

15 **I. Printer specification**

Supplementary Table 1| Table comparing the system developed in this work with the 17 reported open-source extrusion bioprinters. PF127 = Pluronic F127, PAA = polyacrylic **reported open-source extrusion bioprinters.** PF127 = Pluronic F127, PAA = polyacrylic
18 acid, CMC = sodium carboxymethyl cellulose, NaAlg = sodium alginate, PCL = acid, CMC = sodium carboxymethyl cellulose, NaAlg = sodium alginate, PCL = Polycaprolactone, hiPSCs = Human induced pluripotent stem cells, and ECM = extracellular

20 matrix.

- 29 **Supplementary Figure 1|** A summary comparing work reported in this study, and those 30 reported by existing open-source printer platforms (see above Printer specification for 31 literature source).
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34 **Supplementary Table 2| Specifications of the system developed in this study and the** 35 **four commercial 3D extrusion-based bioprinters commonly used in the bioprinting community.** The commercial systems are Allevi 3^{33} (denoted as \ddagger below), Cellink
37 BioX^{34,35} (†) and Envision TEC 3D Bioplotter Starter Series³⁶ (*) and GeSim 37 BioX^{34, 35} (†) and Envision TEC 3D Bioplotter Starter Series³⁶ (*) and GeSim 38 BioScaffolder BS3.3³⁷ (8). It should be noted that, though Printer. HM has a lower BioScaffolder BS3.3³⁷ (§). It should be noted that, though Printer.HM has a lower mechanical resolution and a narrower heating range than commercial systems, soft 39 mechanical resolution and a narrower heating range than commercial systems, soft material printing typically does not require a high temperature (*i.e.* > 60 °C), and the material printing typically does not require a high temperature (*i.e.* > 60 °C), and the print resolution of extrusion soft material printing is in general > 100 µm. As Printer. HM 41 print resolution of extrusion soft material printing is in general $> 100 \mu$ m. As Printer. HM
42 is designed for soft material fabrication purpose, the heating range and the mechanical 42 is designed for soft material fabrication purpose, the heating range and the mechanical
43 resolution adopted in Printer. HM are considerably adequate. resolution adopted in Printer.HM are considerably adequate.

46 **II. Bills of materials**

47 **Supplementary Table 3| Part list and the breakdown costs of the printing platform.**

48 The total cost refers to the associated cost of a platform equipped with 4 printheads,

49 stage and syringe heating systems, a UV module and a camera. * denotes the components
50 used in the electrical circuit.

used in the electrical circuit.

III. Assembly instruction

 Prior to the assembly, the custom-designed parts were 3D printed with PLA or ABS using an Ultimaker S3 3D printer. A syringe holder and a stage holder were custom-made with aluminum. All the custom-designed CAD files are listed in **Supplementary Table 4** and are available on Github.

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 Supplementary Figure 2| a) Overview of the setup. b) 3D printed stages for accommodating different sizes of receiving reservoirs. c) 3D CAD design of the piston - driven extrusion printhead. Add-on d) UV light for photopolymerization and e) camera for *in situ* monitoring.

66 **Supplementary Table 4|** Customised accessory list. The printing time is referred to the 67 time required when a 'fast' print setting is used in Ultimaker.

II.1 Printhead assembly

 Supplementary Figure 3 shows the step-by-step assembly instruction of a printhead. **1.** Before assembling the printhead, the lead screw *(Part C8)* was milled to the designed dimension. (see **Supplementary Figure 3**, step 1). **2.** Next, the stepper motor *(Part C6)* was attached to the 3D printed motor holder using M2.5 screws. **3.** The stepper motor holder was then inserted to an aluminium rail *(Part C2)* and locked in place with the drop in tee nuts *(Part C34)* and M5 screws. **4.** A shaft coupling *(Part C7)* was connected to the motor shaft and tightened using the built-in set screw. **5.** Two 10 cm linear rails *(Part C10)* were tightened firmly on the aluminium rail using M5 screws at a position of ~28 mm from the top edge of the aluminium rail. **6.** A linear guide pillow block *(Part C11)* was inserted to each linear rail. **7.** The modified lead screw was combined with a lead screw nut *(Part C9)*, which was then tightened on a 3D printed lead screw nut mount. **8.** The lead screw was tightened to the shaft coupling, and the 3D printed lead screw nut mount was then linked to the linear guide pillow blocks on the linear rail using M4 screws. **9.** The end of the lead screw was connected to a ball bearing *(Part C12)* that was fit to a 3D printed syringe holder. **10.** The 3D printed syringe holder was then tightened on the aluminium rail using M5 screws and the drop in Tee nuts. **11.** The printhead assembly was finished by placing magnets *(Parts C14 & C15)* to the designed holes of the lead screw nut mounts and the syringe holders using super glue.

