AN INSTRUMENT FOR CONTROLLED, AUTOMATED, CONTINUOUS PULLING OF SUB-MICROMETER FUSED SILICA PIPETTES

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ABSTRACT

Glass micropipettes are drawn glass needles with tip openings of 1 µm or less, useful for a wide range of biological and electrophysiological applications. In the state-of-the-art of fabrication of micropipettes, a skilled individual is able to produce about 2-4 micropipettes per minute. Many labs, which utilize hundreds of pipettes on a weekly basis, would benefit by the increased speed, accuracy, and repeatability of an automated fabrication apparatus. Therefore we have designed, built, and tested a working prototype of a fully automated fused silica micropipette puller. We report on the design and operation of this device along with measurements on the size and deviation of the micropipette tips. A trend has been determined between pulling velocity and tip geometry. This relationship allows for the ability to pull micropipettes with a specified geometry. Such control and automation has previously never been available and saves many hours of skilled labor.

INTRODUCTION

Current manufacturing of micropipettes consists of manually loading previously prepared lengths of capillary glass into a fabrication device and clamping them into place. A program is entered, and the two ends are pulled in opposite directions after a section of the glass is raised to the glass transition temperature [1]. The axial force necessary to pull the capillary apart is usually achieved via a hanging weight, solenoid [2], pneumatic actuator [3], or combination thereof. While this design has proven simple and robust enough to dominate the commercial offering, it is not the most flexible or readily adaptable for continuous automation.

Fused silica, while offering superior mechanical and electrical properties to the traditionally used borosilicate or aluminosilicate glass, is not often utilized for pipettes due to the high melting point compared to other glasses. However, devices do exist which are capable of reaching the greater than 1600°C needed to pull fused silica glass.

Our design relies on automated feeding of a continuous spool of fused silica capillaries via a set of pinch rollers. The capillary is then fixed in place with pneumatic clamps and heated with a gas torch. A stepper motor driven lead screw then pulls one end of the capillary. Our prototype utilizes a gas torch to achieve the necessary temperatures to pull fused silica glass, but subsequent prototypes could easily replace this with other means of heating, such as a CO_2 laser.

For a micropipette puller to be useful it must be able to manufacture tips of 1 µm and have high repeatability. Therefore, we have measured the tips created through runs of different parameters to calculate their average size and standard deviation. Also, this has led to a characterization of the relationship between pull velocity and tip geometry.

METHODS

Heating of the fused silica capillaries is done by a propylene gas jet which burns at about 1980°C; a higher temperature than propane. Based on this temperature, the dimensions of a typical capillary, fused silica properties, and propylene properties a heating model was created. The model consisted of first calculating the Grashof and Reynolds numbers. Since it was found that the Grashof number was much smaller than the Reynolds number, it was seen that forced convection dominates the heating, as was expected. Next, the Nusselt number was calculated using the Churchill and Bernstein correlation. From this the convective heat transfer coefficient was found. This model was used to get an estimate of the heating time required to reach the necessary temperature of about 1680°C to melt fused silica glass.

Pulling of the pipettes required two separate force calculations. It is known that a pull force of about 45 N is necessary to pull the pipettes. The clamping force and pulling force must be great enough to achieve this requirement. The clamps [Festo, EV-20/75-5] are rated to provide a force of 600 N at the maximum stroke length and 600 kPa. These clamping modules are covered in silicon to provide a higher coefficient of friction. Empirically, we determined from four bench tests that the coefficient of friction for this setup is at least 0.22. This results in a clamping force of approximately 132 N.

A lead screw and stepper motor assembly is used to pull the pipettes. Given the torque of the motor, the coefficient of friction between the screw and nut, and the lead of the screw, the axial force was determined to be about 199 N. These calculations have shown that the pull force is much higher than that required to produce pipettes. Also, since the torque is a function of the driving frequency, the pull force was reduced so as to not pull the capillary out of the clamp.

