An Instrument for Controlled, Automated Production of Micrometer Scale Fused Silica Pipettes

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Micropipettes are hollow glass needles with tip openings ranging from less than 1 μm up to 75 μm. Based on the size of the inner diameter of the micropipettes, they can be used for applications such as patch clamping, microinjection, and cell transfer. In the state-of-the-art 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 from the increased speed, accuracy, and repeatability of an automated fabrication apparatus. We have designed, built, and tested a working prototype of a fully automated fused silica micropipette puller. Our device pulls pipettes from a continuous spool of capillary glass, which leads to minimized setup time for the operator and the ability to produce 6 micropipettes per minute. Micropipettes were pulled with average lengths ranging from 6–20 mm and average tip diameters ranging from 18–175 μm. Standard deviations for length and diameter were calculated to range from 0.24-2.9 mm and 3.5–12 μm, respectively. Through measurements of the pulled pipettes, a trend has been determined which shows higher pulling velocity increases tip length and decreases tip diameter. A new model for heat transfer and geometrical analysis for the heating and cooling of the pipettes has been developed and matches closely to this experimental data. This can be used to predict pipette geometry. [DOI: 10.1115/1.4004194]

1 Introduction

Glass micropipettes are used for many applications based on their tip inner diameter and tip length [1]. Once the desired geometry for a given application is known, it is important to be able to consistently reproduce it. Many labs utilize tens or hundreds of micropipettes per week and would greatly benefit from an automated device to produce micropipettes that still meets the demands for reproducible geometry. There are two basic ways of forming micropipettes: (1) chemical etching and (2) heating and drawing. Chemical etching involves dipping the end of a capillary into a solution of hydrofluoric acid (HF), which etches the glass to a fine tip [2]. The geometry of the tips depends on factors such as the organic solvent used with the HF, precise time control, and micrometer increment movement [3]. Current processes, which have only been able to create one micropipette at a time, take ~ 2 h to create a micropipette and involve many manual steps such as removing the polyimide coating, cutting the capillaries to length, and suspending them in solution [2]. Hydrofluoric acid is also extremely difficult to handle because of its corrosive nature, which often leads to rough surface finish. While this process seems to be adaptable to mass production of micropipettes, due to these inherent difficulties, it has not been done to date.

In the heating and drawing fabrication method, micropipettes are formed by first heating a hollow glass tube, and then applying an axial force that causes the tube to neck and break. This process consists of manually loading previously prepared lengths of capillary glass into a fabrication device and clamping them into place, most often by a set of thumbscrews. The two ends are pulled in opposite directions after a section of the glass is raised to the glass transition temperature [4] according to user specified pull force and heating time. The axial force necessary to pull the capillary apart is usually achieved via a hanging weight, solenoid [5], pneumatic actuator [6], or a 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. The operator is involved in loading a precut piece of capillary glass, clamping it into place, selecting a program that will produce the correct pipette geometry, and unloading the finished product. This process takes up to 1 min and only produces one or two pipettes at a time. Further, this production rate can only be achieved after the user is trained in operating the machine and a program is found which yields pipettes of a desired geometry. Both of these processes can take days to weeks, and if a new geometry is required, the operator must spend time creating a new program and measuring the pipettes to ensure they are correct. Clearly, a device capable of consistently and controllably producing identical micropipettes in an automated, continuous fashion would cut down on training and operating time.

The most important property of any micropipette-producing device is repeatability. Repeatability for micropipettes varies based on application. Tip inner diameter and repeatability for various micropipette applications can be seen in Table I.

As seen here, the lowest acceptable repeatability for micropipette inner diameter is 10% for holding pipettes. Therefore, this was determined to be the required repeatability. Also, for our device to be useful it must have a higher production rate than conventional methods. Therefore, our main functional requirements were a minimum of four pipettes per minute, a minimum desired diameter range of 1-100 μm that could be useful for different applications, and the ability to use the three most common micropipette materials: fused silica, aluminosilicate, and borosilicate.

