KKU ENGINEERING JOURNAL 2016;43(S1):158-161 Research Article

KKU Engineering Journal

https://www.tci-thaijo.org/index.php/kkuenj/index

Design and development of a local microelectrode puller for electrophysiology research in Thailand

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Received April 2016
Accepted June 2016

Abstract

Fine glass capillaries (also called pipettes) have been extensively used in electrophysiologic method for decades as either a recording or stimulating microelectrode. Fabrication of the electrode requires a special puller. This work presents the initial design and development of a local glass capillary puller to serve electrophysiology research in Thailand. This instrument model was primarily designed for making bipolar-stimulating electrodes from two-barreled borosilicate glass capillaries, which will be used in the study of neuronal control of vascular tone. The main systems include the heating and the pulling components, which were designed to be operated in adjustable manner by users. The heat and pulling force were governed by the controlling current. The design was validated through experimental results. Appling 19A of heater current and 133 W of power for 60-90 second duration onto Kanthal-heating filament was sufficient to melt the glass. The pulling mechanism is generated by a vertical solenoid puller with adjustable force control system. The electrode feature was acceptable for performing *in vitro* experiment in a large blood vessel (e.g. human tissue). However, similar studies in the rodent of which vascular size is far smaller may require a sharper-tipped electrode. Limitations of the prototype model were also addressed for further improvements, including the limited pulling distance of the solenoid, the firmness of the holders for the heating filament and multi-barreled glass tubing, and the fluctuation of heating current. We anticipate that the invention of sophisticated life-science instrument could enhance multidisciplinary research collaborations in order to reduce overall investment on research instruments for our nation, and may help open a new avenue of production industry of sophisticated instrument in Thailand.

Keywords: Bipolar-stimulating electrode, Electrophysiology, Electrode puller, Vertical electrode puller, Stimulating electrode, Adjustable pulling force

1. Introduction

Electrophysiology supports all levels of neuronal function study from microscopic events (e.g. recording of single ion channel), to macroscopic events (e.g. integrative process of regional synchronization of the tissue) [1-2]. The basic electrophysiology rig is comprised of several devices including vibration isolation table, micromanipulators, microscope, amplifier and data acquisition software, stimulator unit, and microelectrode puller [3]. The puller is used to make microelectrodes, which considered as one of the basic instruments used either for recording or stimulating purpose. By using proper glass capillaries and a suitable puller, a special microelectrode can be fabricated for that particular application. Well-known commercial electrode pullers are from USA, Canada, Germany (David Kopf Instruments, Sutter Instrument Co., Harvard Apparatus, HEKA instruments Inc.), and Japan (Narishige). Most commercial pullers are particularly designed for making such a long and sharp microelectrode for patch clamp or intracellular recordings [4-5]. On the other hand, the choice for making microelectrode with comparatively short and wider tip is still limited. A suitable puller for making is kind of microelectrode does not available commercially nowadays and needs to be custom made, which is costly. This particular type of electrode is required as a stimulating electrode to perform focal nerve stimulation in the study of neural control mechanism of blood pressure [6-7]. Besides, the investment for a fully-equipped electrophysiology station is expensive and need to be imported (approximately 75,000 USD without tax) [3]. It is undeniable that costly research device is a critical obstacle for the research success of our nation, especially in young researchers. Development of domestic technological innovation via multidisciplinary collaboration may overcome this hindrance. In this project, we concentrated our attention on providing some basis rational of design and development of the vertical electrode puller for making bipolar stimulating electrodes from two-barreled glass capillaries. This type of electrode will be used in focal perivascular nerve stimulation allowing neurotransmitter release and vascular contraction, which is a key experiment in identifying the mechanism of sympathetic overactivation in hypertension (Figure 1). The puller design was mainly divided into three parts: mechanical system,

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doi: 10.14456/kkuenj.2016.47

pulling system and heating system. In this article, the puller system design is deliberated in part 2, experimental result is shown in part 3, and conclusion is discussed in part 4.

2. Design of the micropipette puller system

2.1 Micropipette mechanical system

The anatomical structure of the puller is composed of heating filament holders, the upper- and lower- capillary holders, and sliding part. The heating filament holders are used to position the heating filament responsible for passing the heat onto the two-barreled borosilicate glass capillary (World Precision Instrument, USA). It is important to place the glass precisely at the center of the heating element and keep the latter stationary throughout the heating and pulling processes [8]. The upper capillary holder is designed to be stationary. The lower capillary holder is intended to be a moving part and connected to the sliding bar (Figure 2). The moving part can be moved up and down through a sliding bar, which is controlled by the solenoid puller. During the pull, the moving part is moving down as the solenoid puller is supplied energy, and pulling force.

