Patch-Clamp Microchip Testing Circuit Interface

PROJECT PLAN

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1 Introductory Material

1.1 Acknowledgement

This design document was supported by our advisor Dr. Que Long and graduated student Silu Feng. We thank them for their great assistance in this project, although they may not agree with all of the interpretations of this design document.

1.2 problem Statement

Patch-clamp is a technology in biological engineering field which means to catch cell with tiny clamp and observe the bioelectricity behavior of the cell. Our project is focusing on how to build up a set of electric environment and circuit to provide operational interface between the patch-clamp and electron microscope. With patch-clamp, human will observe a clearer picture about cells, which for our project are neurons, the nerve system cells. This could be done with getting a clear electric current square graph of the currents that generated when ions try to enter and leave the membrane of neurons, and this is what we do for our project to build the interface to capture the current graph. These behaviors of neurons will lead biological engineers to look for many possible cures of some serious nerve diseases that could not be cured right now.

We use PC-ONE Patch/Whole Cell Clamp to build the interface. This clamp equipment is also the main tool we use to build our interface.

1.3 Operating Environment

The operational environment of Patch-Clamp Microchip Testing Circuit Interface is biological laboratory with complete safety equipments and professional operators. The operation of Patch-Clamp Microchip Testing Circuit Interface must be done safely under required professional assistance.

1.4 Intended Users and Intended uses

Patch-clamp technique can not only be used in neuroscience but also a huge variety of physiological questions. For this technique is still the laboratory technique, so the user for

this technique should be the experimenters, who want to know the ion current on the membrane when we apply different voltage or we use different solution in the bath. The most commonly use for this technique is drug discovery.

For our project, we will test the different voltage on the neuronal cells to find the way to increase cell viability and make the neuronal cells can split fast. The method can be used on depression treatment, for we can make their neuronal cell more excited than before.

1.5 Assumptions and Limitations

Assumptions:

- The patch-clamp testing circuit can catch two cells at the same time to decrease the testing times.
- The simulation result will be useful to defeat depression .
- The mode can be used multiple times until we finish the project.

Limitations:

- Two semesters to work on this project
- Do not have too much testing samples (neuronal cells). We may fail many times, so it is hard to get the correct answer.

Do not have appropriate material to reduce the room noise

1.6 Expected End Product and other Deliverables

At the end of this semester, we are supposed to deliver a fully setted up patch-clamp circuit, which is operational to catch cells and observe the bioelectricity behavior of the cell. The goal is to build up a set of electric environment and circuit to provide operational and functional interface between the patch-clamp and electron microscope. In addition, before April 2018, we are supposed to measure the ion channel potential of cells and the action potential of cells under external stimulations successful by using the patch-clamp microchip.

2 Proposed Approach and Statement of Work

2.1 Functional requirements

- Patch-clamp could catch neuron.
- Current could be observed through PC-ONE interface.
- Neuron must be alive during operation.

This project will be able to allow operators to easily operate the patch-clamp technique to observe the ionic behavior of leaving and entering the neuron's' membrane with setupped PC-ONE Patch-Clamp Interface. Then through patch-clamp to gain a square current flow phase graph which expresses the ions' behavior when it tried to enter or leave the neuron.

2.2 Constraints considerations

Constraints:

- We could only use PC-ONE as our interface instead of other interfaces on the market.
- We only focused on the Current Clamp method and neuron is the only type of cell we need to observe.
- We would only work with our team members among with advisors.

For project specific requirements, we have above two constraints for now, and each of them is also the goal we need to implement.

Non-functional Requirements:

- We need to strictly follow the equipment manual to operate all operations to fulfill the project requirements.
- We have to strictly follow the safety guide of labolarity to make the experiment process safely.
- We have to cite every sources we used during the development, and ensure that the project is totally independent.

- Plan regular meeting with client to make sure the project is meeting the requirement.
- Timely maintain the project to avoid critical bugs.
- Refresh reports and document on time to meet current process.
- For ethics rules, we need to follow IEEE ethics code(@).

To ensure above requirements, the project will be able to keep up and complete on time with required quality and deliverables.

2.3 Technology considerations

In our project, the electrode material of clamp will need to be found by ourselves to meet the best performance of getting accurate data without leakage. The best choice could be silver, which is one of the best electric conductors for us. The advantages of silver is that it has high electric conductivity. But the downside of this material is that it is expensive and we only have limited budget.

Another technology consideration is the environment to keep neurons alive. We are still trying to find the best inoculum liquid to meet the best performance.

2.4 Safety considerations

For our project, all members should follow the laboratory safety guide to operate patch-clamp and PC-ONE.

2.5 Previous Work and Literature

For our project now, we are basicly using Dagan Company's PC-ONE patch-clamp to build our own operational interface. The literatures we are now using are *A patch-clamp device with integrated actuators for cell selection and positioning*(#), *Patch clamp technique: review of the current state of the art and potential contributions from nanoengineering*(*)

and *PC-ONE Patch/Whole Cell Clamp Operating Manual Ver. 1.1*(&). All literature are used to get better background information about patch-clamp.

2.6 Possible Risks and risk management

The weak organizational management of team, lack of communication between advisor could, lack of background knowledge according to patch-clamp, and technical difficulties may slow or hinder our pan. We will try our bests to overcome these obstructs with more detailed rules and seek any possible help to make them spannable.

2.7 Project Proposed Milestones and evaluation criteria

We will first test the PC-ONE patch-clamp with several parameters to make sure it works properly. Then find the possible inoculum for neurons and possible materials for electrodes. After that, a prototype of connected patch-clamp with neurons and other necessary equipments will be setup to collect data. Finally, the finished patch-clamp testing interface will be setup as deliverable.

