Supporting Information

Hydrogel Bioink with Multilayered Interfaces Improves Dispersibility of Encapsulated Cells in Extrusion Bioprinting

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KEYWORDS: 3D bioprinting, bioink, interfacial retention, gelatin methacrylate, cell sedimentation

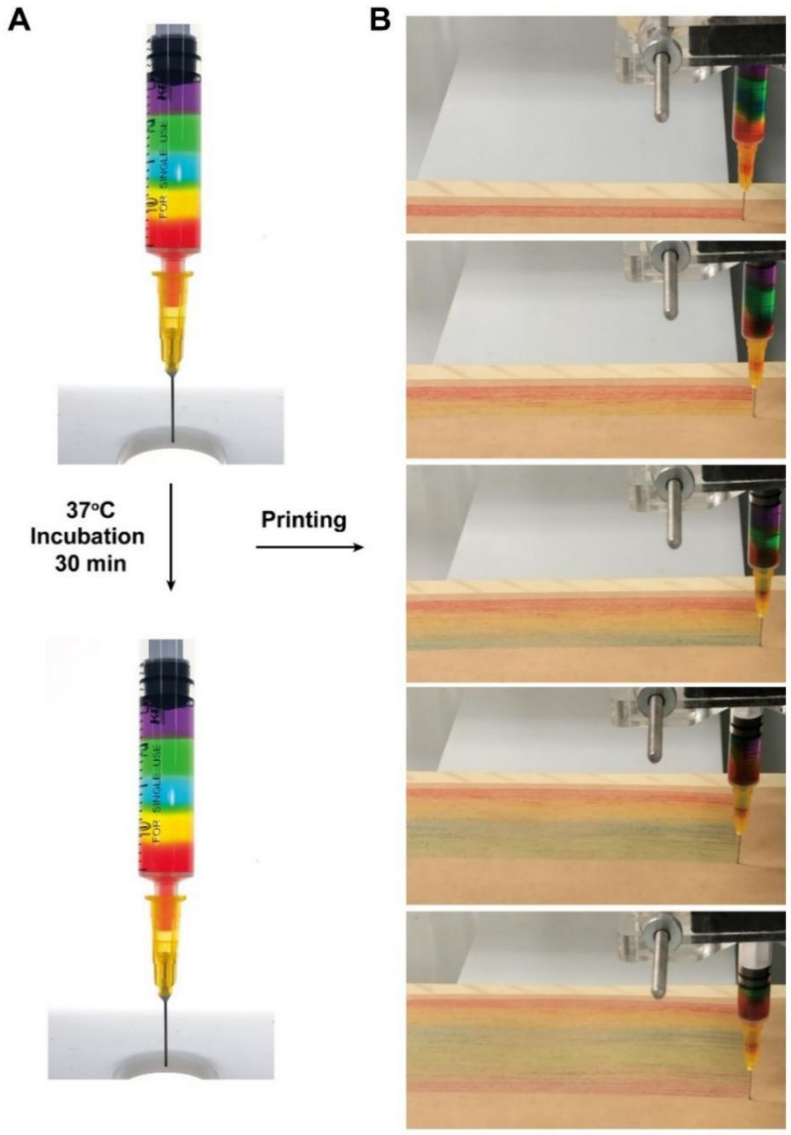


Figure S1. Printing with SF-M-GelMA bioink. A) The SF-GelMA bioinks were stained with food dyes as follows: SF-0.5-GelMA in red, SF-0.75-GelMA in yellow, SF-1-GelMA in blue, SF-1.25-GelMA in green, and SF-1.5-GelMA in purple. The loading of SF-M-GelMA was achieved by a cooling step. Incubation at 37 ℃ for 30 min was performed to detect the stability of the multilayered state. B) The ordered output of the SF-M-Layered GelMA bioink corresponded to that of the loading procedure. The value of M is 5.

The diffusion of SF molecules in GelMA solution (5%, w/v).