2.2 Control System

Figure 3 illustrates the control block diagram of the designed micropipette puller. This designed system includes the heating system, pulling system, 24VDC- supply system, and the main control system. The key systems are heating and pulling system, which are discussed in this section.

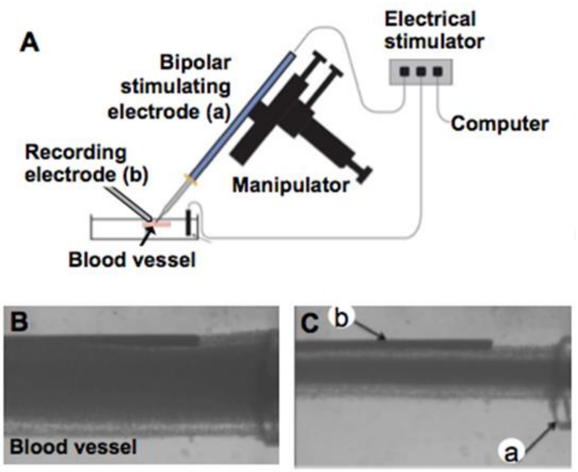

Figure 1 Application of the bipolar stimulating electrode in the study of neural control mechanism of blood pressure. A) Overall experimental setup. The bipolar stimulating electrode (a) consisting of two Ag/AgCl bared wires inserted into a pulled double barrel glass filament placed on the surface of the blood vessel. The wires were connected to a stimulator unit. On the other side, a carbon fiber-recording electrode (b) was also positioned on the blood vessel. To keep the tissue alive, it was perfused with warm-oxygenated Krebs' solution (B). Electrically evoked focal nerve stimulation via the bipolar electrode caused perivascular neurotransmitter release and thereby vascular contraction (C). Neurotransmitter release and vascular reactivity were detected under microscope.

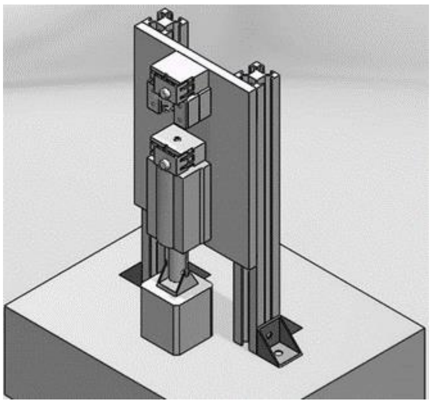

Figure 2 Isometric drawing of the sliding and pulling components of the puller

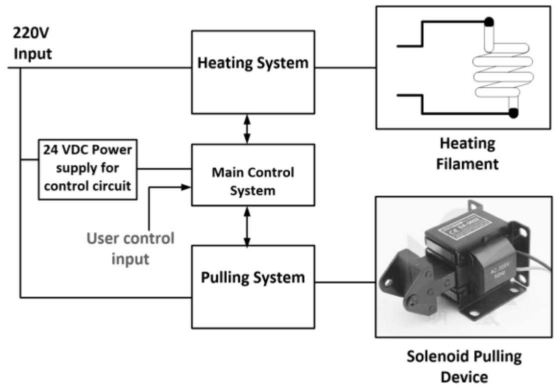

Figure 3 Control system block diagram. The heating system supplies energy to the heating filament. The heat temperature is controlled by the heating current via main control system. The pulling system performs as the pulling control signal is given.

2.2.1 Heating system

The heating system was designed to control the heating temperature generated by the heating filament. The commercial Kanthal filament in spiral shape (PE-21 HD, Narishige, Japan), a resistance-heating alloy, used in comparable pullers was obtained [9]. To pull the capillary, the heating filament needs to be properly surrounded the capillary to provide sufficient melting temperature. The slight movement of either the glass capillary or the heating filament may affect the shape of the pulled electrode. The applied heater current was converted to the heat by a DC-DC converter that can provide 0-12V of output voltage with 25A maximum current and 300 W maximum rated power. The operating temperature was measured by an infrared thermo scan (ThermaCAM™ E45, FLIR System, USA).

2.2.2 Pulling system

An AC-220V solenoid-pulling device was used as the main puller compartment. The solenoid requires adjustable 0-220 V, 50 Hz power supply as the different pulling force was needed. In the pulling system, a DC-AC converter (inverter) was used as the main topology for converting constant direct current to alternating current with variable voltage and frequency. The inverter was controlled through the Pulse Width Modulation (PWM) control method for adjusting amplitude and frequency required.

