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Effect of process parameters of the positive displacement pump for extrusion based bioprinting application

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Abstract

Bioprinting is an emerging technology that provides the ability to fabricate customized tissue constructs. One of the most common methods is Extrusion-Based Bioprinting (EBB) which allows extruding high viscosity materials at a low cost. While currently available EBB methods provide an acceptable extrusion process, several disadvantages remain a challenge, such as low extrusion accuracy in terms of start and stop accuracy. The present study aimed to explore the relationship between the extrusion accuracy and syringe features, including plunger rubber compressibility and the amount of volume inside the syringe. The weighing scale and the flow rate sensor are used to assess the extrusion accuracy. Results show that the plunger rubber has a negative impact on extrusion accuracy due to its compressibility. Likewise, when the amount of compressed volume inside the syringe increases, the start and stop accuracy of extrusion decreases. The time required to reach desired flow rate was measured as 17, 25, 29 seconds (±0.5) for 20, 30 and 40 ml filled syringe volumes, respectively. The findings can contribute to a better understanding of the positive displacement pump and the extrusion accuracy relationship. As a result of these investigations, suggestions for a better extrusion pump were identified for future research.

Keywords: Additive manufacturing, 3D printing, Bioprinting, Extrusion-based bioprinting, Direct ink writing

1. Introduction

Additive manufacturing (AM) or 3D printing has gained significant attention among the hobbyist, academia and industry in the last decade. AM is a layer-by-layer fabrication method from a computerized 3D model using materials in the form of liquid, solid, powder, gel and paste.

Bioprinting, a branch of additive manufacturing (AM), is in demand technology to fabricate tissues and organs due to its advanced controlling features of the fabrication environment [1]. The common methods in bioprinting are Droplet-based bioprinting, laser-based bioprinting and extrusion-based bioprinting (EBB) using biomaterials (hydrogels and/or cells) [2].

EBB attracts great attention due to its ability to extrude high viscous material (range from 300 to 30000 cps) with necessary precision for various applications [3]. In the EBB applications, syringe pump based extruders are the main choice due to their uncomplicated usage and easy to clean attributes. While this method provides acceptable extrusion, several disadvantages remain a challenge, such as material leakage from the nozzle, extrusion precision and cartridge volume restriction.

In the bioprinting literature, a relatively small body of research is concerned with these challenges in terms of controllability of the flow and the effect of extrusion parameters on printability. The printability of hydrogels was studied regarding the effect of material properties and printing speed, and the results of studies evaluated by printed lines and scaffold [3], [4]. In addition, one study suggests that the compressibility of plunger rubber of plastic syringe has a negative effect on extrusion accuracy in terms of start and stop accuracy, which results in delayed extrusion start and material leakage after stopping the extrusion [5]. However, the behaviour of fluid flow still requires more attention to understand other effects stem from the extrusion unit, such as the effect of compressed volume in the syringe.

While the EBB is used by tissue engineering researchers, the same syringe pump based extrusion method is used in various research areas, including the direct-ink-writing (DIW) of ceramic pastes, 3D food printing, microdispensing of conductive inks and adhesive dispensing [6]–[9]. Thus, more detailed research for the controllability of the flow can be found in the related research areas.

One of the most in-depth theoretical fluid flow modelling of the syringe based extrusion was reported by Chen and Kai (see Equation 1) [9]. The relationship between flow rate and plunger velocity can be seen in Equation 1, where Q is the flow rate, X is the plunger position, A_p is the cross section area of the plunger, A_n is the cross section area of the needle, ρ is the fluid density, Ln is the needle length, V_0 is the initial fluid volume in the syringe, B is the bulk modulus, Q_u is the flow rate for a power-law fluid under a unit pressure, depending on the fluid flow behaviour and the needle geometry. The equation shows that the extrusion

accuracy is not only affected by the material properties but also affected by the compressed volume inside the syringe. In addition, it shows that EBB researchers should carefully consider the effect of fluid compressibility (Bulk modulus).

$$\frac{Q(s)}{\dot{X}(s)} = \frac{A_p}{\left[\frac{\rho L_n (V_0 - A_p X)}{B A_n}\right] s^2 + \left[\frac{V_0 - A_p X}{B Q_u}\right] s + 1} \quad (1)$$

The effect of compressed volume can also be understood from the research that compares the syringe based pumps with other methods, including needle valve and progressive cavity pump (PCP). This kind of comparison can be seen in recent articles from the DIW and bioprinting research areas [10], [11]. A recent study by Fisch et al. (2020) compares the performance of a pneumatic syringe based pump and progressive cavity pump (PCP) [11]. It can be clearly understood from the research that the usage of PCP increases the precision and accuracy of the printed object due to the constant compressed volume at the outlet of the pump.