(1) We re-considered the diffusion of SF molecules in GelMA solution. Nauman and his colleagues detected and measured the mobility and diffusion rate of different protein molecules in water in their study.[1] They found that the diffusion coefficient of proteins was related to their molecular mass. The diffusion coefficient of bovine serum albumin (BSA), which the average molecular weight (MW) is 66.5 kDa, is 64-72 $\mu m^2/s$. The diffusion coefficient of Fibrinogen, which the average MW is 339.7 kDa, is 23-34 $\mu m^2/s$. The average MW of SF molecules in our study is about 100 kDa. Based on the results in Nauman's study, the diffusion coefficient of a single SF molecule is between 23-72 $\mu m^2/s$ in water at 25℃. According to the Stokes–Einstein equation, the diffusion coefficient can be calculated as following

$$D = \frac{KT}{3\eta\pi d}$$

, where D means diffusion coefficient, K is the consistency, T is absolute temperature, η is the viscosity of fluid, d is the diameter. It can be concluded that the difference in diffusion coefficient of SF molecules in water and GelMA is determined by the difference in viscosity of these two liquids. Moreover, the diffusion radius can be calculated as following

$$R = \sqrt{6DT}$$

, where R means the diffusion radius, D is the diffusion coefficient, T is the diffusion time. The viscosity of water is 8.9×10^{-4} Pa·s, and the viscosity of GelMA at zero shear rate in our study is higher than 1000 Pa·s. Hence, the diffusion radius of a single SF molecule at 25℃ is about 0.66-1.18 μm in 1h.

(2) Since the diffusion of SF molecule was difficult to be observed directly, we supplemented data to show the diffusion of food dyes at room temperature (25-30℃) as shown in Figure S2. The MW of food dye is about 1 kDa which is much smaller than that of SF molecule (the average of MW is 100 kDa). Hence, the diffusion of SF molecules is much slower than food dyes. The results showed that the diffusion of food dyes in the first 12h was about 1-2 mm. Hence, the diffusion of SF particles could be inferred at a smaller scale. As a result, only very slight variation in concentration would take place in the multilayered system during the printing procedure (even lasting for hours). Hence, the variation in bioink properties which may influence the printing operation and encapsulated cells may be almost ignored.

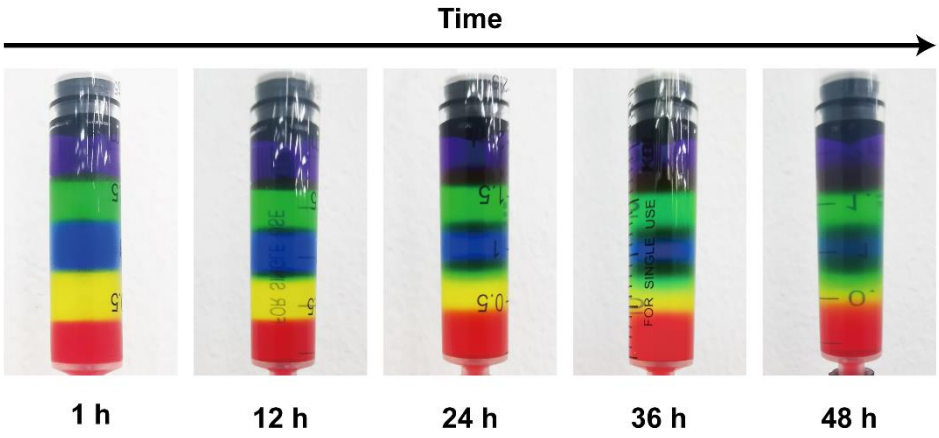


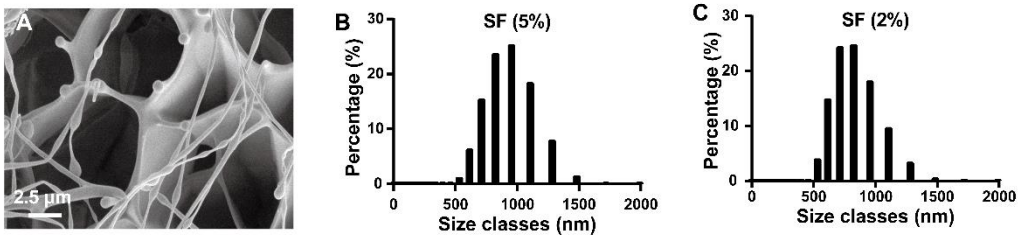
Figure S2. The diffusion of food dyes in multilayered bioink. There was slight diffusion of food dyes in the first 12h at 25 to 30℃. As time went by, more diffusion of food dyes took place. The volume of cartridge is 2 ml.