2 Analysis

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

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Table 1 Repeatability for various micropipette applications [1]

Application	Inner Diameter Range (μm)	Outer Diameter Range (μm)	Repeatability (%)
electrophysiology (extracellular)	1–3	2	33
patch clamp	1–3	2	33
pronuclear injection	0.3–0.7	0.5	29
microinjection	15–25	20	20
intracytoplasmic sperm injection	5–15	10	33
nuclear transfer	5–15	10	33
holding pipettes	20–25	22.5	10
aspiration pipette	15–75	45	40

point compared to other glasses. However, devices do exist which are capable of generating temperatures greater than 1300 °C needed to pull fused silica glass. Currently, there are two methods for reaching this necessary temperature: a CO_2 laser, and a gas flame; typically butane gas. Our prototype employs a propylene gas torch due to the much lower cost and higher burning temperature, but this could easily be replaced with a laser in subsequent prototypes. Furthermore, we chose to focus on pulling fused silica because it is the hardest material to heat. Pulling the other two types of glass is achievable if fused silica can be pulled.

A conceptual model of the theoretical heating curve for pulling a micropipette was created and is shown in Fig. 1. In this model, the fused silica capillary is heated past the glass transition temperature, Tg, to a steady state value in duration t_1, the heating time. Next, the heater is shut off and the motor starts pulling the micropipette after a negligible delay, $t_2 - t_1 \approx 0$. From the time the motor starts pulling to the point at which the glass transition temperature is reached is the cooling time, $tc = t_3 - t_2$. This parameter, tc, determines the tip length and tip inner diameter. Once the glass transition temperature is reached, the two ends of the capillary are pulled apart.

A propylene gas jet heats the capillaries at about 1980 °C [7], a higher temperature than propane or butane. Based on this temperature, the dimensions of a typical capillary, fused silica properties [8], and propylene properties, a model for the heating time was created. The model consisted of first calculating the Grashof, Gr, and Reynolds, Re, numbers. Since it was found that the Grashof number was much smaller than the Reynolds number, forced convection dominates the heating.

Next, the Nusselt number, Nu, was calculated using the Churchill and Bernstein correlation [9] according to

$$\mathrm{Nu} = 0.3 + \frac{0.62\mathrm{Re}^{1/2}\mathrm{Pr}^{1/3}}{\left[1 + (0.4/\mathrm{Pr})^{2/3}\right]^{1/4}}\left[1 + \left(\frac{\mathrm{Re}}{282000}\right)^{5/8}\right]^{4/5} \quad (1)$$

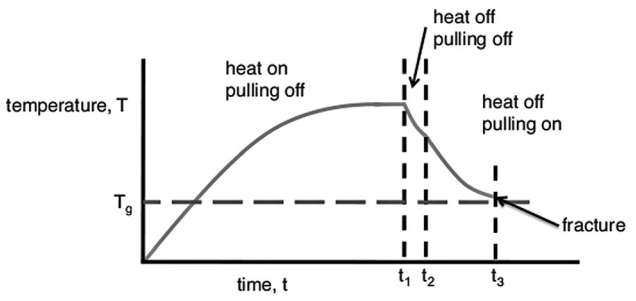

Fig. 1 A conceptual heating and cooling model for pulling a micropipette. The amount of time it takes to cool the pipette back to the glass transition temperature determines the geometry.

where Pr is the Prandtl number. From the Nusselt number, the convective heat transfer coefficient, h, was found using

$$h = \frac{\mathrm{Nu}k}{L} \quad (2)$$

where k is the thermal conductivity, and L is the outside diameter of the capillary. For our system where $k = 1.4$ W/mK, $L = 665$ μm, $\mathrm{Re} = 1.31$, and $\mathrm{Pr} = 0.647$ a convective heat transfer coefficient of 1760 W/m^2 was calculated. A model of the cooling time was also created, and the Nusselt number for cooling was found using the Churchill and Chu correlation [10] according to

$$\mathrm{Nu} = \left\{0.6 + \frac{0.387\mathrm{Ra}^{1/6}}{\left[1 + (0.559/\mathrm{Pr})^{9/16}\right]^{8/27}}\right\}^2 \quad (3)$$

where Ra is the Rayleigh number. The convective heat transfer coefficient for cooling was found as described above with the same parameters except $\mathrm{Ra} = 0.121$ for the prepulled geometry, $\mathrm{Ra} = 0.110$ for the pulled geometry and $\mathrm{Pr} = 0.728$. The heat transfer coefficients for cooling were found to be 1417 W/m^2 and 5190 W/m^2 for the prepulled and pulled geometries, respectively. These two geometries were used as upper and lower bounds since it would have been extremely difficult to create a heating model for the actual process of pulling. These three convective heat transfer coefficients were used in a COMSOL finite element analysis model to get an estimate of the heating time required to reach a steady state temperature of 1950 °C, and the amount of time it takes to cool from that temperature to the glass transition temperature of 1300 °C. For a fused silica capillary of 665 μm o.d. and 300 μm i.d., and the convective heat transfer coefficient found earlier, the time to reach steady state was found to be 4.01 s. The time to cool from that temperature to 1300 °C was found to be 0.059 and 0.031 s for the prepulled and pulled geometries.

