**SUPPLEMENTAL MATERIALS**

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**Appendix 1: Open source STL files**

Below are the sites for downloading the 3D models used in this work.

|  |  |
| --- | --- |
| **3D Models** | **Download Sites** |
| Bio-extruder and Bio-printhead | <https://www.thingiverse.com/thing:5398780> |
| Right Coronary Artery Tree  (NIH 3D Print Exchange model 3DPX-011751 by user awfeinberg) | <https://3dprint.nih.gov/discover/3DPX-011751> |
| Maple Leaf  (Thingiverse #25938 by user MakerBot) | <https://www.thingiverse.com/thing:25938> |

**Appendix 2: Detailed steps to convert a Creality Ender 3 V2 3D printer into a bioprinter**

**Preparation**

1. 3D print all the bio-extruder and bio-printhead parts using PLA or other suitable plastic materials (See Appendix S1 for the STL files).
2. Unmount the original extruder and printhead.
3. Unplug the heater from the mainboard to allow safe operation. Note that thermistor should not be unplugged as the firmware would disable printing if it were disconnected.

**Steps to convert**

Follow the steps below to assemble the bioprinter. A figure is provided below to show how the parts are assembled.

1. Remove the stepper motor from the original extruder and mount the stepper motor on the 3D-printed bio-extruder body via three M3 x 10 mm screws.
2. Fix gear 1 on the shaft of the stepper motor.
3. Connect gear 2 and 3 via a M8 x 40 mm screw and mount them on the extruder body via a 608 bearing.
4. Fix a Terumo SS-30S 30 ml syringe on the bio-extruder body.
5. Replace the original plunger with our 3D-printed plunger.
6. Place the 3D-printed plunger in a way that the stepper motor could rotate to push it via gear 1, 2 and 3.
7. Adjust the tightness of the plunger via a M3 x 10 mm screw.
8. Connect the syringe to a 100 mm 22 Gauge blunt end needle via a 40 cm plastic tube and a luer lock adaptor.
9. Insert the blunt end needle into the bio-printhead and mount it on the X-axis carriage of the 3D printer.

C:\Users\TCY\OneDrive - The Chinese University of Hong Kong\PLKLFC\03 ECA\00 Edu Research\3D Bioprint\01 JMBE submission\Submission\revise\Final\Appendix 2.tif

**Appendix 3: Slicing 3D models for bioprinting**

Slicing means to convert a 3D model into G-code which specify instructions for the 3D printer. We used Ultimaker Cura 4.12.1, which is a free slicing software. The 3D files in STL format are input into Cura and specific Cura setting is used for slicing the 3D models for bioprinting. Below are three special settings that are required when slicing models for bioprinting using Cura.

1. Firstly, the default start and end G-code in Cura should be modified before slicing. The Z axis need to move up before and after printing, so that the printhead could avoid hitting the petri dish at the beginning and the end of printing. In addition, the extruder should be programed to extrude a small amount of bioink before printing, so that the needle could be filled up with bioink. To modify the default start and end G-code, go to Setting > Printer> Manage printers> Machine setting> Printer, input the below G-code and press “Close”.

|  |  |
| --- | --- |
| **Start G-code** | **End G-code** |
| ; Ender 3 Custom Start G-code  G92 E0 ; Reset Extruder  G28 ; Home all axes  G1 Z50.0 F3000 ; Move Z Axis up  G1 F200 E1; Extrude material to fill the nozzle  G1 X110 Y110 F3000.0 ; Move to XY start position  G92 E0 ; Reset Extruder  M302 S10  G1 Z0.3 F3000.0 ; Move to Z0 | G91 ;Relative positioning  G1 E-2 F2700 ;Retract a bit  G1 E-2 Z0.2 F2400 ;Retract and raise Z  G1 X5 Y5 F3000 ;Wipe out  G1 Z10 ;Raise Z more  G90 ;Absolute positioning  G1 X0 Y{machine\_depth} ;Present print  M106 S0 ;Turn-off fan  M104 S0 ;Turn-off hotend  M140 S0 ;Turn-off bed  M84 X Y E ;Disable all steppers but Z |