Reference

1. Nauman, J. V.; Campbell, P. G.; Lanni, F.; Anderson, J. L., Diffusion of insulin-like growth factor-I and ribonuclease through fibrin gels. Biophysical journal 2007, 92 (12), 4444-4450.

The morphology and size distribution of SF particles

The product manual described it as aqueous solution of silk fibroin (https://www.advancedbiomatrix.com/fibroin-silk-solution-2/fibroin-silk-solution/). Indeed, the initial solution was suspension of SF particles. We tested the size classes of the initial solution (5%) and the diluted solution (2%) (**Figure S3**). Additionally, we performed SEM to show the morphology of silk fibroin in GelMA hydrogel. The results showed that the silk fibroin was presented as particles (Figure S3A). The distribution of size classes in these two solutions was similar. The peak value of the size distribution in the initial solution (5%) was found at 955 nm, while the peak shifted to 825 nm in the diluted one (2%) (Figure S3B and S3C). It indicated that there may be more aggregation of SF molecules in initial solution (5%) than the diluted solution (2%).



Figures S3. The morphology and size distribution of SF particles. A) The morphology of SF particles in cross-linked GelMA hydrogel. B) Zeta-sizer data showing the size distribution of SF particles in initial SF solution (5%). C) Zeta-sizer data showing the size distribution of SF particles in diluted SF solution (2%).

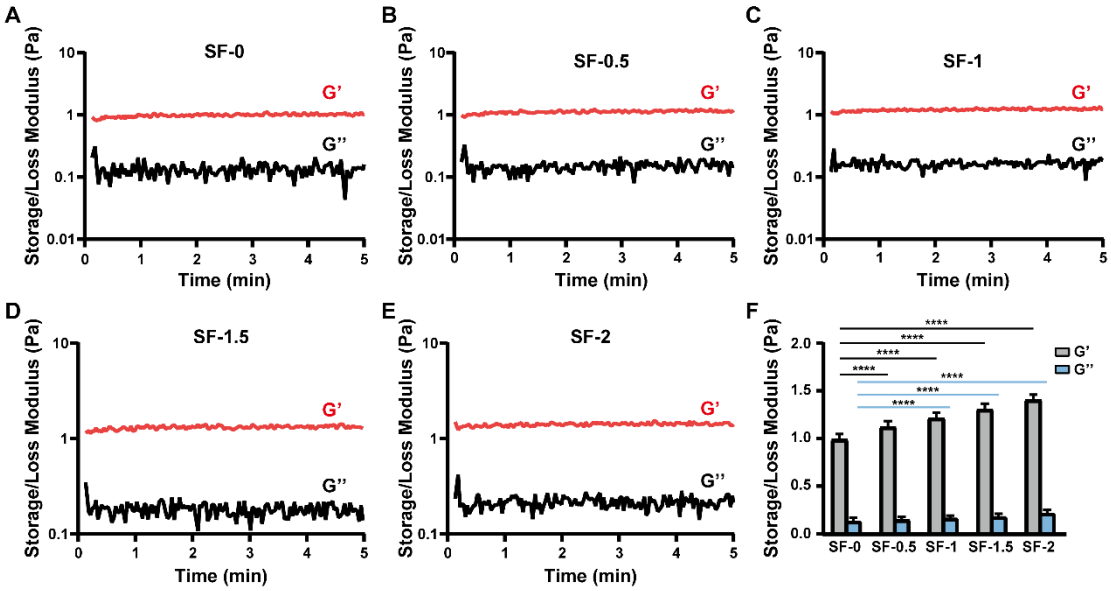


Figure S4. Storage/Loss modulus of different SF-GelMA bioinks. A) Storage/Loss modulus of pristine GelMA bioink. B) Storage/Loss modulus of SF-0.5-GelMA bioink. C) Storage/Loss modulus of SF-1-GelMA bioink. D) Storage/Loss modulus of SF-1.5-GelMA bioink. E) Storage/Loss modulus of SF-2-GelMA bioink. F) The results of Storage modulus (G') and Loss modulus (G'') of different blends.

