Fabrication of a Pipette System using Rapid Prototyping

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Abstract

The purpose of this research is to explore alternative pipetting methods in order to give biochemists tools which will accelerate biochemical research. The practices of biochemists and laboratory devices were examined in order to develop a pipette system which can fill all wells of an 8x12 microplate simultaneously while delivering a different volume to each row. A twelve tip precursor device was made using rapid prototyping to test the effectiveness of the pipetting method. The device functions using air displacement to create a vacuum which aspirates liquid into standard disposable sterile tips with which the device is compatible. The device contains 12 rows of spring-loaded syringes limited by a tilted beam to vary the volume delivered in a linear fashion from row to row. The pipetting approach allows a researcher to set a dial for an upper and lower volume limit, which adjusts the height of each end of the beam. Ninety-six well microplates are frequently used in cell biology and combinatorial chemistry research, but filling such plates is slow and tedious. It is anticipated that this research will lead to reduced shoulder and thumb overuse injuries such as carpal tunnel syndrome and tendonitis, prevalent in lab technicians who operate pipettes. Furthermore, it has the potential to increase throughput by simultaneously delivering different volumes of a solution to each row of a 96-well plate and increase experimentation effectiveness by allowing simultaneous delivery of accurate reagent concentrations.

1. Introduction

The objective of this research is to use rapid prototyping to evaluate a new pipetting method which can accelerate biochemical research. Observation of the biological and chemical laboratory setting reveals that this can be achieved by creating a device to deliver a different amount of solution to each row of a 96-well microplate simultaneously. The pipetting method would save time and money as well as prevent overuse injuries in laboratory technicians. It would also enable researchers to more accurately study time-dependent reactions. Technology pertaining to mechanical engineering and rapid prototyping can be applied to the biochemical field to aid researchers by improving laboratory methods.

1.1. pipettes and ninety-six-well miroplates

In bioscience laboratories, micropipettes are used to deliver small quantities of precisely measured liquid . Many operate using air displacement to aspirate, or draw up, liquid and to dispense it. Nearly all micropipettes operate using disposable tips to prevent cross contamination [1]. To operate a micropipette with an attached disposable tip, a researcher first depresses a button on the end of the device to evacuate air from the tip. Then the point of the disposable tip is submerged in a tray of solution. While the tip is submerged, the button is released, drawing liquid into the disposable tip only. Liquid is dispensed when the button is depressed, and the disposable tip is ejected with the push of a second button.

Ninety-six-well microplates are used in laboratories worldwide to organize cell cultures and chemical reactions. Consisting of 96 miniature cups, they keep experiments together and allow them to be moved easily. The wells are arranged in an 8x12 rectangle, and the plates come in one internationally standard size [2].

1.2. existing devices

Single pipettes deliver fluid to one well, while 8 or 12-tip pipette wands deliver a uniform amount of fluid to an entire row of a microplate. A device also exists which can deliver a uniform amount to each well of a 96-well microplate simultaneously [3]. However, many experiments call for a varying amount of a reagent to be delivered to each row [4]. This can be accomplished using a pipette wand, but the wand cannot deliver solution to all rows simultaneously.

Simultaneous filling is crucial to research of time-dependent reactions, and it also reduces error rates and the time to set up experiments. Robotic microplate fillers are capable of delivering a different amount of solution to each well simultaneously, but only the largest laboratories like those of a pharmaceutical company can afford robots [5]. Thus, researchers at average laboratories like those of a hospital or university are required to pipette one well or row at a time.

1.3. afflictions of lab technicians

Occupational Safety and Health Administration (OSHA) named cumulative trauma disorders (CTD's) to be the largest single category of injuries in the 1990s [6]. Expenses from CTD's including compensation, lost time, and lost productivity total approximately $120 billion per year [7]. Biochemical research laboratories are not without these disorders. In addition to muscular overuse injuries in the hand, wrist, arm and shoulder, five types of tendonitis inflict lab technicians [8]. Repetitive pipetting has also been linked to an increased risk of carpal tunnel syndrome [9]. In 1996, the National Institute of Occupational Safety and Health (NIOSH) called pipetting a "biomechanical hazard" [10].