From this heating model, a basic conservation of volume calculation can be used to predict pipette tip geometry. A simple model for the tip length can be created where the tip length is half the product of the pulling velocity and cooling time, as given by

$$L(v) = \frac{vt_c}{2} \quad (4)$$

where v is the velocity of the pulled pipette and t_c is the cooling time. The factor of one half is to account for the two tips created by each pull. From here, conservation of volume can be used to calculate the resulting diameter as seen in the following equation

$$V = \frac{\pi d_1^2}{4}L_0 = \frac{\pi d_2^2}{4}(L_0 + \Delta x) \quad (5)$$

where V is a constant volume, L_0 is the original length, Δx is the change in length, d_1 is the original diameter, and d_2 is the final diameter. Solving for diameter in terms of velocity yields

$$d(v) = \sqrt{\frac{L_0 d_0^2}{vt_c/2}} \quad (6)$$

where d_0 is the original diameter.

Pulling of the pipettes required two separate force calculations. It is known that a pull force of about 45 N is used by other devices to pull the pipettes [11]. We used this criterion as a baseline for designing the clamps for our device. The pneumatic clamps chosen [Festo, EV-20/75-5] are rated to provide a force of 600 N at the maximum stroke length and a pressure of 600 kPa. These clamping modules are covered in silicone to provide a higher coefficient of friction. Empirically, we determined from four

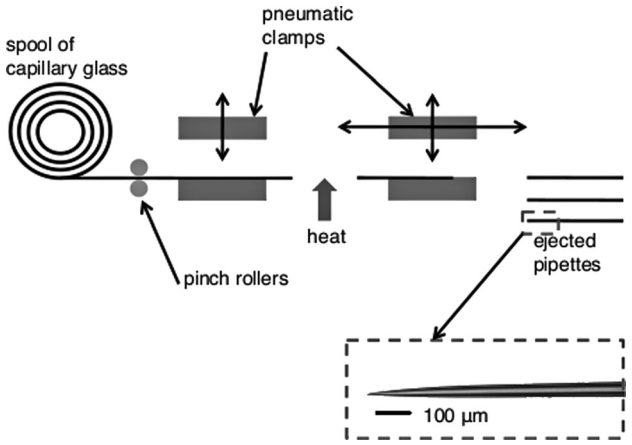

Fig. 2 The basic operation of a fully automated micropipette puller. The inset shows a microscope image of a pulled micropipette manufactured using our machine.

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 according to

$$F = \frac{2T}{d_m}\left(\frac{\pi d_m - \mu l}{l + \pi \mu d_m}\right) \tag{7}$$

where T is the motor torque. For values of $T = 1.0$ Nm, $d_m = 5.8$ mm, $\mu = 0.1$, $l = 1.0$ in/rev [12]. 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.

3 Detailed Design

3.1 Mechanics. As shown schematically in Fig. 2, 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. The finished micropipettes are ejected into a protective case with an elastomer pad, and the nonpulled end of the capillary is cut off.

Our device has six mechanical systems based on pneumatics and stepper motors. Feeding of the capillary spool, pulling of the pipettes, and the ejecting tray are powered by stepper motors. As mentioned previously, the clamps used to hold the capillary as it is being pulled are pneumatic clamps. A pneumatic piston is used to actuate the cutting wheel, and the same pneumatic line is used to blow the capillary into the ejection tray. A detailed photograph of these operations can be seen in Fig. 3.

The capillary is constrained between a vee-groove and the pneumatic clamp as shown in Fig. 4. The vee groove, with 90° angle, is a kinematic constraint for the cylindrical capillary with the normal forces acting through the centroid. This design allows for a wide range of diameters of capillary to be used.

Stepper motors are used because of their high torque and angular position control. The resolution of our stepper motors is 0.18° per step. This results in the capability to pull any length of micropipette within a 39.9 μm increment, a pulling displacement resolution of 12.7 μm, and a pull velocity up to 1.36 m/s.

The machine is fabricated from a rigid aluminum base and an acrylic exterior, as shown in Fig. 5. The aluminum base provides a rigid yet lightweight structure to allow very repeatable pulls and still maintain a bench top scale.

