

An Extrusion System for Printing Hydrogel

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Abstract

This research delves into the transformative potential of extrusion-based bioprinting in tissue engineering and regenerative medicine. The study employs a system specifically designed for printing hydrogels, utilizing additive manufacturing principles to fabricate three-dimensional structures that closely resemble natural tissues. The process involves the layer-by-layer extrusion of bioinks, often hydrogel-based materials laden with cells, enabling the precise creation of complex biological constructs. The study's objectives encompass the design of a printing shape using Inkscape and its conversion to Gerber file format, the creation of an extrusion mechanism to be integrated with a current CNC machine for 3D bioprinter development, and the establishment of a bioprinting system capable of printing patterned biopolymers. The research successfully designed a geometric shape using Inkscape software, configured settings to display X, Y, and Z coordinates, and saved the file in G-code format for the printing system. An extrusion mechanism was created using a silicon tube, linking the controller to the extruder component and integrating it with an existing commercial CNC machine. The final objective involved the creation of a bioprinting system that can print patterned biopolymers, focusing on circular or cylindrical shapes to accurately replicate organ architecture. These geometric forms, first created in 2D, were piled using customized G-code to create 3D structures. The findings underscore the significant potential of extrusion-based bioprinting in advancing healthcare solutions.

1. Introduction

3D bioprinting is an additive manufacturing process that emerged as a potential regenerative strategy for tissue engineering which relies on computer-assisted manufacture that precisely aligns the cells to form three-dimensional constructs that simulate native microenvironment imparting specific biological function. 3D bioprinting has immense application in the fabrication of tissue models or organoids, replacement of damaged tissue and drug development [1]. Bio-inks, which is the essential component of 3D bioprinting, consist of biomaterial that are specially designed to encapsulate cells or to incorporate growth factors. In the scaffold-free printing methods, 3D tissue constructs are printed using bio-inks that consist of biomaterial and cells, and the scaffold-free approach deposit tissue spheroids in desired patterns to grow into large functional tissue structures [2].

2. Methodology

This section describes the methods used to achieve the goals set for the development of the extrusion-based 3D bioprinting system. The experimental procedures are depicted and presented using a block diagram, an explanation, an image, and a flowchart. This work began with the design and development of the extrusion bioprinting controller and the system development flowchart, as shown in Fig. 1. The electronic and mechanical components of the extruder system were developed separately. The g-code for the system was programmed in Inkscape and then simulated using NC viewer in the electronic part. Following the design of the system using the Geber controller, the hardware was created based on the shape created in Inkscape. This chapter includes and explains the controller's circuit. This chapter also discusses the development of Bioprinting system, Stepper motor speed verification, Geometric shape printing design for 3D bioprinting, Design of construct and G-code, Gelatine Bioink. Preparation for investigating bioprinting process parameters such as flow rate, printing speed, and bioink concentration was discussed, as well as printing patterns designed using G-codes. This chapter also elaborated on the preparation of bioink samples for each characterization, including the degradation test. Each experiment was carried out in accordance with the research objectives.