These disorders stem from the posture, force, and repetition of the pipetting motions, and decreasing any of these factors will reduce the risk of CTD's [11]. Pipette manufacturers have made improvements in ergonomics to improve technician posture and reduce the force required to operate pipettes but very few have sought to reduce overuse injuries by reducing pipetting repetitions. It has been found that some laboratory technicians pipette as much as 12,000 times per day [12]. This research aims to reduce the number of repetitions lab technicians must make by allowing the entire microplate to be filled simultaneously rather than row by row or well by well. This could potentially spare the lab technician 95 repeated motions each time a microplate is filled.

1.4 need for new method

A new pipetting method which enables a researcher to deliver a different amount of solution to each row of a microplate simultaneously will increase the rate at which chemical and biological research is conducted by decreasing the amount of time researchers spend pipetting. It will also enable researchers to perform more accurate time-dependent experiments by allowing reactions with varying amounts of reagent throughout the microplate to begin simultaneously. Furthermore, the proposed method is versatile, with the capability to deliver a uniform amount to all wells of the microplate, an even more common task in laboratories than varying the volume [13]. David Mitchell, lab manager of the biochemistry department at the Medical College of Wisconsin, emphasized the importance of such a method to aid in the department's ongoing cancer research [14]. Because the method will allow lab technicians to fill entire microplates at once, experiments can be performed faster and more data can be gathered in the same amount of time, improving the rate and quality of research performed.

It has been established that a device with the capability to deliver liquid simultaneously to all wells while delivering a different amount to each row does not currently exist in an affordable price range for most laboratories. One of the largest providers of pipettes, Titertek, would have to custom-build such a device at a cost of $38,000 for a manually-operated device or $70,000 for a robotic device [15]. It is estimated that the implementation of the method developed by this research would cost around $1000 to produce [16]. As a result of this relatively low cost, the technology and time-saving capacity are made available to all laboratories.

1.5 rapid prototyping

Rapid prototyping is a technology which is used to create three-dimensional solid objects from computer aided design (CAD) files on a computer. All rapid prototyping in this research project was conducted at the Rapid Prototyping Center (RPC) of the Milwaukee School of Engineering (MSOE). The two types of rapid prototyping utilized in this research project are the Stereolithography Apparatus (SLA) and Selective Laser Sintering (SLS) process. The SLS process, shown in figure 1, uses a CO_2 laser and scanner to selectively sinter, or fuse, a Duraform™ polyamide powder layer by layer to build up an object. Once a layer has been sintered, the object is lowered and a roller is used to spread a fresh layer of powder on top of the object. Each layer is sintered to the layer below it, and the surrounding powder supports the object while it is being built. In this way, 3D objects of any geometry can be created [17].

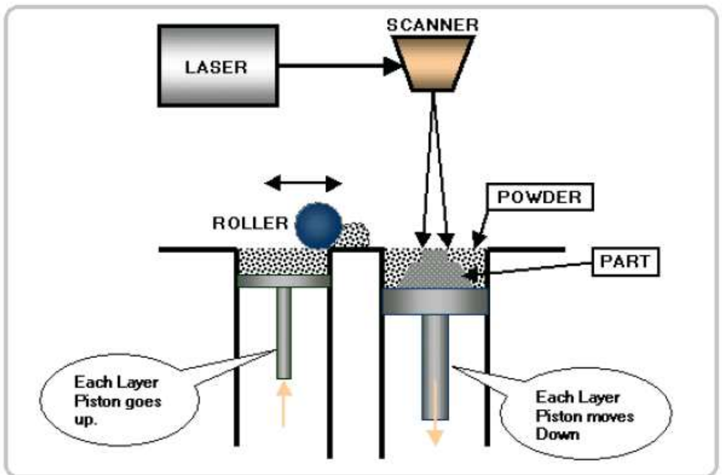

Figure 1 SLS process

The SLA operates in a similar manner to the SLS, with a UV laser curing an object layer by layer within a vat of photopolymer. Each time a layer is cured, the part is lowered and a layer of uncured photopolymer is applied to the top. The SLA is extremely accurate, building to within ±.005 inches, and known for its capability to handle intricate part geometries. The SLS on the other hand fabricates parts that are much stronger than the SLA but are not as accurate [18]. Due to the varying properties of the parts produced, both rapid prototyping methods were utilized in this research project.

