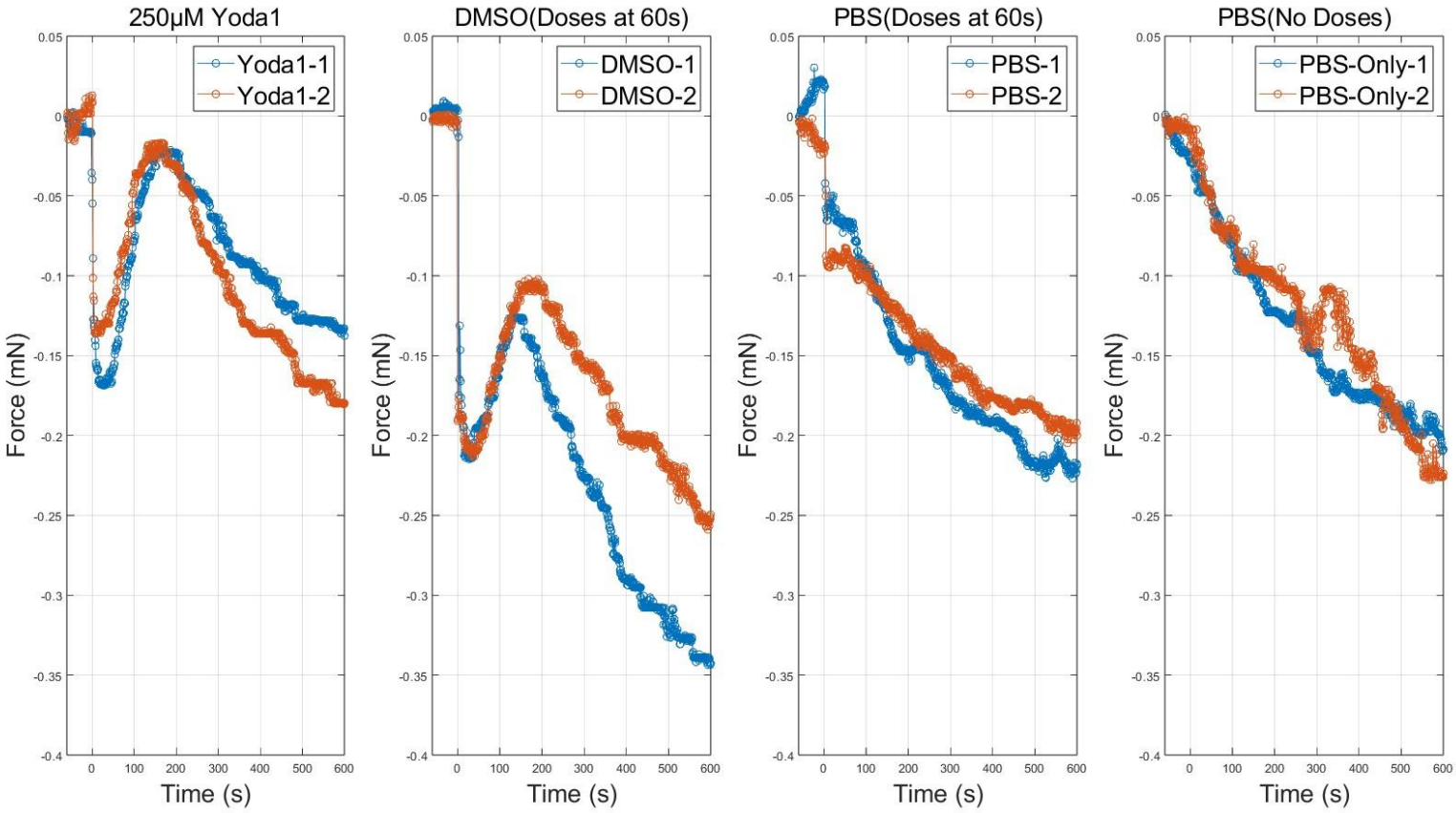

Figure 8. *The result of the one-dose experiments for 250μM Yoda1, DMSO (as 250μM Yoda1 volumes), and PBS (as 250μM Yoda1 volumes); as well as the result of the no-dose PBS alone experiments.*

5 Discussion

5.1 Force Drops and Relaxation

From the results, it can be concluded that, overall, all force readings are dropping; this indicates that the rings are relaxing through a period of time since all ending force readings are lower than the starting force readings. This may be because the rings are stretched under the normalized condition at the beginning of the experiment; thus, the rings are relaxing over a period of time.