3 Experimental results and discussion

3.1 Experimental setup

In the test, all sub-systems were tested individually and then they were integrated into the final system so that the final functional tests were ultimately assessed.

The test setup is shown in Figure 4, which includes 1) the designed micropipette puller system and 2) the LeCroy oscilloscope used for measuring current and voltage signals. These two sub-systems need to communicate with each other and need to be controlled to assure that the system can work as needed. The designed main control system offers these required tasks by providing control signals to all sub-systems.

3.2 Experimental results

The experimental goal is to determine sufficient heat for melting a two-barreled glass capillary. The solenoid pulling force was set at maximum level throughout the experiment, while various degree of the heater currents (3-20A) were applied onto the Kanthal-heating filament for 60-second duration. Then, the temperature yield and glass-melted ability were measured. The result is shown in Table 1. The two-barreled glass capillary began to be melted at 19-20 A of the heater current.

3.3 Fabrication results of the microelectrode puller

The rational of this experiment is to determine the impact of heating temperature on the shape of micropipettes. We hypothesized that increasing the heat current would contribute to higher heating temperature, thereby a smaller and micropipette tip. As the variation of the heater current measured by a current meter was ±1A, the heater current was applied at the two levels (19-20A, and 20-21A). Besides, three heat durations were applied (60, 90, and 120 seconds) prior to the pull. The shape of a glass capillary after fabricated is shown in Figure 6. Next, the quantitative data were obtained to verify the reproducibility of the puller. After separation, the upper- and lower parts of the pulled micropipettes were taken for the outer diameter measurement under a microscope as shown in Figure 7. At lower operating temperature (19-20A), the upper part diameter of capillary was smaller than the lower part. However, when the heater current was increased to 20-21A, there was no significant difference in tip diameter between the upper and lower pieces. From our observation, there was the recoiled force generated by the supported spring located on the sliding bar (Figure 5). This reverting force affected the glass separation, thereby the tip diameter and electrode shape. It was also observed that there was very slight movement of the heating filament during high-peaked temperature leading to slightly bended tip. Last, the capillary holder frequently failed to hold the two-barrel pipette in place during the pull causing incomplete separation. Wrapping around the multi-barreled glass with white-sealing tapes increased the holder tightness and temporally solved the issue (Figure 6A). However, at 120 seconds of heating duration for 20-21A current, the cushion tape was melted down and caused incomplete glass separation.

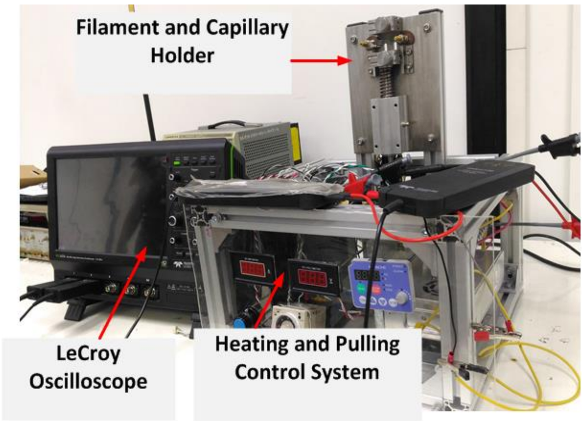

Figure 4 Experimental setup

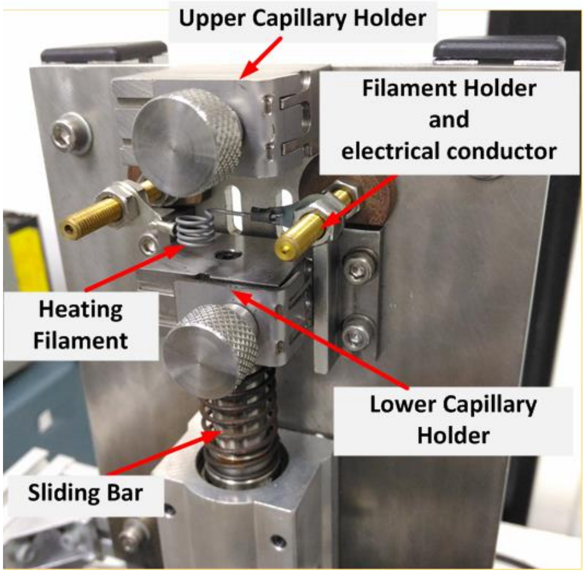

Figure 5 Completed assembly of the heating and pulling components

Table 1 Contribution of the heater currents in temperature yield and glass-softening ability. The heating duration was set at 60 seconds.