2. Methodology

2.1. pipette mechanics

Critical elements of pipettes were researched in literature as well as by disassembling a number of existing pipettes to observe the inner mechanisms. In this way, a general understanding of pipette mechanics was obtained. Pipettes are incredibly simple in design and structure, inspiring the simple mechanical methods employed in this research. The vast majority of pipettes use air displacement from piston/cylinder combinations to draw up and to dispense liquid. Springs allow the button of a pipette to come back up after the user releases it. Dials linked to screw mechanisms are used to adjust the stroke length of the pistons, thereby adjusting the volume delivered by the pipettes.

2.2. twelve tip trial method

Before the method of delivery to all 96 wells of a microplate was attempted, a variable-volume 12-tip device was created to test the effectiveness of the proposed method. A beam limits the range of 12 spring-loaded pistons so that the volume delivered varies linearly. A linear arrangement of volumes is a common practice for researchers, and considerably simplifies the method.

Figure 2 depicts the proposed method. The 12 tips are carefully modeled to be compatible with common disposable pipette tips. Each tip then corresponds to one of 12 cylinders. The air displacement mechanism of the pipette is accomplished by pistons which move in and out of the cylinders, drawing fluid in and out of the attached disposable tips. The seal of the piston is maintained by an o-ring embedded in the cylinder and held in place by an internal snap ring. Each of the pistons is attached to a piston head which guides the piston and ensures alignment with the cylinder by residing within a track on the inside of the casing. Each piston is surrounded by one of 12 identical springs which cause the piston to be at its highest allowed position. The highest allowed position of the piston is limited by a tilted beam which is the truly revolutionary aspect of this method. The beam provides the linear variation of volumes along the length of the pipette. The springs also cause the beam to always be at its highest allowed position which is limited by the dials and screws. The dials provide an easy way for the user to specify the highest and lowest volume delivered by limiting the height of the beam and in turn the height of the pistons. When the user depresses the button, the rod will push the beam down. The beam will in turn depress the pistons until they are all fully lowered and all of the liquid has been released. The pin joint between the rod and beam allows the beam to swivel, being angled when it is in contact with the dials and horizontal when it is fully depressed. In order to deliver a uniform amount of liquid to all wells of the plate, both dials could be set to the same volume.