This research aims to investigate the effect of plunger rubber compressibility of a plastic syringe and the effect of the amount of compressed volume inside the syringe on extrusion accuracy.

2. Material and methods

2.1. Materials

Tap water was used as a calibration material for syringe pumps, and it was used to validate in-house built large volume syringe pump (LVSP).

Pharmaceutical grade glycerol (100%v/v) was purchased from Boots (UK). It was selected due to the similar viscosity to biomaterials used in bioprinting. Glycerol viscosity ranges from 644.2 to 1487 cps at the temperature ranges from 19.74 to 29.44 °C and the suitable viscosity range for extrusion-based bioprinting is defined as the range from 300 to 30000 [3], [12].

2.2. Syringe pumps

The Harvard Apparatus PHD2000 syringe pump has ±1% accuracy and repeatability. It has a built-in control board, and by adjusting the target volume and flow rate according to syringe size, the required volume is extruded.

In-house built LVSP was made by laser cutting and FFF type of additive manufacturing material processing methods. It contains NEMA 23 stepper motor and the Duet 2 Wi-Fi control board to send G-codes via computer for controlling the stepper motor.

2.3. Syringes and needles

The 5ml and 60ml BD plastic syringe, and 50ml glass syringe (SAMCO) were used. The glass syringe was used to eliminate the rubber compressibility effect on the flow rate.

The tapered plastic needle was used with an internal diameter of 0.41 mm.

2.4. Flow sensor

Sensirion SLF3S-0600F liquid flow sensor was used in the flow rate measurement experiment. It can measure the flow rate with ±5 % accuracy and repeatability.

The sensor was calibrated to water, isopropyl alcohol and acetone. Therefore, the required calibration for glycerol was made by measuring the weight of extruded volume and comparing the sensor data.

2.5. Weighing scale

Kern PFB 200-3 precision balance was used to measure the accuracy and precision of extrusion. It can measure with ±0.001% accuracy.

2.6. Experimental setup

There are two experimental setups (see A in Figure 1) with the LVSP and the PHD2000 pump. Due to the PHD2000 cannot handle the 60ml syringe, the LSVP was used in the extrusion quality test to see the effect of volume on extrusion. Therefore, the PHD 2000 was used to validate the accuracy and precision of the LSVP by experimenting with extruded volume measurement.

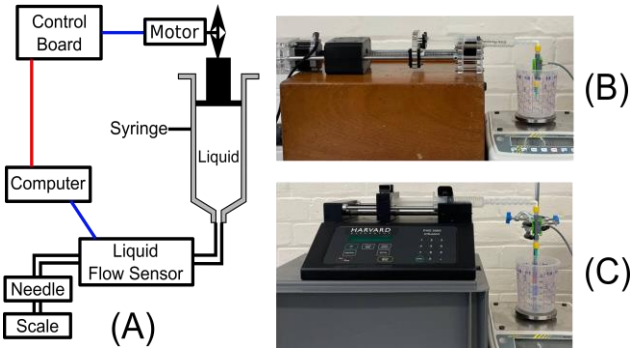

Fig 1. Diagram and demonstration of experimental setups. A) Experimental setup diagram for the LVSP and Harvard Apparatus PHD2000 Infusion syringe pumps. Blue and red lines show the connections and, the red line exists only in the LVSP. B) The LVSP with the sensor and weighing scale. B) The PHD 2000 with the sensor and weighing scale.

2.7. Validation of the LVSP

The experiment process parameters can be seen in Table 1. Syringe pumps were configured using these parameters.

The experiment begins with filling the 5 ml syringe with water and removing the air bubbles kept inside the syringe. Subsequently, the syringe was placed in the used pump and theoretical volume extruded, then measured with the precision scale. Experiments were conducted ten times for each pump and at 28 °C ±1.

Table 1. The experimental parameters of the LSVP validation.

Parameter	Value
Flow rate	0.5 ml/min
Theoretical extruded volume	0.5 ml
Needle diameter	0.41 mm
5 ml plastic syringe diameter	12.06 mm

2.8. The effect of plunger rubber compressibility of a plastic syringe on extrusion accuracy

The effect of plunger rubber compressibility on extrusion was investigated using the LVSP experimental setup. The experiment process parameters can be seen in Table 2.

The experiment begins with filling the used syringe with 20 ml glycerol and removing air bubbles kept inside the syringe. Subsequently, the used syringe was placed in the pump and theoretical volume extruded, then measured with the precision scale. Experiments were conducted three times for each pump and at 28 °C ±1. Subsequently, average values were calculated and multiplied by the calibration value (2.47) by fitting the sensor reading with the theoretical flow rate.

Table 2. The experiment parameters of the effect of plunger rubber compressibility of a plastic syringe on extrusion.