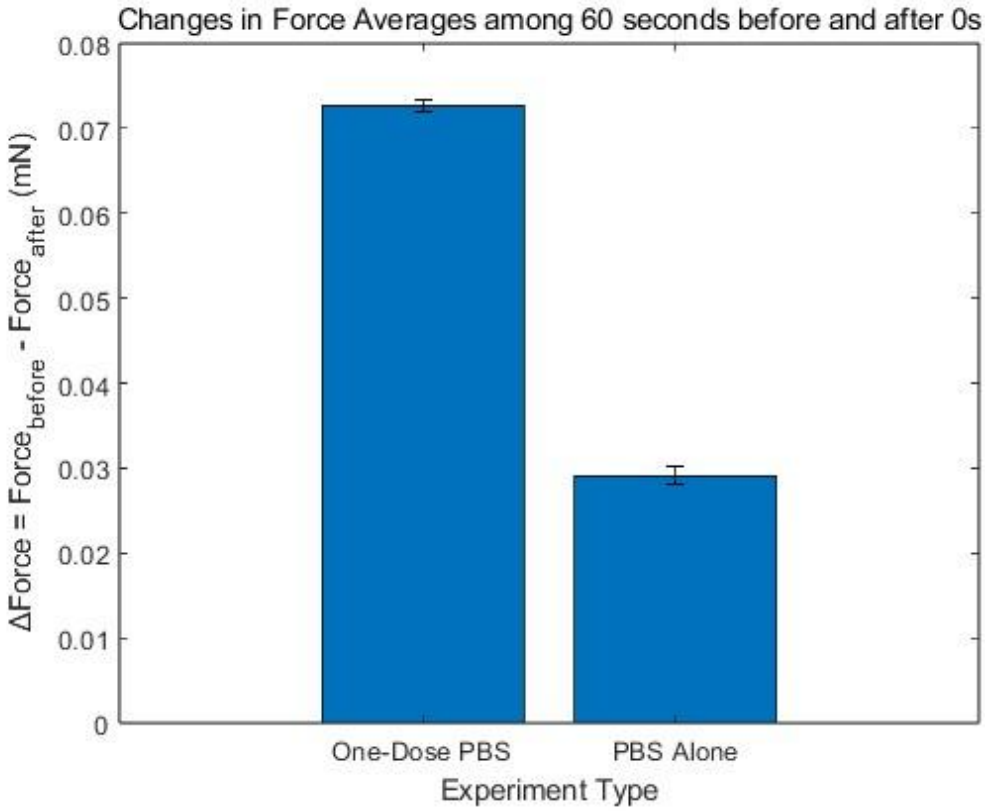

Figure 9. *The difference (F_{before} - F_{after}) between the averages among 60 seconds before and after 0s in one-dose PBS experiments and no-dose PBS experiments.*

By comparing the average force readings among 60 seconds before 0s and average force readings after 0s between one-dose PBS and no-dose experiments in **Figure 8**,

there is a drop in force readings at 0s in one-dose PBS experiments. The force drop in the one-dose is significantly higher than the smaller drop in no-dose experiments with a p-value of 0.00044834 by T-test, as indicated by **Figure 9**.

While all results showed a certain drop of force readings at 0s except in no-dose experiments, it suggests that the force drop is because of the action of dose addition.

5.2 Force Rises and Piezo1 Activation

It can be noticed that the force readings tend to increase after the drops at 0s. This may suggest the effect of Yoda1 opens the Piezo1 channels and allows Ca^{2+} to enter the cell; thus allowing the vessel to contract.