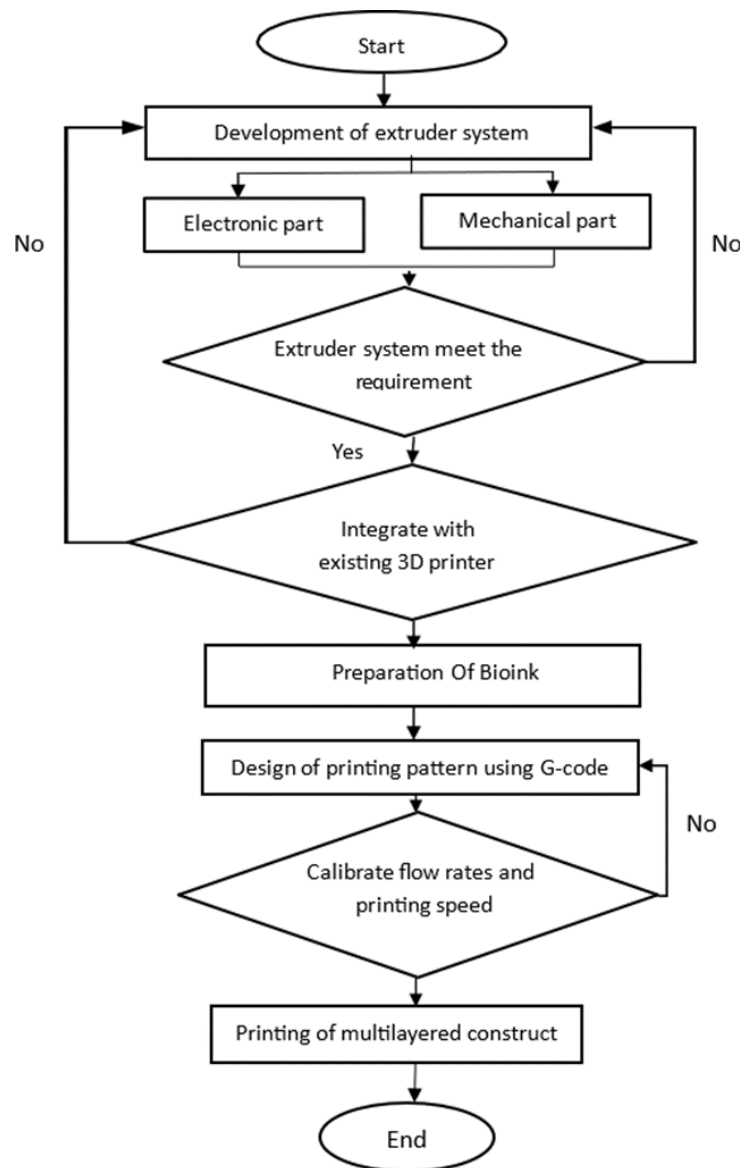


Fig. 1 Overall flowchart of the work

2.1 Designing geometric shapes for an extrusion printing

To verify the viability of printing 3D biostructures, four different geometric shapes were chosen. These shapes include the circles in Fig. 2 (a), the straight lines in (b), and the mesh in (c), which are related to the structure of our blood vessels and epithelium layer. These geometric forms were first created in 2D and then piled using the

customized G-code to create 3D structures. The circle shape was the one that was printed on the study materials in this research. Based on previous research, many organs in the body, such as blood vessels, intestines, and airways, have tubular structures. Bioprinting circular or cylindrical shapes is necessary for accurately replicating the architecture of these organs.

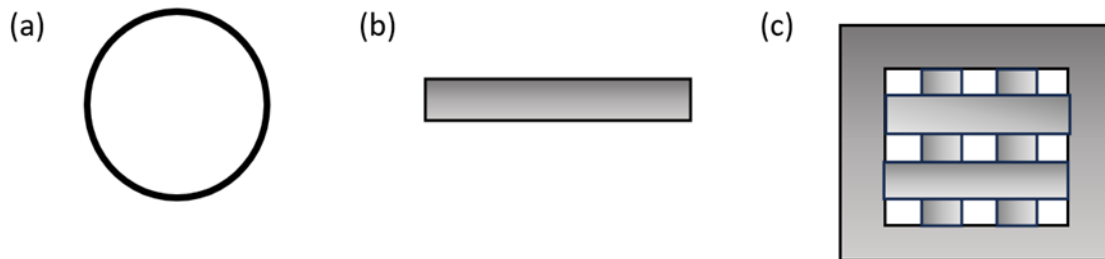


Fig. 2 Shape of (a) circle, (b) straight lines and (c) mesh

2.2 Design of constructs and G-code

In the beginning, the structures were created using the open source Inkscape software version 0.92.4. Users can use this software to create any form of shape and even convert photos into G-code files. Three-dimensional (3D) or two-dimensional (2D) build designs are also possible. The 3D printer can then accept the design when it has been saved and transformed into a G-code file. An update to Geber Control Software version 0.8 will be made to the G-code file. Inkscape software can be used to create a basic two-dimensional circular form. Altering the X and Y axes in the tool library will change the design's position. The design parameters are shown in Fig. 3 as a green box that can be adjusted to suit individual preferences.