1. Secondly, to allow accurate calculation of extrusion speed by the software, one would need to modify the compatible material diameter. Go to Setting > Printer> Manage printers> Machine setting> Extruder, input 24.5 mm as the compatible material diameter and press “Close”. 24.5 mm is the diameter of the 30 ml syringe.
2. Thirdly, special printing condition is needed to be set for slicing 3D bioprint models. The default printing condition by Cura cannot be used because the conditions for plastic printing and bioprinting are different. For example, the printing temperature in bioprinting needed to be set as equal or lower then 25°C due to the absence of heater in the bioprinter. The print speed also needed to be slowed down to enhance print quality. Below is the condition that we used for bioprinting using the DIY bioprinter. Among various parameters, we believe that the flow is the most critical parameter that affects the print quality. It determines the extrusion speed of the bioink. According to our experience, 8% of flow would result in the best print quality.

|  |  |
| --- | --- |
| **Preset Printer** | Creality Ender-3 Pro |
| **Material** | Custom |
| **Nozzle Size** | 0.5 mm |
| **Profile** | Custom (based on Standard Quality - 0.2mm) |
| **Layer Height** | 0.2 mm |
| **Wall Thickness** | 1 mm |
| **Wall Line Count** | 2 |
| **Print Thin Wall** | yes |
| **Horizontal Expansion** | 0.0 mm |
| **Top/Bottom Thickness** | 0.8 mm |
| **Top Thickness** | 0.8 mm |
| **Top Layers** | 4 |
| **Bottom Thickness** | 0.8 mm |
| **Bottom Layers** | 4 |
| **Infill Density** | 30% |
| **Infill Pattern** | Cubic |
| **Printing Temperature** | 0°C |
| **Build Plate Temperature** | 37°C |
| **Flow** | 8% |
| **Print Speed** | 10 mm/s |
| **Travel Speed** | 30 mm/s |
| **Enable Retraction** | No |
| **Enable Print Cooling** | yes |
| **Minimum Layer Time** | 5.0 s |
| **Minimum Speed** | 2.0 mm/s |
| **Generate Support** | no |
| **Build Plate Adhesion Type** | None |

**Appendix 4: Troubleshooting Q&As**

Q: Why no bioink is coming out from the nozzle during printing?

A: The nozzle might have been clogged. You might need to unclog the nozzle by inserting a needle into the nozzle. Move the needle back and forth carefully until the clog is removed.

Q: Why does my bioprint look bulkier than the digital model?

A: The extrusion rate might be too high. Too much bioink is extruded. You might need to decrease the percentage of flow in the slicing setting.

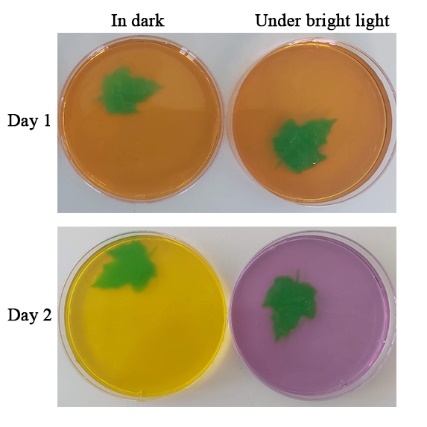
Q: Why is my bioprint so fragile?

A: The extrusion speed might be too low. The layers might not be firmly connected together, making the bioprint easy to disintegrate. You might need to increase the percentage of flow in the slicing setting.

Q: Why are there strings on the surface of the bioprints?

A: This is caused by bioink oozing out of the nozzle while the extruder is moving between different sections of the print. You could enable retraction in the slicing setting to reduce stringing. Retraction means withdrawing the bioink back into the nozzle after printing one section of the model. You could also increase the travel speed to reduce the time of oozing.

**Appendix 5: Bioactivity of the algae-laden artificial leaf**



To visualize the bioactivity of the algae-laden artificial leaves, two of them were submerged in petri dishes contanining 30 ml of hydrogencarbonate indicator. One of them was put in dark environment and another one was put under bright light for a day. Photosynthesis under bright light resulted in a net consumption of carbon dioxide and turned the indicator into purple, while respiration in the absence of light released carbon dioxide and turned the indicator into yellow. The principle of this work is similar to a previously described learning activity (11).