2.8 Project tracking procedures

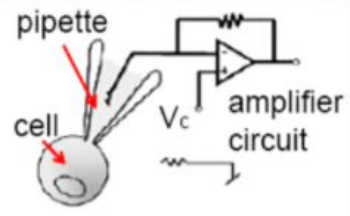
We will use every week's weekly meeting among group and advisor to make sure the procedures are tracked. Basically, when we meet every week, we will conclude the week's works and to check the assignments that were assigned to each team member. Then during the meeting, we will assign the following jobs to each of our team members.

2.9 Objective of the task

We will be able to deliver a working patch-clamp microchip testing interface with functionalities of obtain the proper ionic current generated by the entering and leaving neuron's membrane behavior through oscilloscope and generate a square current graph.

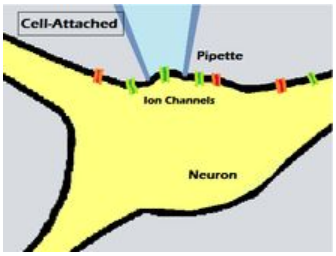
2.10 Task approach

Describe any possible methods and/or solutions for approaching the project at hand. You may want to include diagrams such as flowcharts to, block diagrams, or other types to visualize these concepts.



Graph 2.10 patch-clamp circuit

Schematic of the conventional patch-clamp technique using glass pipette for capturing a cell for characterization of cellular ion channels and its operational process



Graph 2.11 Whole cell attach

2.11 Expected Results and Validation

What is the desired outcome?

Ideally the seal resistance with a cell should be in the range of GigaΩ for recording from a single ion channel. The pulse wave of ionic current, which record by the oscilloscope.

How will you confirm that your solutions work?

Compared the results with assumption and advisor Dr. Que Long and graduated student Silu Feng will check the result.

3 Estimated Resources and Project Timeline

3.1 Personnel effort requirements

Table 3.1: Personnel effort requirements

Task	Estimate schedule (hours)
Fully understand the documents and manual about patch-clamp microchip	30
Setting up the equipments	6
Test:Capacitance compensation	4
Test:Electrode resistance test	3
Test:Junction potentials-search mode	5
Test:Patch sealing resistance	3
Test:Recording during voltage clamping	5
Test:Current clamping	6
Test:Series resistance compensation	5
Test:Leakage resistance compensation	7
Test:the PC-one voltage clamp mode	18
Test:the PC-one in current clamp mode	22
Measure the ion channel potential of cells.	24
Measure the action potential of cells under external simulations.	30

3.2 other resource requirements

Identify the other resources aside from financial, such as parts and materials that are required to conduct the project.

The neuron cells we are going to observe and corresponding documents about how to store these cells and keep them alive.

3.3 Financial requirements

There is no other financial resources required for our project expect the neuron cells we are going to buy. The financial fund is ready.

3.4 Project Timeline

table 3.3: Project Timeline

Task	Start time	End time	Task description
Project begin	Week 1	Week 3	Held the first group meeting and give task to every group member
Website setup	Week 2	Week 3	Set up the website, upload member's information. Start to upload weekly report.
Gather information and research	Week 3	Week 5	Read documents and corresponding background knowledge about patch-clamp. Read the manual of the commercial amplifier and probes.
Setup and operation	Week 6	Week 13	Capacitance compensation Electrode resistance test Junction potentials-search mode Patch sealing resistance Recording during voltage clamping Current clamping Series resistance compensation Leakage resistance compensation
Complete the electrical testing setup	Week 14	Week 16	Finish setting up the patch-clamp testing circuit interface
First semester Presentation	Week 15	Week 16	Present the component of setup and the testing plan for next semester

Testing circuit	Week 17	Week 22	Testing the PC-one voltage clamp mode Using speed to simulate patch currents Testing the PC-one in current clamp mode
Measure the data under different simulations	Week 23	Week 30	Measure the ion channel potential of cells. Measure the action potential of cells under external simulations. Gather the results of the measurements by microscope and summarize the information get from the experiments.
Comprehensive final report	Week 31	Week 32	Present the whole project and the achievements in this project.

4 Closure Materials

4.1 CONCLUSION

Our project is focusing on how to build up a set of electric environment and circuit to provide operational interface between the patch-clamp and electron microscope. With patch-clamp microchip, people could observe a clearer picture about cells, which for our project are neurons, the nerve system cells. This could be done with getting a clear electric current square graph of the currents that generated when ions try to enter and leave the membrane of neurons. We are going to measure the ion channel potential of cells and the action potential of cells under external stimulations after we setting up the patch-clamp microchip successfully. Meanwhile, during the process of experimental measurements, we should figure out a method to store these neuron cells to keep them alive.

4.2 REFERENCES

&: Dagan Corporation, PC-ONE Patch/Whole Cell Clamp Operating Manual Ver. 1.1, www.DAGAN.com.

*: Zhongcheng Gong, Krithika Nagarajan, Siva Penmetsal, David Millsl, and Long Quel, *A patch-clamp device with integrated actuators for cell selection and positioning*, Institute for Micromanufacturing, Louisiana Tech University, USA, School of Biological Science, Louisiana Tech University, USA.

#: Y Zhao*1, S Inayat*1, D A Dikin*2, J H Singer*3, R S Ruoff*4, and J B Troy*1 , *Patch clamp technique: review of the current state of the art and potential contributions from nanoengineering*, 08 June 2009, DOI: 10.1243/17403499JNN149.

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@: IEEE Code of Ethics, *https://www.ieee.org/about/corporate/governance/p7-8.html*

4.2 Appendices

N/A