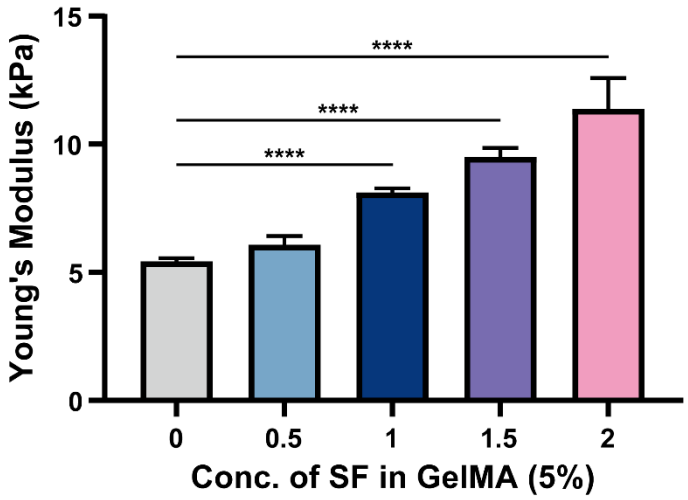


Figure S5. Young's modulus of different SF-GelMA bioinks. With the increasing concentration of SF mixed in GelMA, the Young's modulus was found to increase significantly (SF-0-GelMA vs. SF-1-GelMA, ****P < 0.0001, SF-0-GelMA vs. SF-1.5-GelMA, ****P < 0.0001, SF-0-GelMA vs. SF-2-GelMA, ****P < 0.0001, n=4).