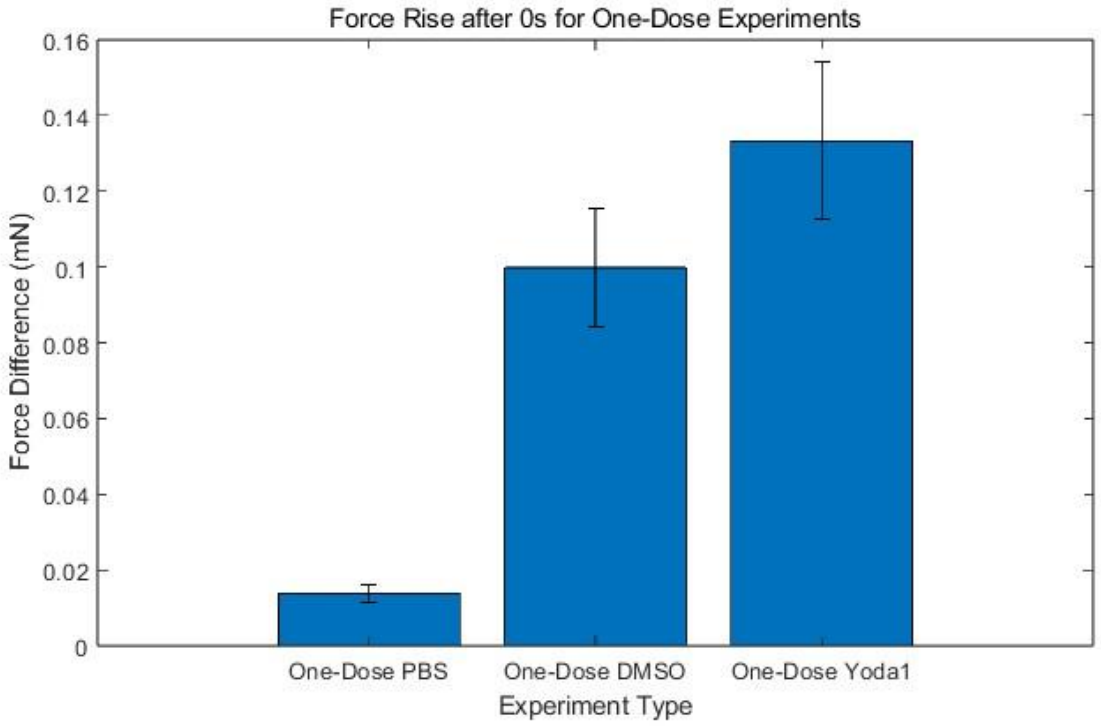

Figure 10. *The force difference (force rise) in one-dose PBS experiments, DMSO experiments, and one-dose 250μM Yoda1 experiments.*

Figure 10 shows the force difference of different conditions in one-dose experiments, with the formula:

Force Difference = Minus Local Minima after 0s

Plus Local Maxima after the Local Minima

The force difference in one-does Yoda1 experiments and in one-dose DMSO experiments are not significantly different with a p-value = 0.20828 by T-test, but they are both significantly higher than the force difference in one-dose PBS experiments with a p-value = 0.016137 and a p-value = 0.014774 respectively by T-test. These results suggest that while DMSO and Yoda1 have similar effects, they are causing significant changes than PBS alone in this type of experiment.