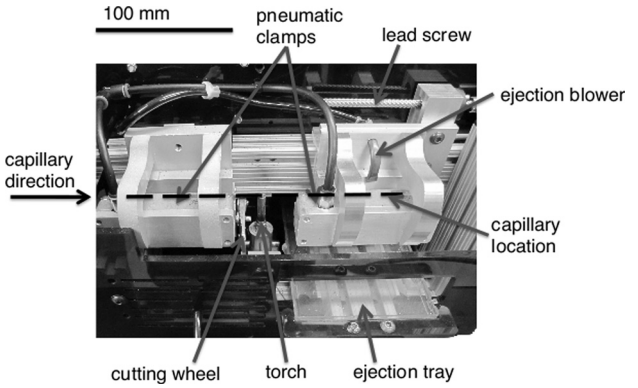

Fig. 3 Photograph of the main mechanical and thermal components of a fully automated micropipette puller

3.2 Thermal. As mentioned previously, there are two ways of reaching the 1300 °C glass transition temperature of fused silica glass: a CO_2 laser and a gas flame. Resistive wire elements composed of platinum and iridium or nichrome [Sutter, P30] are used for glass with lower melting temperatures but cannot be used with fused silica glass. Our initial concept for heating relied on a tungsten wire in an inert nitrogen environment to reach the required temperatures.

This was chosen at first because it could easily reach the melting temperature of fused silica, and tungsten wire is relatively inexpensive. The inert environment was necessary because of immediate oxidation that occurs when tungsten reaches the necessarily high temperatures. After initial bench testing; however, it was determined that the alignment of the capillary and tungsten wire, as well as the difficulty in maintaining an inert environment made a resistive wire too difficult. Therefore, a gas flame was chosen because of low cost and simplicity. The gas flame is manually adjusted with a needle valve when the machine is first turned on. After this, the flame is no longer adjusted by the operator and simply actuates in and out of the capillary's range to apply heat.

3.3 Control Hardware and Software. The prototype is controlled via a microcontroller [Arduino, AT Mega 328]; this allows a vast range of input and output capabilities. Parameters are entered via a touch screen panel, that is, both efficient and user-friendly. As we develop better controls for the machine the

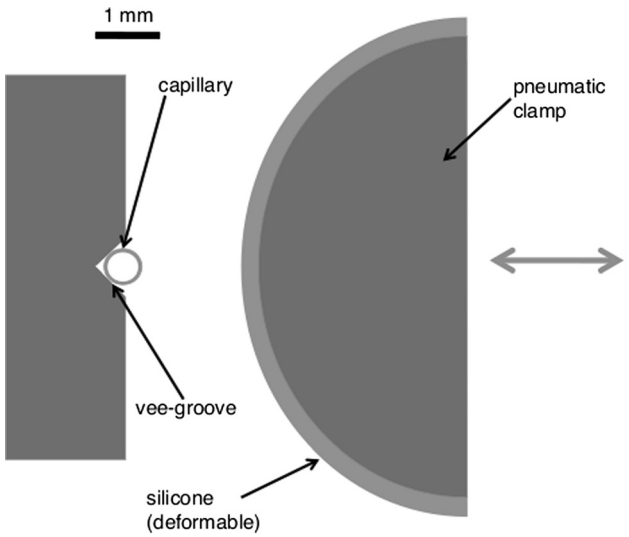

Fig. 4 Diagram of pneumatic clamp and vee-groove used to secure both ends of the capillary for pulling

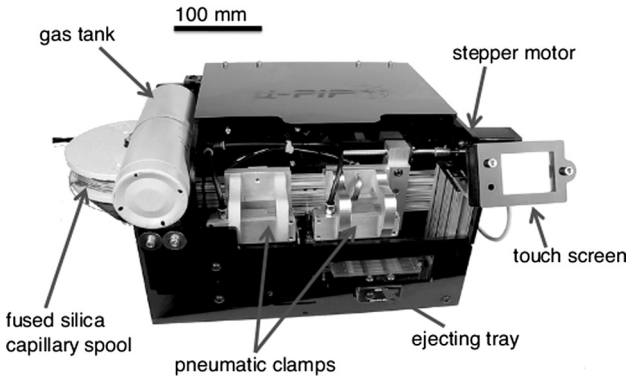

Fig. 5 Photograph of an instrument for controlled, automated, continuous production of fused silica micropipettes

microcontroller can be quickly updated to provide end users with the most up-to-date system.

