

1 **Electronic Supplementary Information to:**  
2 **Ice modulatory effect of the polysaccharide FucoPol in directional freezing**

3 Bruno M. Guerreiro<sup>1,2</sup>, Leo T. Lou<sup>3</sup>, Boris Rubinsky<sup>3\*</sup>, Filomena Freitas<sup>1,2\*</sup>

4 <sup>1</sup>UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, School of Science and Technology,  
5 NOVA University Lisbon, Caparica, Portugal

6 <sup>2</sup>Associate Laboratory i4HB - Institute for Health and Bioeconomy, School of Science and Technology, NOVA  
7 University Lisbon, Caparica, Portugal

8 <sup>3</sup>Department of Mechanical Engineering, University of California at Berkeley, Berkeley, CA, USA

9 \* Corresponding authors.

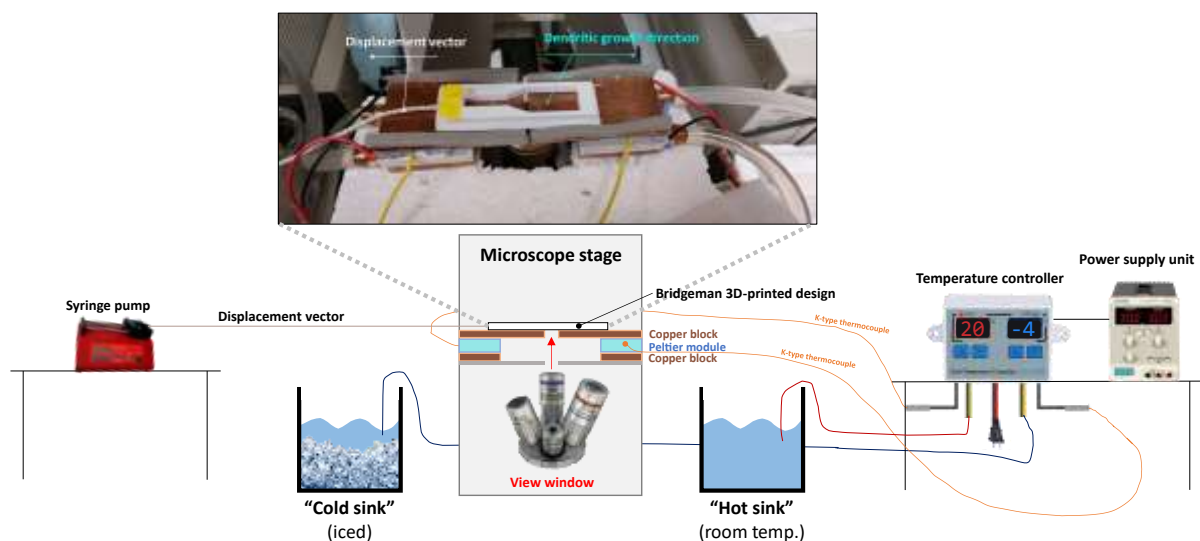
10

11

12

13 **SI.1. Directional freezing stage**

14



15

16 **Figure SI.1. Complete directional freezing setup schematics.** A power supply unit connected to a temperature  
17 controller measure the temperature of two water baths, of boundary temperatures. Then, the effective temperatures  
18 at the directional freezing stage are modulated by Peltier modules, connected to a controller by a K-type  
19 thermocouple, to establish a thermal gradient. To avoid thermal lag, the Bridgeman 3D-printed design is in direct  
20 contact with copper blocks to improve thermal conductivity. A syringe pump is responsible for the displacement  
21 of the Bridgeman shape, the driving force for the liquid solution to generate unidirectional dendritic growth, by  
22 gradually exposing the sample holder to all slices of the thermal gradient. A 5 mm microscope view window  
23 exists, from which dendrite image acquisition was performed.

24

25 **SI.2. 3D-cryobioprinting of FucoPol as proof-of-concept**

26 Here we show that a 0.5 wt.% FucoPol solution, responsible for creating the highly  
27 symmetrical, aligned and shape-defined tubular dendrites could also be used as a biocompatible  
28 matrix. **Table SI.1** shows the optimal parameters acquired in a previously described 3D-  
29 cryobioprinter (*Bioprinting* **27** e00225 (2022)) while attempting to extrude a 0.5 wt.% FucoPol  
30 aqueous solution. **Figure SI.2** shows preliminary photographs of the 3D-cryobioprinted 0.5  
31 wt.% FucoPol solution, before and after structural freezing. The photographs are not  
32 representative of what a final 2D waffle-like matrix would look like, but it does present  
33 conceptual proof that FucoPol has the mechanical robustness to be extruded and intentionally  
34 structured.

35 **Table SI.1.** Optimal printing parameters for a 0.5% FucoPol solution to verify POC printability.

Parameter description	Optimal value
Immersion bath contents	45% aqueous ethylene glycol (EG)
Print plate temperature	-12 °C
Nozzle gauge	18 G
Distance from nozzle to print plate	0.7 mm
Extrusion velocity	E-3 with F200
Bioprinted layer height	2.4 mm

36



37

38 **Figure SI.2.** Photographs demonstrating the preliminary POC printability of a 0.5% FucoPol solution. The left and right panels  
39 correspond, respectively, to a printed FucoPol structure before and after complete structural freezing of the liquid fraction.