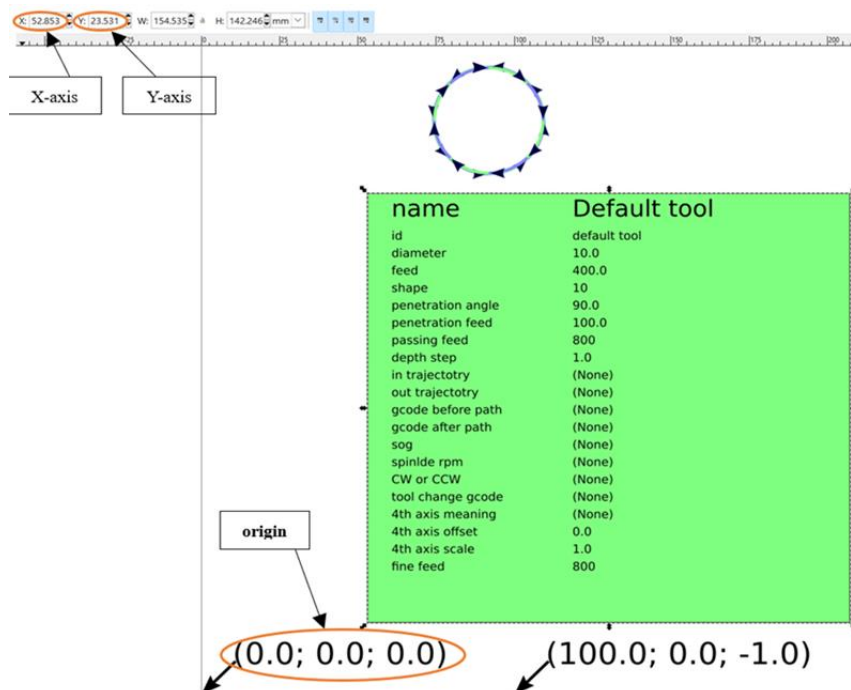


Fig. 3 A 2D circle shape design in Inkscape software

G-code is required in the work for instructing the 3D printer on the direction to move, the printing speed, and even when to start or stop the operation. One programming language that can be used to instruct computerized machine tools is called G-code. G-code consists of the end code, the pattern code, and the start code. To generate the desired structures, one might modify the G-code. For convenience in changing the code, NC Viewer, a G-code viewer and simulation program, was utilized.

The beginning G-code code shown in Fig. 3 is typically exactly the same for every code instruction. The 3D printer's spindle was turned on by using the M3 setting. It was necessary to declare the unit of measurement used in the design, such as G21 to stand for millimeters (mm). Following that, the printer head was moved to the required X, Y, and Z axis coordinates using the positioning code. Normally, the F value would represent the feed rates of the filament that the 3D printer extrudes, however in this case, the F value was called to start the machine's

printing speed. The feed rates or flow rates of bioink in this research were programmed by the microcontroller in the controller board rather than the G-code because of the modification of the nozzle tip for the bioink extrusion.

```
%
(Header)
(Generated by gcodetools from Inkscape.)
(Using default header. To add your own header create file "header" in the output dir.)
M3
(Header end.)
G21 (All units in mm)
```

Fig. 4 The starting of G-code

The pattern code that was set up to use a 3D printer to manufacture the intended structure is displayed in Fig. 4. It is called the traverse move, G00. In this concept, the printer head was moved quickly and without using any cutting motion from a starting point to a specific direction. G01 will start the machine's circular motion and the G03 to go in an anticlockwise direction. When the printing process is ready to begin, it will travel to the designated location. The speed rate in millimeters per minute was defined by the F value in the code. The code "G03 X79.334217 Y202.099378 Z1.000000 I-0.000000 J-17.638671 F400.000000" in Fig. 5 indicated that the platform would descend by 1 mm and the nozzle would move in the designated direction at a speed of 50 mm/s to the coordinates of X=79.334217 Y=202.099378

```
G00 Z5.000000
G00 X91.806641 Y207.265625
G01 Z-1.000000 F100.0(Penstrate)
G03 X79.334217 Y202.099378 Z-1.000000 I-0.000000 J-17.638671 F400.000000
G03 X74.167969 Y189.626950 Z-1.000000 I12.472426 J-12.472426
G03 X79.334417 Y177.155707 Z-1.000000 I17.635349 J0.000005
G03 X91.806641 Y171.990230 Z-1.000000 I12.472224 J12.474572
G03 X104.278866 Y177.155708 Z-1.000000 I0.000000 J17.640047
G03 X109.445310 Y189.626950 Z-1.000000 I-12.468898 J12.471242
G03 X104.279063 Y202.099378 Z-1.000000 I-17.638681 J0.000000
G03 X91.806641 Y207.265625 Z-1.000000 I-12.472420 J-12.472415
G01 X91.806641 Y207.265625 Z-1.000000
G00 Z5.000000
```

Fig. 5 The coding to print the circle structure

3. Result and Discussion

This section discusses the parameters that must be optimized for the bioprinting technology, and it is about to develop bioprinting system to printing the design. The investigation is begun with do research and learn about the effect of nozzle diameter on printability of ink, the relationship between the flow rates and motor speed. The effect of printing speed and flow rates to width of the printed filament and the influence of nozzle distance to the printability of bioink Printability of multilayer structure.