Our pipette puller design relies on constant pull velocity rather than constant pull force. This is a much better control scheme than prior art because pull velocity directly controls the factors that cause 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. Furthermore, controlling the pulling velocity has a direct impact on the geometry as described previously. Given a cooling time and velocity, a tip length and diameter can be predicted. Varying the delay between consecutive steps of the stepper motor changes the velocity of the pull. There are 200 delays per revolution, which equates to a travel distance per delay of 127 μm. This resolution was found to be high enough to produce a smooth pulling operation.

Through the touch screen, the user enters the number of pulls, N, pipette length, heat time, t_H, slow pull speed, v_s, slow pull distance, P_s, fast pull speed, v_f, fast pull distance, P_f, and delay, t_d. The slow pull is the initial pull that begins to neck the capillary after it is heated, and the fast pull finishes the necking and breaks the capillary into two pipettes. The delay is the time between these two steps. Future versions of the software will have inputs for capillary diameter, material, and pipette geometry based on empirical models. A diagram of the sequence of events that the program runs can be seen in Fig. 6. The software runs open loop for simplicity. The program proceeds in a linear fashion, repeating itself for the number of pulls the user has entered. The other user inputs modify how fast and how long the motors are on, and how much of a delay there is between the slow and hard pull.

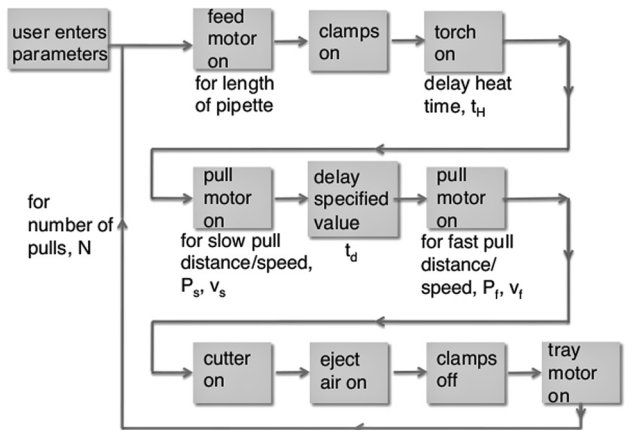

Fig. 6 The sequence of events that the pipette pulling program runs. The user inputs the number of pipettes, *N*, length, heat time, t_H, slow pull speed, v*s*, slow pull distance, P_s, fast pull speed, v_f, fast pull distance, P_f, and delay, t_d.

4 Experimental Results

Characteristic dimensions of micropipettes include tip length and inner diameter. Therefore, we have measured the tips created through runs of different pull velocity. For each run of micropipettes produced under a set of parameters we computed the mean and standard deviation of length and diameter. This has led to a characterization of the relationship between pull velocity and tip geometry. This relationship leads to the ability to eliminate the time consuming process of reprogramming a pipette puller for new tip geometries. Measurements were performed using a microscope [Leica, MZ16 F] and a stage micrometer with 10 μm resolution.

Our prototype has a maximum production rate of 6 micropipettes per minute. This triples the production rate of current nonautomated pullers and is also significantly faster than etching micropipettes. 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, varying pull velocity between each set. The heating time was set to 14 s after experimental testing showed that anything below about 10 s would yield poor results. This is a reasonable fit to our heating model, which says that greater than 4 s of heating is needed to reach a steady state temperature that provides a sufficiently long cooling time to pull pipettes. We did not verify the force of the pulls, but we did not notice any slip of the capillaries in the clamps. The inner diameter can be measured because the transparent glass allows visualization of the inside of the pipette tip. In addition to this, three micropipettes from each of the indicated samples shown in Fig. 8 were analyzed under a SEM [FEI, Nova Nanolab 200 FIB/SEM] to verify measurements and observe tip geometry. The results for length and diameter can be seen in Figs. 7 and 8, respectively.