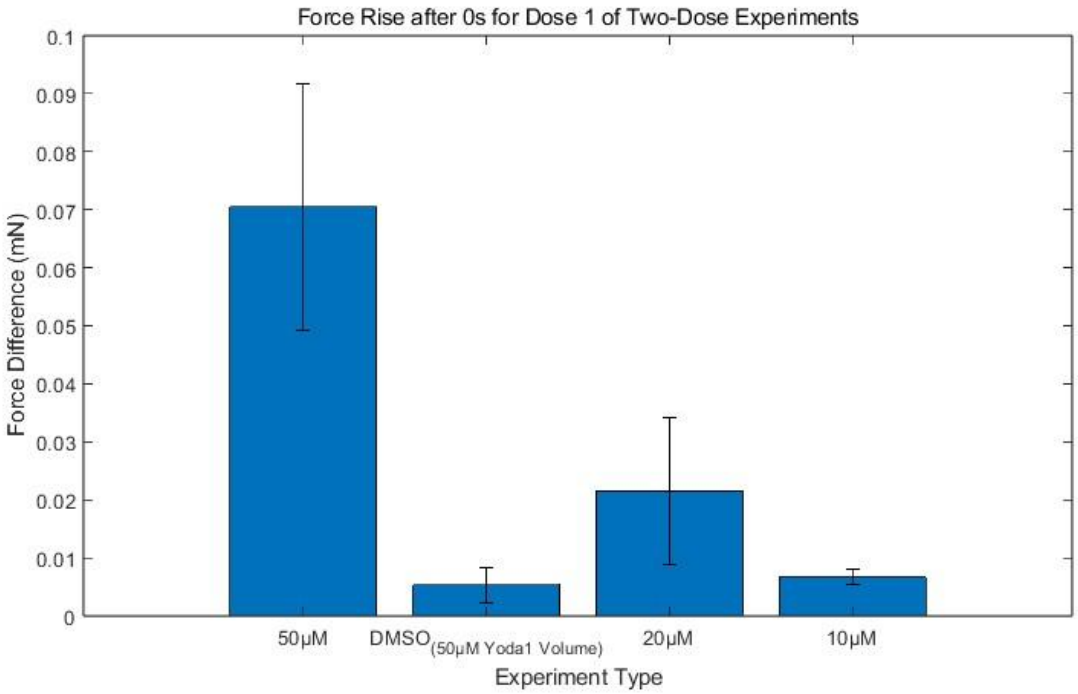

Figure 11. *The force difference (force rise) in dose 1 of two-dose 50μM Yoda1 experiments, DMSO (50μM Yoda1 volume) experiments, 20μM Yoda1 experiments, and 10μM Yoda1 experiments.*

To further investigate how different concentrations affect the force rise, the force rises of the first dose in the two-dose experiments are compared as shown in **Figure 11.**

The force difference in dose 1 of two-dose 50μM Yoda1 experiments is significantly higher than the same volume used in dose 1 of the two-dose DMSO experiments with a p-value = 0.0062714 by T-test; it is also significantly higher than dose 1 of the two-dose 20μM Yoda1 and 10μM Yoda1 experiments with a p-value = 0.024379 and a p-value = 0.006567 respectively by T-test; However, the force difference in dose 1 of two-dose 20μM Yoda1 experiments is not significantly different from the force difference in dose 1 of two-dose 10μM Yoda1 experiments with a p-value = 0.13813 by T-test.

Another comparison is made between the 250μM Yoda1 experiments and the first dose of the 50μM Yoda1 experiments by T-test. With a p-value of 0.046806, the force rise in the 250μM Yoda1 experiments is significantly higher than the force rise of the first dose in the 50μM Yoda1 experiments.

These results suggest that the higher concentration of Yoda1 is causing a higher force rise in larger concentrations, such as 250μM and 50μM; However, a smaller concentration, such as 10μM and 20μM, may not give distinguishable effects. Besides, DMSO in the volume of 50μM Yoda1 is causing significantly smaller effects than 50μM Yoda1.

5.3 Comparing the Effect of Dose 1 versus Dose 2

To compare the difference of force rises between dose 1 and dose 2, the force rise of

the first dose and the second dose in the two-dose experiments are compared as shown in **Figure 12** by the following formula:

Force Difference (Dose 2) = Minus Local Minima after 600s

Plus Local Maxima after the Local Minima

From the results, the force differences in dose 1 and dose 2 are not significantly different in any conditions (50μM Yoda1, DMSO, 20μM Yoda1, and 10μM Yoda1) with p-values = 0.13103, 0.51108, 0.88754, and 0.49327 respectively by T-test.