 The heating printhead was assembled similarly using different 3D printed parts of syringe holders *(Parts D17 & D18)* that were custom-made to fit an aluminium barrel *(Part D19)*. The aluminium barrel was wrapped with a Nichrome wire as the heating wire *(Part C30)* and a K- type thermocouple *(Part C24)* as the temperature sensor using a high temperature tape *(Part C31)*.

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II.2 Installation of printheads to the frame

 The frame of the dispensing module is made of six aluminium rails, and was assembled using hidden tee nuts and L brackets *(Parts C35 & C36)* as linkages according to **Supplementary Figure 3**. The frame was then fastened on the aluminium breadboard by means of the customised 3D printed adapters. In total, 4 printheads were built. They were firmly installed to the frame using the 3D printed adapter plates.

Supplementary Figure 3| Printhead assembly.

II.3 Installation of 3D printed stage to the robotic arm

 All 3D printed stages were designed to mount to the uArm with an adapter piece obtained from the laser engraving kit of the uArm. To connect the stage to the uArm, first, the adapter piece was disassembled from the laser engraving kit (see **Supplementary Figure 4a**, Step 1). The adapter piece was then attached to the 3D printed stage using M3 screws (Step 2), inserted and fastened to the uArm using the hand screw of the uArm (Step 3). The uArm was locked firmly on an aluminium breadboard *(Part C4)* using clamps *(Part C5)* (Step 4).

 For the heating stage, an aluminium holder *(Part D28)* was made for fitting a 3.5 mm petri dish. The aluminium holder was then wrapped with a heating coil *(Part C30)* with a K-type thermocouple *(Part C24)* placed on the holder using a high temperature tape *(Part C31)*. The holder was then clamped by two 3D printed parts *(Parts D26 & D27)* to form the heating stage (**Supplementary Figure 4b**). The installation of the heating stage to the uArm was the same as the procedure mentioned above.

 Supplementary Figure 4 | a) Assembly procedure of the stage to the uArm. **b)** A detailed view of the heating stage.

II.4 Electrical circuit of the printheads

 Supplementary Figure 5 shows the circuit diagram of a printhead. The stepper motor of the printhead is controlled by an Arduino board. Before connecting the stepper motor to the Arduino board, a current limiting procedure was carried out with the motor drive to limit the loaded current. This procedure was to prevent the loaded current from exceeding the rated current of the stepper motor, hence avoiding overheating of the stepper motor. The procedure 128 was carried as follows³⁹. First, the current limit of the motor drive, I_{max} , and the reference voltage of the motor drive, *Vref*, were calculated using the below equations. The actual current limit of the stepper motor, *Irating*, is usually 70% of the driver current limit, *Imax. Irating* is 670 131 mA according to the stepper motor specification⁴⁰ and R_{cs} is the current sense resistance of the 132 motor drive, which is $0.068 \Omega^{39}$.

$$
I_{\text{max}} = \frac{I_{\text{rating}}}{0.7} = \frac{0.67}{0.7} = 0.96 \text{ A}
$$
 (1)

$$
V_{ref} = 8 \times I_{max} \times R_{cs} = 8 \times 0.96 \times 0.068 = 390 \text{ mV}
$$
 (2)

 Next, an Arduino script was written for running the motor drive in full step mode by setting the logic levels of the MS1, MS2 and MS3 pins to 'LOW'. After ensuring the motor was not connected to the motor drive, the script was uploaded to the Arduino board. A multimeter was then used to measure the voltage, *Vref*, between the potentiometer and the GND pin connecting to the Arduino board (see **Supplementary Figure 5**). *Vref* was tuned to approximately 390 mV by rotating the potentiometer using a screwdriver.

 Supplementary Figure 5| Circuit diagram for connecting a stepper motor with Arduino and a motor drive. In the current limiting procedure, the stepper motor was detached from the circuit. *Vref,* was measured between the potentiometer and the GND pin to the Arduino board, as indicated by the red crosses.

147 After the current limiting procedure, the stepper motor was connected to the motor drive using the arrangement shown in **Supplementary Figure 5**. Overall, a single Arduino mega board and a power supply were used to control and operate four printheads. **Supplementary Figure 6** shows the overall circuit diagram. As an Arduino mega board has 54 digital pins and the control of each printhead requires 8 digital pins, more printheads can be incorporated into the system if needed.