3.1 The Correlation Between Motor Speed and Time(s)

A mild linear slider that serves as an extruder for the bioink was added to the extrusion system. Selecting an output rotational speed from a list that corresponds to the linear slider's time is necessary to initiate the system motion. The stepper motor's rotation speed dictates the extruder's flow rate, and the stepper motor is managed by a stepper motor driver. To regulate the speed, the stepper motor was programmed using the Arduino IDE.

In Fig. 6 presents the graph about the print speed using the extruder that have combined with the controller. Based on the graph shows that the more time recorded, the more the speed limit decreases which mean the speed limit declines as more time is spent on it.

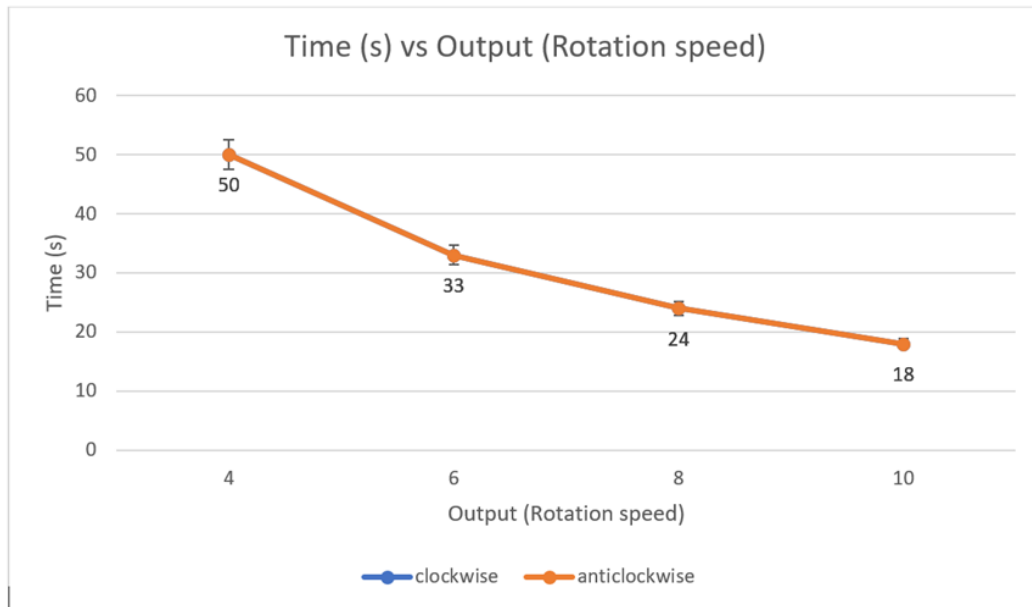


Fig. 6 Graph by print speed (time(s) as an expression output (rotation speed))

In Fig. 7 shows the display of stepper motor controller in clockwise mode while in Fig. 8 is display of stepper motor controller in anticlockwise mode which is using the SMC02 version with the details. Table 1 and Table 2 data that have been collected from the observation, that shows the data that received is the same.



Fig. 7 Display of stepper motor controller in clockwise

Table 1 Print speed by stepper motor controller clockwise

Output	4	6	8	10
Time (s)	39	32	24	15



Fig. 8 Display of stepper motor controller in anticlockwise

Table 2 Print speed by stepper motor controller anticlockwise

Output	4	6	8	10
Time (s)	39	32	24	15

3.2 The Impact of Nozzle Distance on the Printability of Bioink

One of the factors that can affect how well a 3D bioink structure prints is the distance of the nozzle tip (Fig. 9) from the glass substrate or platform. The nozzle Z-offsets that were examined in this experiment were 1 mm, 2 mm, and 3 mm.