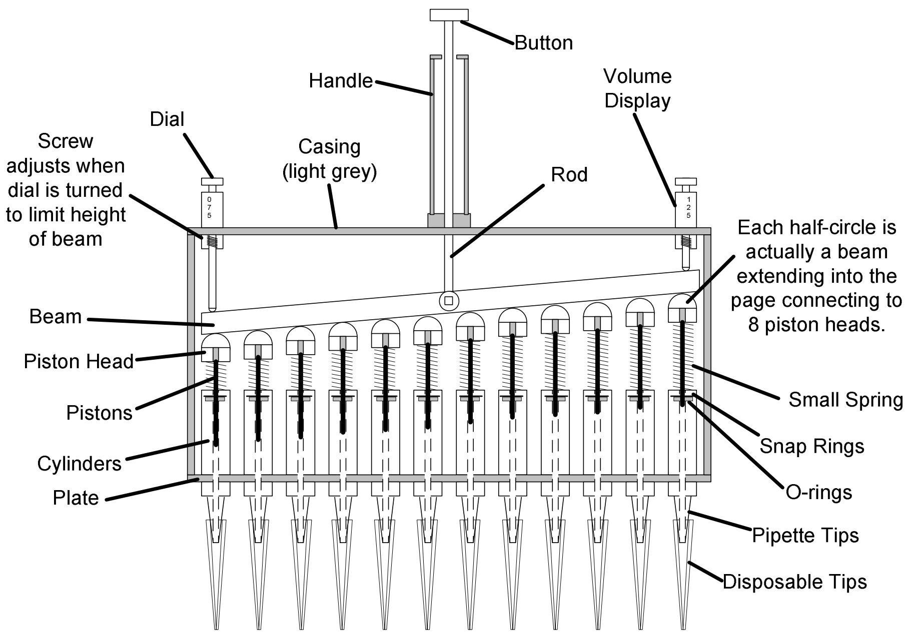

Figure 2 Labeled drawing of pipetting method

The twelve-tip device was modeled in Solidworks, as shown in figure 3, and the majority of parts were created by the RPC at MSOE. The SLA was used to fabricate one part consisting of the tips, cylinders, and plate while the SLS process was used to fabricate the casing, piston heads, beam, and rod of the device. The SLA was used for its incredible accuracy. Accuracy was needed to accommodate disposable pipette tips as well as the o-rings and snap rings housed within the cylinders. The SLS technology was needed for the strength of the parts produced, as the casing, rod, and beam experience the highest loads during operation. The pistons of the device were machined from stainless steel and polished to reduce friction as much as possible. All other parts of the device were purchased from an outside vendor.

Shrink tubing and superglue connect the stainless pistons to the SLS piston heads. Two layers of tubing provided some flexibility, enabling the pistons to be self-aligning, and the porous SLS material absorbed superglue so that only the outer layer of shrink tubing was rigidly anchored. Figure 4 shows the completed prototype.

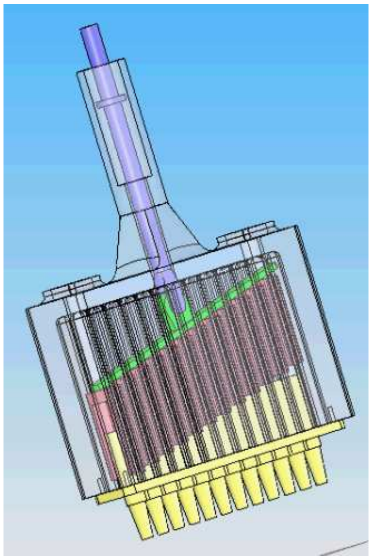

Figure 3 Solidworks model of twelve tip device

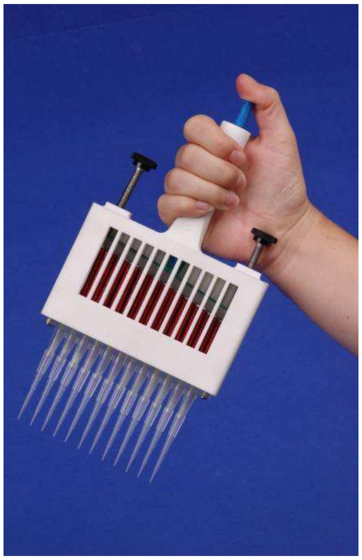

Figure 4 Twelve tip device fabricated using rapid prototyping

2.3. evaluation of twelve tip method

The twelve-tip device was assembled and does in fact deliver varying volume from each tip. However, it is extremely difficult to operate due to the twelve springs used to hold the pistons up. With refinements in the spring constant, the device might become easier to operate. Also, the entire configuration of the device might be changed so that pipetting force could be exerted by other parts of the hand.

2.4. ninety-six tip method

Though the twelve tip method needs improvement, a preliminary 96 tip method was conceived. The method consists of a 96-tip pipette with an 8x12 array of tips and pison/cylinder pairs, one for each well of a microplate. In each row, all eight pistons are connected to a single piston head, ensuring that all tips in each row will deliver a uniform amount simultaneously. Two springs are used to independently spring-load each row to ensure that the piston head stays horizontal and is in constant contact with the beam. The method also includes a stand to help in correctly aligning the pipette with all 96-wells of a plate.

Again the physical concept for the method was modeled in Solidworks and can be seen in figure 5. The proposed method is theoretically effective, but requires improvement if the method is to benefit the biochemical research community.