Parameter	Value
Flow rate	500 µl/min
Extruded amount	500 µl
Needle diameter	0.41 mm
Syringes	60 ml Plastic and 50 ml Glass

2.9. The effect of filled syringe volume on extrusion accuracy

This experimental plan was designed to show the effect of compressed volume inside the syringe and its effect on the flow rate. The LVSP experimental setup was used, and the process parameters of the experiment can be seen in Table 3.

The same experimental procedure as the previous section was applied, and 50 ml, 40 ml, 30 ml and 20 ml filled syringes were used. All experiments were conducted three times, and average values were calculated and multiplied by the calibration value (2.47) by fitting the sensor reading with the theoretical flow rate. The experiment was carried out at 28 °C ±1.

Table 3. Extrusion quality experimental plan.

Parameter	Flow Rate
Flow rate	500 µl/min
Extruded amount	250 µl
Needle diameter	0.41 mm
Syringes	60 ml plastic and 50 ml glass
Filled volume	50, 40, 30 and 20 ml

3. Results and discussion

3.1. Validation of the LVSP

The purpose of this experiment was to validate the LVSP. The comparison of accuracy, precision and repeatability of the PHD2000 and LVSP pumps are shown in Table 3.

The average of measured values for the PHD2000 pump is 0.4845 ml with a standard deviation of 0.005212. While the theoretical extruded volume was calculated as 0.5 ml according to parameters given in Section 2, measured values are below the theoretical value. Similar results were observed for the LVSP as the average measured value of 0.4862 ml with a standard deviation of 0.005281.

Results show that the LVSP can perform just as well as the PHD200 pump, and both pumps can provide repeatable extrusion with precision and accuracy. Therefore, there is no inconvenience to use the LVSP for the following experiments.

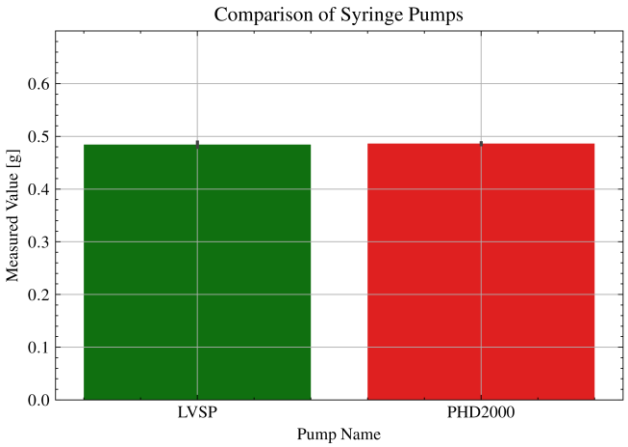

Fig 2. The weight measurement of the LSVP and PHD2000 pump.

3.2. The effect of plunger rubber compressibility of a plastic syringe on extrusion accuracy

The correlation between the rubber compressibility and the extrusion accuracy was tested in this experiment. Figure 3 shows the result of the flow rate measurement for the plastic and glass syringes with the same process parameters.

The flow rate was set to 500 µl/min and 500 µl glycerol extruded. The sensor readings multiplied by 2.47 to fit the graph with the flow rate and a sensor reading below 5% µl/min of measured value disregarded according to the sensor accuracy datasheet [13].

As Figure 3 shows, there is a significant difference between the two syringes in terms of the required time to reach the desired flow rate and to complete extrusion. The required time to reach the desired flow

rate was measured as 60 s ±0.5 and 7 s ±0.5 for plastic and glass syringes, respectively. Similar behaviour was observed during the completion of extrusion as 48 s ±0.5 and 11 s ±0.5 for plastic and glass syringes, respectively.

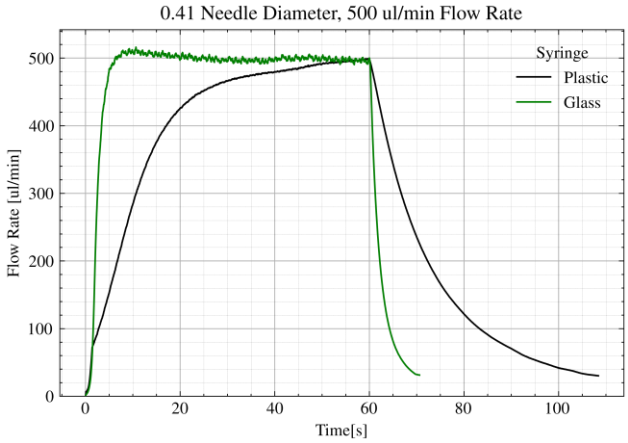

Fig 2. The flow rate measurement of the plastic and glass syringes to see the effect of plunger rubber compressibility.