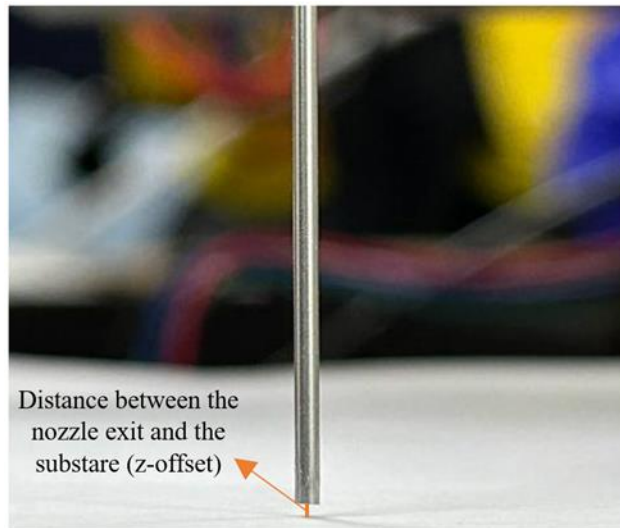


Fig. 9 Distance between the nozzle exit and the substrate (z-offset)

The nozzle distances of 1 mm in Fig. 10 (a) and 2 mm in Fig. 10 (b) resulted in printed filament with good resolution, as seen by the printed filament's ability to keep the specified circular shape. When the distance was raised to 3 mm, the printability and accuracy of the manufactured filament began to deteriorate (Fig. 10 (c)). At a nozzle distance of 3 mm, the structure failed to maintain its circular shape and lost its edges. When the nozzle distance was increased, the printed filament produced a poor big strand of bioink that did not attach to the surface accurately. Furthermore, nozzle distances less than 1 mm were found to be challenging for the nozzle to move smoothly because they were closer to the bioprinter surface. This demonstrated that the nozzle height may be used to control the direction of the bioink dispensed, which influences the printability of the produced filament. To achieve good printability of the bioink printed filament, a nozzle distance of 1 mm was chosen in this study. The dashed line in Fig.10 serves as the printed filament's reference line.

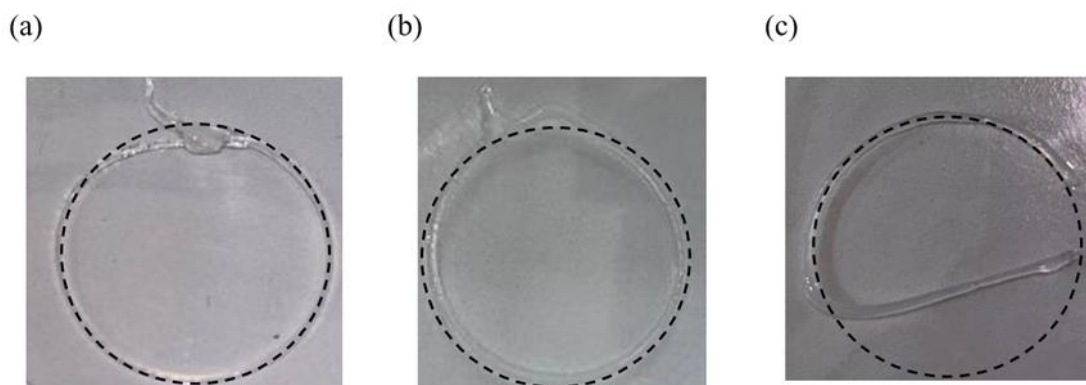


Fig. 10 The manufactured filament at nozzle distance (a) 1, (b) 2, and (c) 3mm (Scale bar: 3mm)

4. Conclusion

A 3D bioprinting technology based on extrusion has been created to replicate a microenvironment that resembles biological structures. The first objective of the work which is to design the printing shape with Inkscape and convert to Gerber file format has been successfully. The geometric shape was drawn using Inkscape software.

Next, configure some settings so that the X, Y, and Z coordinates as well as the details of the origin coordinate may be seen. Lastly, save the file in G-code format so that the printing system may use it.

The second objective is to creating an extrusion mechanism to be added to the current CNC machine in order to create a 3D bioprinter. Using a silicon tube, the controller was linked to the extruder component and integrated with the already-existing commercial CNC machine. A potentiometer that may be adjusted to vary the rotating speed output, a forward button, a reverse button, a forward indicator, a reverse indicator, a delay time or cycle, and a rotating speed make the interface for the extruder's controller. Additionally, the drill head can move in the X, Y, and Z coordinate system in accordance with G-code commands.

The last objective is to create a bioprinting system that can print patterned biopolymers. Circle, straight line, and mesh structures are among the shapes of biopolymers that are related to the structure of our blood vessels and layer of epithelium. These geometric forms were first created in 2D and then piled using the customized G-code to create 3D structures. The circle shape was the one that was printed on the study materials in this research. Bioprinting circular or cylindrical shapes is necessary for accurately replicating the architecture of these organs.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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