 Supplementary Figure 6| Overall circuit diagram of the dispensing module composed of four printheads.

II.5 Electrical circuit of the heaters

 Supplementary Figure 7 shows the circuit diagram of the heating module. The syringe heater was connected to a power adapter with rating of 9 V and 2 A, whereas the stage heater was powered by a power adapter with a 12 V and 500 mA rating. An Arduino nano board and a MOSFET were employed to control each heating module because of their compactness in size.

Supplementary Figure 7| Circuit diagram of a heating module.

II.6 UV module and camera

 A UV light source can be easily mounted onto the aluminium breadboard when needed. Here, a low power UV light torch (5 W*, NSUV365, Nightsearcher*) with wavelength of 365 nm. A camera unit *(C922, Logitech)* was added onto the breadboard for *in-situ* monitoring and recording the printing process.

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180 **IV. Maximum build volume**

 The maximum build volume is estimated by subtracting the weight of the customised stage from the payload capacity of the robotic arm (see **Supplementary Table 5** which provides the estimated maximum build volume when different built stages are in use). The estimated 184 maximum build volume is $\sim 490 \text{ cm}^3$ (assuming the density of the material is equal to 1g/cm³, i.e. the approximate density of most soft materials).

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187 **Supplementary Table 5|** Estimated maximum build volume when different built stages 188 are in use.

Stages	Weight of the customised	Estimated max. build
	stages (g)	volume $(cm3)$
35 mm petri dish stage	8	492
55 mm petri dish stage	15	485
90 mm petri dish stage	15	485
30 mm rectangular container		493
stage		
38 mm rectangular container	11	489
stage		
75x25 mm glass slide holder	10	490
35 mm petri dish heating stage	20.9	479

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191 **V.Theoretical resolution of the printhead**

Steps per mm = $\frac{1}{n^{1+\alpha}}$

192 The theoretical volumetric resolution per step of the printhead when using a sixteenth micro-193 stepping resolution was calculated using below equations²³. A microstepping stepper motor 194 drive *(A4988, Pololu)* was chosen as it enables five microstep resolutions (full, 1/2, 1/4, 1/8 and $1/16$ step)³⁹. The selected stepper motor and the threaded rod have a step angle of 1.8° 195 196 and a pitch spacing of 2.54 mm^{40,41}.

197

198 Step per revolution =
$$
360/\text{step}
$$
 angle = $360/1.8 = 200$ steps per revolution

$$
\frac{1}{2}
$$

199 **Steps per mm** = $\frac{1}{\text{pitch spacing}} \times \frac{1}{\text{micro}-\text{stepping resolution}} \times \text{steps per revolution}$ = $\frac{1 \text{ revolution}}{254 \text{ mm}} \times \frac{1}{1} \times \frac{200 \text{ steps}}{1 \text{ Borelutio}}$

$$
= \frac{1 \text{ revolution}}{2.54 \text{ mm}} \times \frac{1}{16} \times \frac{200 \text{ steps}}{1 \text{ revolution}} = 1260 \text{ steps/mm}
$$

$$
201 \t\t\t\tDispensing resolution = 1 mm/1260 steps = 0.8 \t\t\t\t\t\mu m per step
$$

203 **VI. Printing demonstration**

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205 **Supplementary Figure 8|** Fabrication of a) perfusion moulds made of SE1700 and b) a 206 3D nose model made of Ecoflex using Printer.HM. Both constructs were printed using
207 CAD files as the geometry inputs. The SE1700 ink was printed with a 25G needle, using 207 CAD files as the geometry inputs. The SE1700 ink was printed with a 25G needle, using
208 a stage speed of 2 mm/s and an extrusion flowrate of 825 uL/h. The SE1700 construct 208 a stage speed of 2 mm/s and an extrusion flowrate of 825 μ L/h. The SE1700 construct 209 was then cured at 70°C overnight. For the ecoflex printing, the ecoflex ink was prepared 209 was then cured at 70° C overnight. For the ecoflex printing, the ecoflex ink was prepared 210 following a similar formulation reported in a previous study, where Part A ecoflex 00-210 following a similar formulation reported in a previous study, where Part A ecoflex 00-
211 30 was mixed with Part B Ecoflex 00-30 (with $1.2w/v\%$ Slo-io and $1.2w/v\%$ Thivex) at 211 30 was mixed with Part B Ecoflex 00-30 (with $1.2w/v\%$ Slo-jo and $1.2 w/v\%$ Thivex) at 212 1:1 wt. ratio with the addition of a drop of oil-based light orange color ink for 1:1 wt. ratio with the addition of a drop of oil-based light orange color ink for 213 visualisation. The ink was then embedded printed in a $6 \frac{w}{v\%}$ fumed silica – mineral oil 214 support bath. The printing was performed with a 27G needle, extrusion speed of 825 215 μ L/h and a stage speed of 2 mm/s. The printing time was around 30 min. After printing, 216 the printed construct was left at room temperature for 2 days for curing before collecting 216 the printed construct was left at room temperature for 2 days for curing before collecting
217 it from the bath. Scale bars = 5 mm. it from the bath. Scale bars $= 5$ mm.