Current (Ampere)	Power (Watt)	Temp (º C)	Glass Melted
3	6	125	No
6	12	>275*	No
9	18	>275*	No
12	48	>275*	No
15	60	>275*	No
16	112	>275*	No
17	119	>275*	No
18	126	>275*	No
19	133	>275*	Yes
20	140	>275*	Yes

*Thermo scan could not report the temperature higher than 275ºC

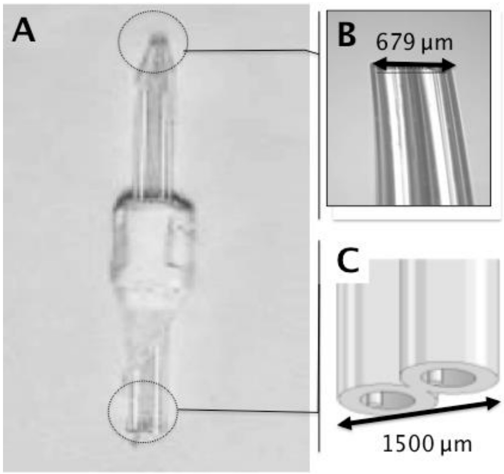

Figure 6 A capillary feature after heated at 19-20 A of heating current for 60 seconds and vertically pulled by the solenoid (A). Magnification of the capillary tip under the microscope (B) compared to the original terminus (C).

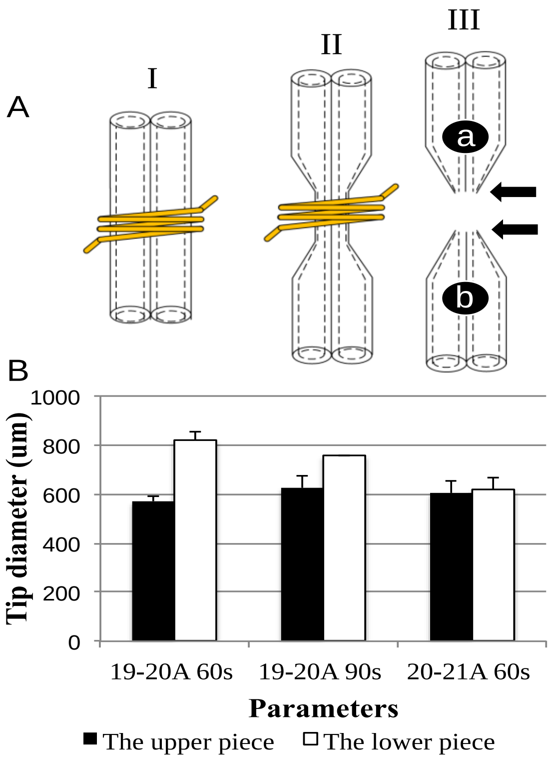

Figure 7 Capillary structure before and after fabrication. A) A drawing sketch of a capillary at three stages during its fabrication. B) Measurement of tip diameter after separation by indicated parameters. The upper (a) and lower (b) pieces were taken for measuring the outer diameter under a microscope. Data are mean ± s.e., n=1-5.

4. Conclusions

This work has proposed the design and fabrication of the first local multi-barreled borosilicate capillary puller. The proposed system was designed to operate in flexible manner, adjustable heating temperature and controllable pulling force. The fixing elements and pulling structure were designed and fully installed. With 20 mm sliding distance, the system can reproducibly pull and form a two-barreled glass capillary with short tip. The average tip diameter is ranged between 665±90µm depending upon the temperature and timing parameters. The upper part seemed to reach the target of further electrode make, as its tip diameter was quite smaller (600±24 µm versus 730±83µm). The average tip diameter of the upper part micropipette could fit the perivascular nerve stimulation experiment in large blood vessels including human tissue (Figure 1). However, the similar study in smaller vessels of rodents may require far smaller tip size in range of 100-200 µm. It was observed that several variables affected the pipette shape during pull and they should be improved in our next model—1) the heating filament holders should not have moved, especially during the high operating temperature applied, as a slight movement of the heating filament leads to bended tip. Reducing the distance between the two holders may improve the stationary issue. 2) The glass holders need to be improved to hold the slippery multi-barreled glass throughout pull. 3) The solenoid pulling distance should be increased to avoid the reverting force, allow fabrication of smaller-tipped pipettes, and to permit the operation at lower temperature to prolong the lifetime of the costly heating filaments.

5. Acknowledgements

This project was supported by the Faculty of Medicine, Thammasat University (MTU-DS-3-133/57). We also would like to express our sincere thanks to Wantha Jenkhetkan for her assistance with the microscopic measurement, Panudech Jareanwong, Sakdithach Tongnuemai, and Siwat Wongkuan for their invaluable help with the test setup.

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