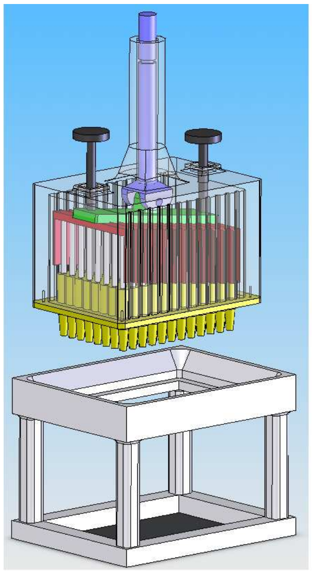

Figure 5 Solidworks model of 96-tip pipette and stand

3. Accuracy of linearly varying volumes

The linearly varying volumes of the twelve tip method, seen clearly in figure 6, were tested to determine the accuracy of the prototype. Linear variation and reproducibility are extremely important in the proposed pipetting method because both are critical to biochemical research.

Twelve eppendorf tubes were used to receive liquid from the twelve tips of the device so that distilled water could be pipetted and weighed. First the eppendorf tubes were numbered and individually massed on an Ohaus® Galaxy™ 160D analytical balance. Then the twelve tip device and disposable tips were used to repeatedly pipette distilled water into the eppendorf tubes. After each of four pipetting repetitions, the eppendorf tubes were massed to determine how much water had been added with each repetition. Dividing the difference in mass for each repetition by the density of water yielded the volume delivered in each iteration from each tip.

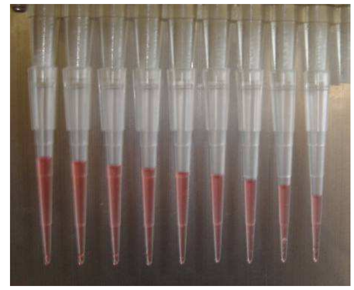

Figure 6 Twelve tip method and visible linear volumes

The experiment yielded four sets of data, each representing a single pipetting repetition. Figure 7 shows a plot of the volume delivered and a line of best fit for one of the iterations. Volumes between 20-100µL were delivered in this experiment, but other linear volume ranges are possible, including a uniform distribution. The equation of the line is also shown on the plot, and can easily be found once the method has been reduced to practice and calibrated. From the specified lowest and highest volumes, the researcher can determine the equation of the volume distribution line and thereby the volume delivered to each well.

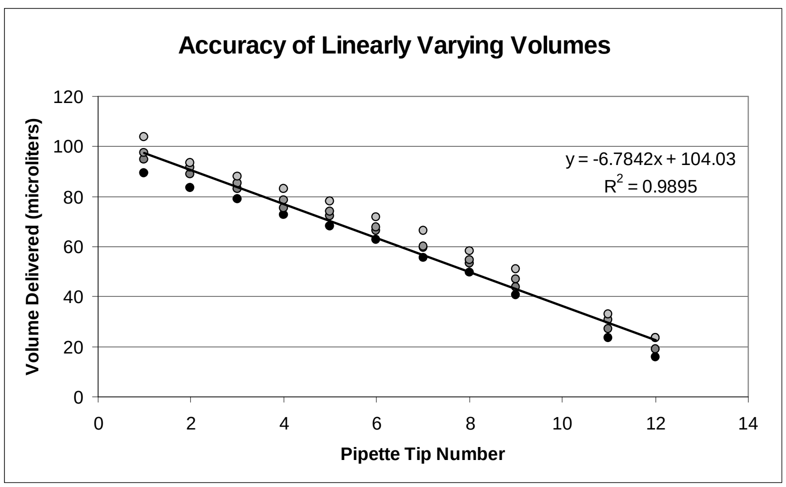

Figure 7 Linear regressions of four pipetting trials with the twelve tip device

Overall, the very high correlation coefficient reveals that the data adheres closely to a linear distribution of volumes. The close proximity of the four data sets also indicates accurate repeatability of volumes. The dials of the device were left in the same position for all iterations of the experiment, but may have shifted slightly from repeated contact with the beam. As a result, the four data sets are not completely coincident, but repeatability will improve with calibration.