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220 **VII. Theoretical linewidth calculation**

221 The cross-sectional area, *A,* of the printed filament, when it is printed at over-extrusion

222 condition (see **Supplementary Figure 9**), can be calculated using the following equation:

$$
A = \frac{Q}{v_{\text{dragging}}}
$$

224 where $Q =$ Extrusion flowrate and $v_{dragging} =$ dragging speed of the filament, which can be

225 assumed as the speed of the stage, v_{stage} . Therefore,

$$
A = \frac{\pi d^2}{4} = \frac{Q}{v_{stage}}.
$$

227 And, the theoretical line width, *d*, can be calculated as

$$
d = \sqrt{\frac{4Q}{\pi \cdot v_{stage}}}.
$$

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231 Supplementary Figure 9 Schematic showing the over-extrusion condition, where the diameter of the filament, d_2 is greater than the nozzle diameter, d_1 (die swell ratio > 1).

diameter of the filament, d_2 is greater than the nozzle diameter, d_1 (die swell ratio > 1).

VIII. Codes for the printing operation

 Below shows the user-defined inputs in the Python programme templates for different geometry inputs. The codes are available on Github. Users only need to define the inputs in the programme and the operation process will be executed automatically.

Supplementary Figure 10| The required inputs in the Python template for using coordinates as the geometry input

Supplementary Figure 11| The required inputs in the Python template for using equations as the geometry input.

- **Supplementary Figure 12|** The required inputs in the Python template for using G-code generated from CAD as the geometry input. The conversion of CAD to G-code was made
- using 3D Slic3R, an open-source 3D slicing software.

Supplementary Figure 13| The required inputs in the Python template for using picture -

 generated G-code as the geometry input. The conversion of picture to G-code was made using Inkscape, an open-source graphics software.

IX. Conversion of pictures to G-code

 Printing with picture input was enabled by the 'Gcodetools' extension on Inkscape (https://inkscape.org/), which was an extension designed for CNC machines. The extension was installed on Inkscape. To covert a picture into printing paths, the workplace size on 260 Inkscape was first adjusted according to the size of the stage by clicking 'File \rightarrow Document 261 properties \rightarrow Custom sizes'. The acceptable form of pictures can be photos of hand-drawn sketches, pictures created by any drawing software or drawings created on Inkscape. For photos of hand-drawn sketches or pictures created by other drawing software, the pictures were imported to Inkscape and were converted to a vector path using the following procedures: 1) 265 Convert the image to a binary image ('Filters \rightarrow Color \rightarrow Greyscale') and 2) Trace the 266 centreline of the image ('Extensions \rightarrow Images \rightarrow Centerline Trace 0.8a \rightarrow Select 'Replace image with vector graphics' and 'Trace bright lines'). For drawings created on Inkscape, the 268 drawing was converted to paths by clicking 'Path \rightarrow Object to path'. After converting the picture to path, the path was then placed at the centre of the workplace and was converted to a 270 G-code file by applying the following step: 1) Navigate to 'Extensions \rightarrow Geodetools \rightarrow 271 Orientation points' and select '2-points mode', 2) click 'Extensions \rightarrow Gcodetools \rightarrow Tool 272 library' and select 'Cone'; and 3) Select the path, navigate to 'Extensions \rightarrow Gcodetools \rightarrow Path to Gcode' and save the file by clicking 'Apply' under the 'Path to Gcode' tab with the file name defined in the 'Preferences' tab. The generated file was then imported to the Python programme for the picture input (See Supplementary Figure 12), which was designed for reading the G-code files generated by this extension.

278 **X.Ink preparation**

279 **Supplementary Table 6| Formulations of the inks and support baths used in this** 280 **work.** Concentration is expressed as w/v% unless specified.

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XI. Supplementary videos

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