MATERIALS

The machine is fabricated from a rigid aluminum base and an acrylic exterior, as shown in Fig.1. The aluminum base provides a rigid yet lightweight structure to allow very repeatable pulls and still maintain a bench top scale. The prototype is controlled via an Arduino micro controller; this allows a vast range of input and output capabilities. Parameters are entered via a 3.5” touch screen panel that is both efficient and user-friendly. As we develop better controls for the machine the Arduino can be quickly updated to provide end users with the most up-to-date system.

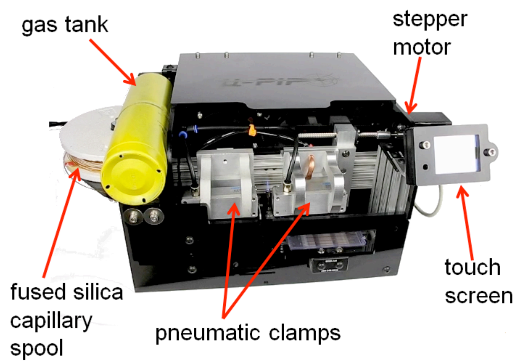

FIGURE 1. Photograph of a fully automated fused silica micropipette puller.

Our machine works off the principle of controlling pull velocity rather than specifying a fixed pull force. This is a much better control scheme because it allows direct control over what causes the capillary to break. The heated glass is a viscoelastic material that will yield when a certain strain rate is reached. By controlling the velocity of the pull the strain rate can be controlled.

EXPERIMENTAL RESULTS

Our device allows for many micropipettes to be created using the same parameters with no human interaction. Using this capability, we pulled seven sets of ten micropipettes each with varying pull velocity between each set. The length and diameter were then measured for each micropipette. The results for length and diameter can be seen in Fig. 2a and 2b respectively.

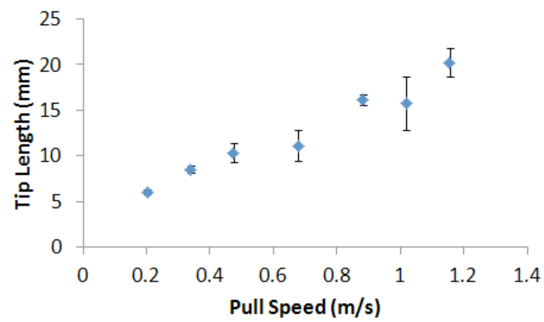

FIGURE 2a. Relationship between pull speed and tip length, with standard deviation.

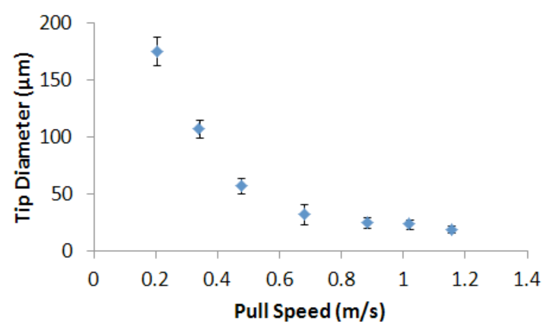

FIGURE 2b. Relationship between pull speed and tip diameter, with standard deviation.

As can be seen, the repeatability varies from 0.24 mm to 2.9 mm for the length measurement, and 12 µm to 3.5 µm for the diameter measurement. These plots show that a relationship can be established between both heating time and length, and heating time and diameter.

Another set of pulls was performed to try to obtain the necessary geometry of a useful pipette. The heating time was increased, and the result can bee seen in Fig. 3.

FIGURE 3. Microscope image of a micropipette pulled on our machine with a desirable tip geometry of less than 1 μm.

CONCLUSION

A device has been built to automate the manufacture of fused silica micropipette tips. It has been shown to be able to make the specified geometry required for electrophysiological experiments with relatively little deviation. A relationship between heating time and tip geometry has been determined. These results show that a device like this can be programmed to produce a specific geometry of micropipette tip, something that has never previously been accomplished. A user is able to input the capillary material, inner, and outer diameter. The machine is able to produce as many tips as necessary of a specified geometry based on this relationship. Such a device saves many hours of skilled labor.

REFERENCES

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