Inaccuracies were expected to a certain degree due to the difficulty of operation and the slight incompatibility of the prototype with disposable tips. Furthermore, the beam of the prototype is visibly warped, and the plot of volumes reveals a slight curve in the same shape as the beam. A stronger beam would produce a strictly linear volume distribution. Also, tip number ten of the prototype is nonfunctional and was therefore not included in the data collection.

4. Conclusion

The spring-loaded pistons and limiting beam of the pipetting method provide an effective mechanism for linearly varying volume and simultaneous delivery while dials also allow the volume delivered to be easily adjusted.

The pipetting method is extremely simple, harnessing mechanical concepts for a biochemical laboratory application. The simplicity provided by the internal beam causes the method to be much more affordable than any robotic or custom-built devices available, making it available to nearly all laboratories. Simultaneous filling and improved accuracy of time-dependent experiments made possible by this pipetting method will save researchers time and money as well as reducing the number of CTDs in laboratories of all sizes. Improvements in ergonomics and ease of operation need to be made before the method can be readily used in laboratories, but it has the potential to bring advanced pipetting options to smaller laboratories and accelerate the rate of biochemical research and development. Pipetting methods have not kept pace with the explosion of biochemical research taking place today but with this type of improved laboratory method, research will continue to grow and accelerate.

5. Recommended Future Work

In order to improve the pipetting method, attention needs to be paid to ergonomics, ease of operation, and sustained calibration. Refinements should be made in the shape of the tips so less force is required to attach disposable tips, and a tip-ejection mechanism should also be included. Since the twelve tip prototype is so difficult to operate, a mechanical or powered assist should be added so that the 96 tip device improves pipetting methods in the biochemical community.

With concentrated research of the biochemical laboratory setting, different versions of the device could be created with improved performance in specific applications. This research also allows expansion for a 384-tip device to deliver variable volumes to each row of a 384-well microplate. The improvements could extend beyond a linear progression of volumes such that a user could dial a volume for every single row of a microplate. Also, “beams” of varying geometry might allow quadratic or exponential variation of volumes across a microplate. A plane and four dials might also be used to limit 96 independently spring-loaded pistons if a variation of volumes in two directions is desired.

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7. References

1. Interview with Dr. Vipin Paliwal, July 18, 2006
2. Ibid.
3. V&P Technologies, “The 96 Channel Manifold Microplate Filler VP 177AD”, http://www.vp-scientific.com/plate_filling_systems.htm, June 8, 2006
4. Interview with Dr. Vipin Paliwal
5. Ibid.
6. Michael Wagner, “Applying Ergonomics for Injury-free Pipetting,” http://www.vistalab.com/pdf/BST102005.pdf, July 13, 2006
7. Ibid.
8. Kevin J. Costello, “Tendonitis: An Often Painful Occupational Condition for Laboratorians,” www.LaboratoryEquipment.com, July 13, 2006
9. Vistalab Technologies, www.ovationpipette.com/commoninjuries.asp, July 10, 2006

10. Wagner, “Applying Ergonomics.”
11. Mar-Lee McKean et all, “Pipette Design and Cumulative Stress Disorders: Radical Ergonomic Improvements Needed to Prevent Injury,” http://www.vistalab.com/pdf/PittConPoster1.pdf, July 13, 2006
12. Wagner, “Applying Ergonomics.”
13. Discussion with Dr. Vipin Paliwal, June 2006
14. Correspondence with David Mitchell, June 8, 2006
15. Phone call to Marc DeLuca, Regional sales representative of Titertek, July 14, 2006
16. Discussion with Dr. Vipin Paliwal
17. Rapid Prototyping Consortium, Milwaukee School of Engineering, “Rapid Prototyping Machines – Selective Laser Sintering,” http://www.msoe.edu/rpc/sls.s html, July 20, 2006
18. Rapid Prototyping Consortium, Milwaukee School of Engineering, “Rapid Prototyping Machines – Stereolithography Apparatus,” http://www.msoe.edu/rpc/sla.shtml, July 20, 2006