This result confirms the effect of rubber compressibility on the flow rate claimed by the recent research on the development of an extruder for the bioprinting application [5].

3.3. The effect of compressed syringe volume on extrusion accuracy

This experiment aims to show the effect of compressed syringe volume on extrusion quality. The result of the flow rate measurement for the glass syringe filled with 50 ml, 40 ml, 30 ml and 20 ml are shown in Figure 4.

Data filtering methods were not used when plotting the graph to clearly see the effect of the compressed syringe volume. The flow rate was set to 500 µl/min and 250 µl glycerol extruded. The sensor readings multiplied by 2.47 to fit the graph with the flow rate and a sensor reading below 5% µl/min of measured value disregarded according to the sensor accuracy datasheet [13]. Therefore, the graph was plotted with the sensor readings above the 10.1 µl/min flow rate.

As can be seen from Figure 4, the time required to reach the desired flow rate and to complete extrusion differs significantly depending on the compressed syringe volume. While the extrusion of 20 ml filled volume was reached the desired flow rate in 17 s ±0.5, the extrusion of 50 ml filled volume could not reach the desired flow rate, and it stayed below 40 µl/min. The required time to reach desired flow rate for 30 ml was 25 s ±0 and 29 s ±0.5 for 40 ml. Similarly, the completion of the extrusion process was measured as 51, 61, 75 and 83 seconds (±0.5) for 20, 30, 40 and 50 ml filled volumes, respectively.

These results suggest that there is a strong correlation between the compressed syringe volume and the flow rate. This finding is consistent with the proposed flow rate mathematical model in the introduction section (see Eq. 1.) [9]. It also explains why the shutter valve-based extruder and the PCP performs better than syringe based extruder in terms of time required to reach the desired flow rate and to complete the extrusion process [10]. This better extrusion quality can be attributed to the valve closing mechanism in the shutter valve and fixed compressed volume at the outlet in the PCP.

Bioprinting technique generally uses 1 ml to 10 ml syringe volume for extrusion. While the result shows the considerable effect of large volume on compressibility, a similar effect can be observed in small volumes with less delayed time at the beginning and the end of the extrusion. This inaccuracy and a possible solution were mentioned in the introduction section; in the research, the PCP performed better than the syringe pump in terms of the extrusion accuracy and precision in the bioprinting application [11].

These results help to explain why the PCP performs better than the syringe pump by showing the effect of compressed volume. The PCP pump has recently been used in the additive manufacturing area, and it produces promising results; however, the cleaning difficulties, scalability of the pump and high cost can be considered as the main drawbacks of this technology [11], [14]. Therefore, it is clear that the adaptation of PCP technology for the bioprinting application with a low-cost and easy to clean attribute can help move this technology forward.

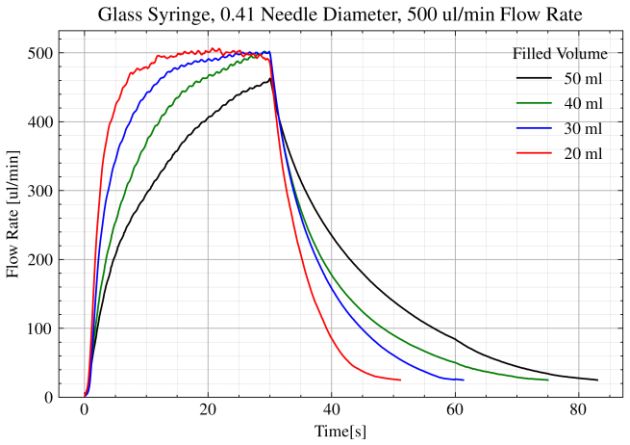

Fig 3. The flow rate measurement of the glass syringe with 50 ml, 40 ml, 30 ml and 20 ml filled volume.

4. Conclusions

The main aim of the present research was to examine the effect of plunger rubber compressibility of a plastic syringe and the effect of compressed syringe volume on extrusion accuracy. The LVSP was developed and validated to perform the experiments with 50 ml glass and 60 ml plastic syringes.

The start and stop accuracy comparison of a plastic and glass syringe was presented. This study shows the strong negative relationship between the effects of plunger rubber compressibility of a plastic syringe on extrusion accuracy. Therefore, the result confirmed the suggestion of the previous research [5].

The research has also shown that there is a negative relationship between the amount of compressed volume and the extrusion accuracy. This study can be considered as the experimental confirmation of the flow rate mathematical model of positive displacement pump research [9].

By taking these findings into account, the EBB can benefit from a better extrusion pump, and a PCP is the strongest candidate according to the previous research [10], [11], [14]. Future studies on the development of a PCP for the bioprinting application is recommended to improve extrusion accuracy.

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Author's statement
Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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