Supporting information

Optimizing extrusion-based 3D bioprinting of plant cells with enhanced resolution and cell viability

Dezhi Zhou(周德志)[1, 3, 4, #], Peixi Li(李佩锡)[1, 3, 4, #], Shuang Yu(余爽)[1, 3, 4], Zhenhua Cui(崔镇华)[1, 3, 4], Tao Xu(徐弢)[5], Liliang Ouyang(欧阳礼亮)[1, 2, 3, 4, *]

1. Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, China

2. State Key Laboratory of Tribology in Advanced Equipment, Tsinghua University, Beijing, 100084, China

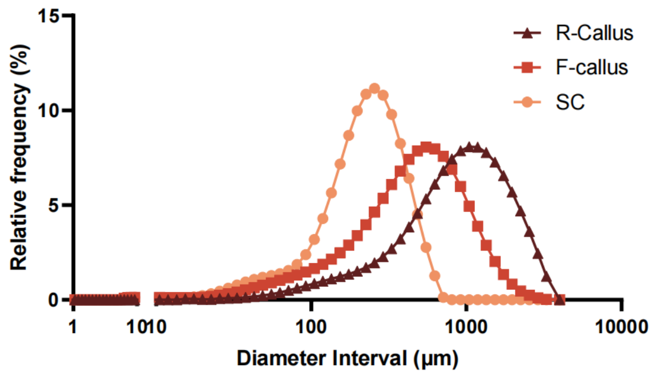
3. Biomanufacturing and Rapid Forming Technology Key Laboratory of Beijing, Beijing 100084, China

4. Biomanufacturing and Engineering Living Systems Innovation International Talents Base (111 Base), Beijing 100084, China

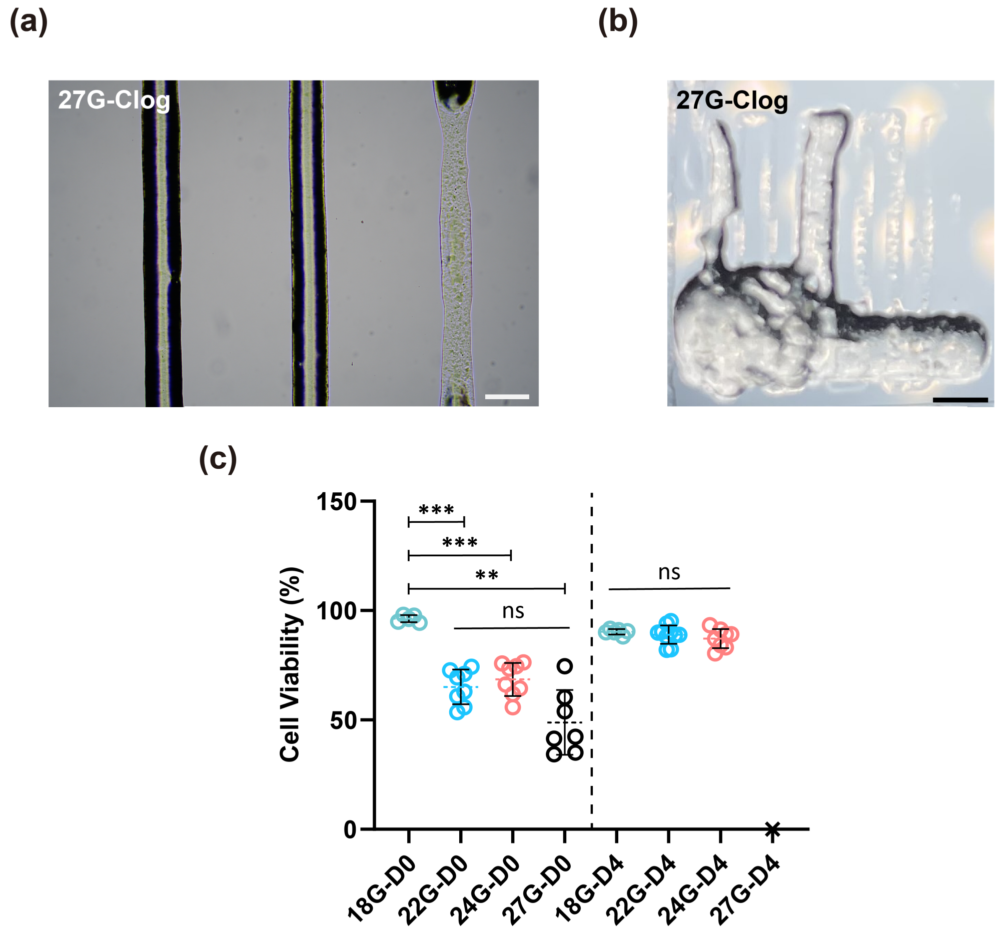
5. Center for Bio-intelligent Manufacturing and Living Matter Bioprinting, Research Institute of Tsinghua University in Shenzhen, Shenzhen 518057, China