As can be seen in Fig. 8, the pulling velocity has a direct impact on the micropipette geometry. For a low velocity, the capillary cools below the glass transition temperature before the machine finishes pulling it. Therefore, the tip fractures, leading to a jagged edge, inconsistent pulls, and unusable micropipettes. However, with increased pulling velocity, the capillary is pulled before it cools down below the glass transition temperature, and a smooth, consistent, usable micropipette is achieved. The repeatability varies from 0.24 to 2.9 mm for the length measurement, and 12 to 3.5 μm for the diameter measurement. This results in repeatability to be within approximately 16% and 9% for the diameter and length, respectively. These plots show that the relationships

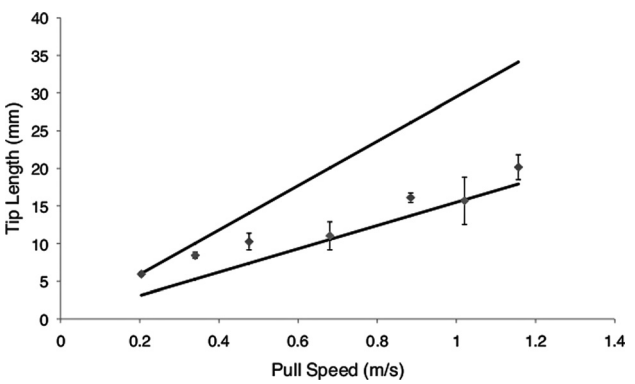

Fig. 7 Relationship between pull speed and micropipette tip length, with standard deviation. $N = 10$, $t_H = 14$ s, $v_s = 0.27$ m/s, $P_s = 2.54$ mm, $P_f = 22.9$ mm, $t_d = 70$ ms, and a varied v_f as indicated. The solid lines indicate the upper and lower bounds achieved using our thermal model and Eq. (4).

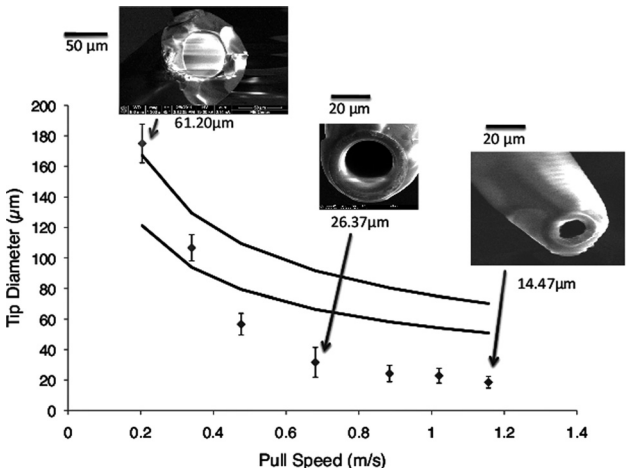

Fig. 8 Relationship between pull speed and tip diameter, with standard deviation. $N = 10$, $t_H = 14$ s, $v_s = 0.27$ m/s, $P_s = 2.54$ mm, $P_f = 22.9$ mm, $t_d = 70$ ms, and a varied v_f as indicated. Electron microscope images of three micropipettes verify the tip diameter and also show the results of velocity on micropipette geometry. The solid lines indicate the upper and lower bounds achieved using our model and Eq. (6). Deviation between the models and experimental results may be attributed to the complex nature of cooling, and the simplifications used in our conservation of volume analysis, but the model fits well.

previously determined for heating time and tip geometry are a good fit for the experimental data. Based on the cooling time and pull velocity, tip length and diameter can be reasonably predicted. The large error of the less heated pipettes is due to the small number of pulls performed and because low heating time resulted in unpredictable fracture.

As mentioned previously, desirable micropipettes are in the range of less than 1 μm up to 75 μm. Although we could not consistently achieve micropipettes with inner diameters of less than 1 μm, we were able to achieve useful pipettes. These can be used for a variety of procedures including microinjection, nuclear transfer, and aspiration. Furthermore, improvements to the heating method should result in more repeatable geometries and greater decrease in inner diameter.

5 Conclusions

An instrument has been designed, built, and demonstrated 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 standard deviations of 0.24 to 2.9 mm and 3.5 to 12 μm for the length and diameter, respectively. It has been shown to have the ability to produce 6 micropipettes per minute with geometries in the range of 6 to 20 mm for the length and 18 to 175 μm for the diameter. This represents repeatability of about 16% for the diameter and 9% for the length. A novel relationship between pull velocity and tip geometry has been developed using a heat transfer model and conservation of volume. This has been verified experimentally for both tip length and inner diameter. These models are based on a constant pull velocity, something that other machines cannot achieve. Also, if a nonconstant pull velocity was required for a different geometry, this could easily be achieved with our machine. These results show that a device like this can be programmed to produce a specific geometry of micropipette tip in an automated fashion, something that has not previously been accomplished. Such a device saves many hours of skilled labor.

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