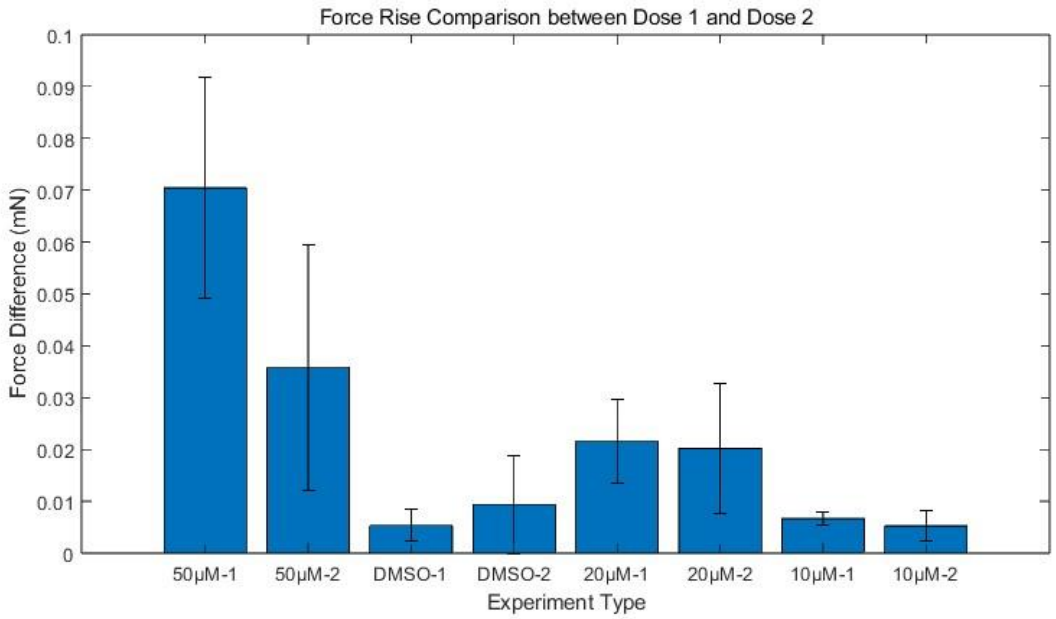

Figure 12. *The force difference comparison between dose 1 and dose 2.*

5.4 Conclusions and Future Directions

From the results and discussion above, it can be concluded that a higher concentration of Yoda1 is causing a higher force rise, which may be because of the activation of Piezo1 channels that allows the influx of Ca^{2+} ions resulting in muscle

contraction. However, such effects in low concentrations of Yoda1 may not be clear.

Additionally, DMSO also causes an effect that allows the force readings to rise. Although such an effect is sometimes significantly lower than the effect of Yoda1, its ability to cause contraction that is similar to Yoda1 has an unknown reason behind it.

Furthermore, when adding the same amount of Yoda1 the second time, the effect of force rise is not significantly different from the first addition, even though the result solution has a doubled concentration. This may indicate that the force rise has a one-time effect, which the previous additions do not contribute to future force rises.

Since there are still questions that remain unknown, there are many future directions to consider:

(1) It may be worth trying another buffer instead of PBS that contains more Ca^{2+} to allow more ions to enter the cell, which may contribute to a larger force rise and may distinguish the effect difference between Yoda1 and DMSO.

(2) It may be helpful to image the Ca^{2+} signaling so that the effects of Yoda1 on the Piezo1 channel can be linked to Ca^{2+} signaling at the molecular level.

(3) The temperature may be a factor that influences force readings. It may be worth conducting experiments in a physiological temperature such as 37°C.

6 Acknowledgement

I want to thank Dr. Jessica Wagenseil for introducing this research rotation project and providing me with guidelines on the design and progress of experiments.

Besides, thanks to my post-doctoral mentor Dr. Garrett Easson who helps me with the procedures of myograph mounting, guides me to handle the work in the animal facility, and helps me with the training of confocal microscope.

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Finally, I want to express my appreciation to all members of Dr. Wagenseil's lab for creating such a positive research atmosphere. Their combined encouragement has played a pivotal role in the success of my research rotation.

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