Co-first authors

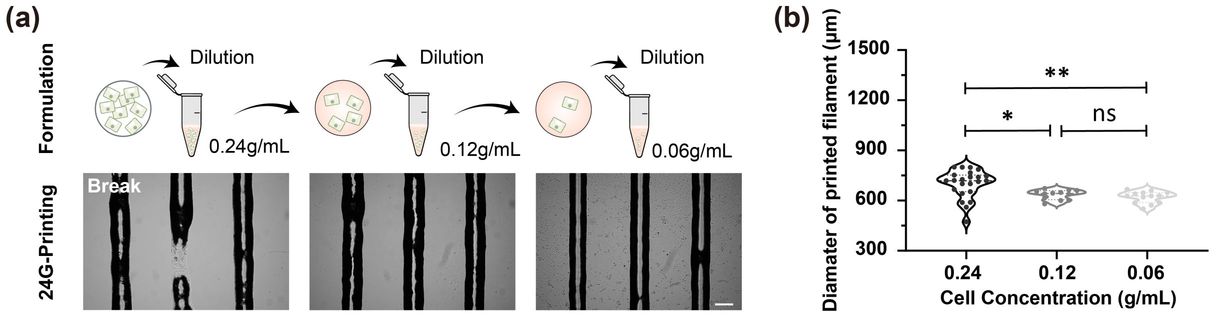
* Corresponding author: ouy@tsinghua.edu.cn



Supplementary Figure 1. The size distribution of regular callus (R-Callus), fragmented callus (F-Callus), and suspension cells (S-Cell) was measured by the laser particle analyzer.

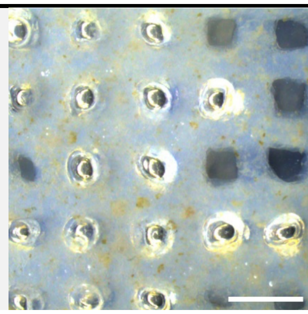
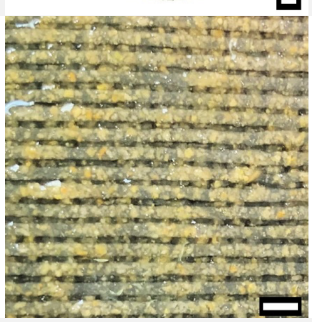


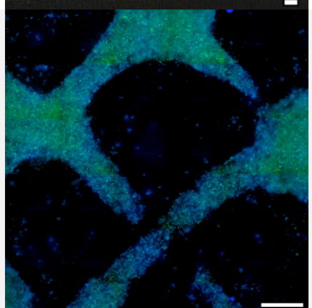
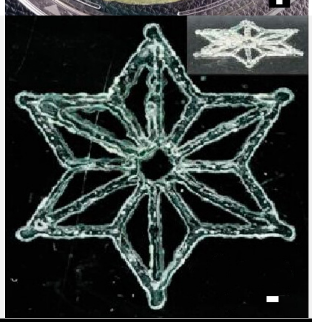
Supplementary Figure 2. (a) The optical images of extruded filaments and (b) the continuously printed scaffold demonstrated the clog of printing S-Cell bioink with a 27G nozzle. (c) Quantitative analysis of cell viability after bioprinting with 18G, 22G, 24G and 27G nozzles. Kruskal-Wallis test, ns, not significant; $**P < 0.01$, $***P < 0.001$. Scale bars: 500 μm (a), 2 mm (b).



Supplementary Figure 3. Influences of the S-Cell concentration on bioprinting and cell viability. (a) The representative images of extruded filaments composed of 0.24, 0.12 and 0.06 g/mL S-Cell formulations by using a 24G nozzle. (b) Quantitative analysis of filament width. Kruskal-Wallis test, ns, not significant; *P < 0.05, **P < 0.01. Scale bars: 500 μm (a).

Suppletmatry Table 1. Existing studies on the 3D bioprinting of land plant cells. Row 1 [1]: Reproduced from [1], © IOP Publishing Ltd. All rights reserved. Row 3 [3]: Reprinted from [3], Copyright (2017), with permission from Elsevier. Row 4 [4]: Reprinted from [4], Copyright (2019), with permission from Elsevier. Row 6 [6]: John Wiley & Sons. © 2021 Wiley-VCH GmbH. Row 7 [7]: Reprinted from [7], Copyright (2020), with permission from Elsevier. Row 8 [8]: Reproduced from [8]. CC BY 4.0. Row 9 [9]: Reproduced from [9]. CC BY 4.0.

Cell Source	**Nozzle Diameter** (mm)	**Representative Bioprinting Structures** (Scale bars: 2 mm)	**Quantitative Cell Viability After Bioprinting**	**Quantitative Cell Growth After Bioprinting**	**Year of Publicaton**	**Ref.**
Basil cells (Ocimum basilicum L)	0.610		N/A	~4-Fold after 20-day culture	2017	[1]
Basil cells (Ocimum basilicum L)	0.250 0.840	N/A	N/A, but more dead cells in the narrower nozzle group	N/A	2020	[2]
Lettuces cells (Valerianella locusta)	0.838		50-60% at day 0	N/A	2019	[3]
Carrots cells (Daucus carota L.)	1.0		N/A	2 to 3-Fold after 35-day culture	2020	[4]
Protoplast (Arabidopsis thaliana and Soybean)	Arabidopsis thaliana: 0.159; Soybean: 0.413	N/A	50-60% at day 0; ~30% at day 5	N/A	2022	[5]

Cell Source	Nozzle Diameter (mm)	Representative Bioprinting Structures (Scale bars: 2 mm)	Quantitative Cell Viability After Bioprinting	Quantitative Cell Growth After Bioprinting	Year of Publicaton	Ref.
Rice cells (Oryza sativa)	0.840		N/A	N/A	2021	[6]
Zinnia cells (Zinnia elegans)	N/A		-35% at day 2	N/A	2021	[7]
Zinnia cells (Zinnia elegans)	N/A		-50% at day 2	~4-Fold after 3-month culture	2022	[8]
Tobacco BY-2 cells (N. tabacum)	0.770		N/A	4 to 5-fold after 7-day culture	2024	[9]

Supplementary Table 2. Printing parameters for various nozzles

Nozzle	**Nozzle diameter** (μm)	**Extrusion flux** (mm^3/s)	**Moving speed of printer** (mm/s)	**Height of Each Layer** (mm)
18G	840	2	5	0.80
		1.5		
		1		
22G	410	0.75		0.40
		0.5		
		0.25		
24G	310	0.6		0.30
		0.4		
		0.2		
27G	210	0.25		0.25

Supplementary Reference

[1] Seidel, J., et al., *Green bioprinting: extrusion-based fabrication of plant cell-laden biopolymer hydrogel scaffolds.* Biofabrication, 2017. **9**(4).

[2] Emmermacher, J., et al., *Engineering considerations on extrusion-based bioprinting: interactions of material behavior, mechanical forces and cells in the printing needle.* Biofabrication, 2020. **12**(2).

[3] Vancauwenberghe, V., et al., *3D printing of plant tissue for innovative food manufacturing: Encapsulation of alive plant cells into pectin based bio-ink.* Journal of Food Engineering, 2019. **263**: p. 454-464.

[4] Park, S.M., H.W. Kim, and H.J. Park, *Callus-based 3D printing for food exemplified with carrot tissues and its potential for innovative food production.* Journal of Food Engineering, 2020. **271**: p. 109781.

[5] Van den Broeck, L., et al., *Establishing a reproducible approach to study cellular functions of plant cells with 3D bioprinting.* Science Advances, 2022. **8**(41).

[6] Varma, A., et al., *Immobilization of transgenic plant cells towards bioprinting for production of a recombinant biodefense agent.* Biotechnology Journal, 2021. **16**(10).

[7] Beckwith, A.L., J.T. Borenstein, and L.F. Velásquez-García, *Tunable plant-based materials via in vitro cell culture using a Zinnia elegans model.* Journal of Cleaner Production, 2021. **288**: p. 125571.

[8] Beckwith, A.L., J.T. Borenstein, and L.F. Velásquez-García, *Physical, mechanical, and microstructural characterization of novel, 3D-printed, tunable, lab-grown plant materials generated from cell cultures.* Materials Today, 2022. **54**: p. 27-41.
[9] Wang, Y.J., et al., *Advancing Engineered Plant Living Materials through Tobacco BY-2 Cell Growth and Transfection within Tailored Granular Hydrogel Scaffolds.* Acs Central Science, 2024. **10**(5): p. 1094-1104.