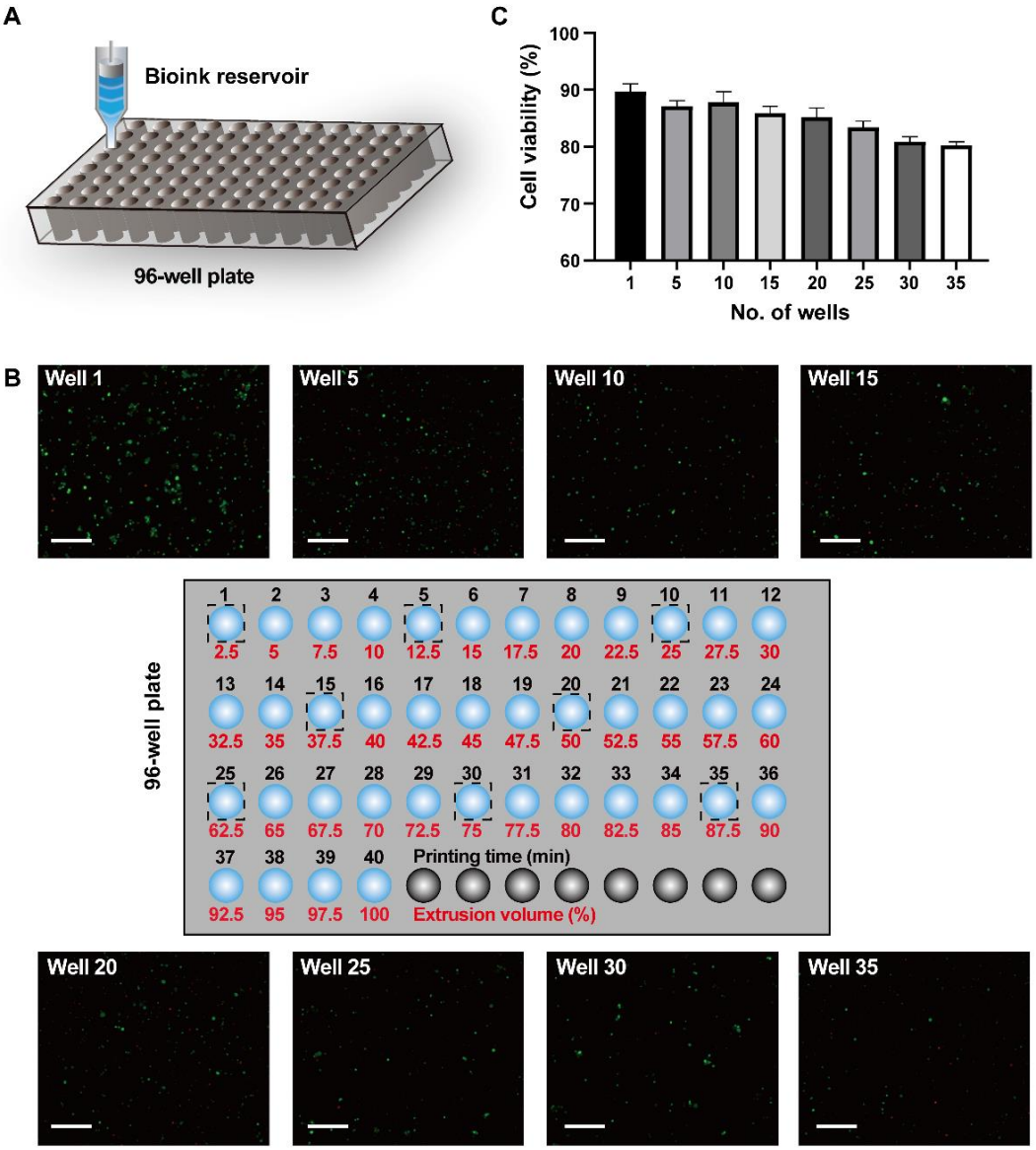


Figure S6. The Live/Dead assay was performed to detect the cell viability in multilayered-bioink. A) Schematic showing the printing procedure using multilayered bioink in the 96-well plate. The feeding rate was 50 μl/min. B) Live/Dead stain of different wells corresponding to different layers in the multilayered system. C) Cell viability in different layers. The total viability was higher than 80% after printing in the multilayered bioink (scale bar = 500 μm).

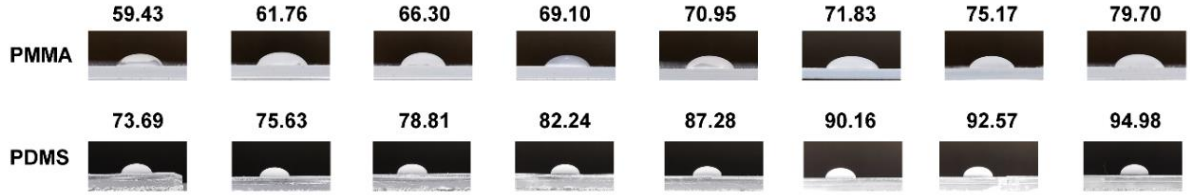


Figure S7. Contact angles of SF-GelMA bioinks on polymethyl methacrylate (PMMA) and polydimethylsiloxane (PDMS). The contact angles increased with increasing concentrations of SF particles.

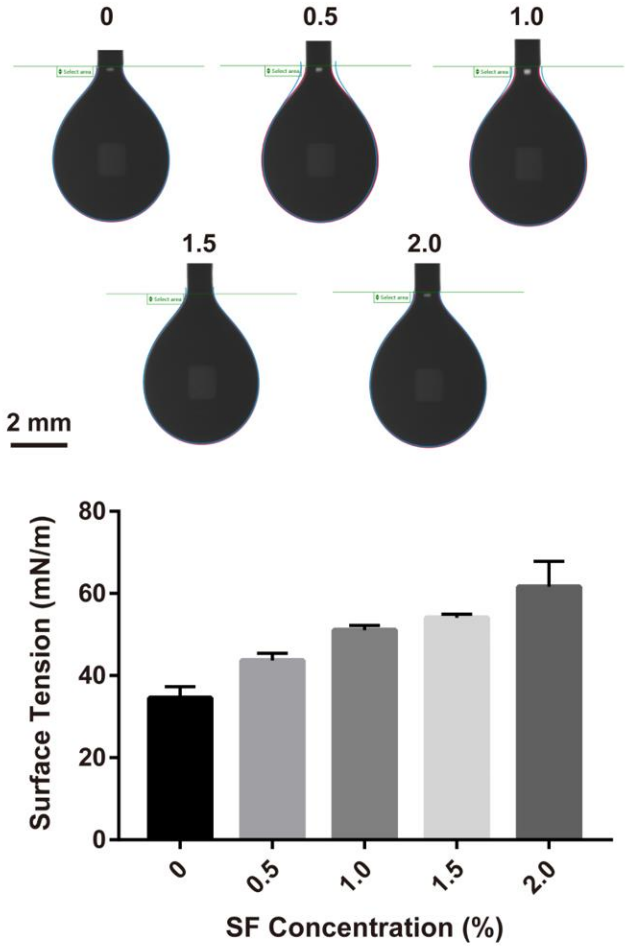


Figure S8. The surface tension of different SF-GelMA bioinks to the air was detected by the pendant drop method. The surface tension increased with the enhancement of embedded SF particles.

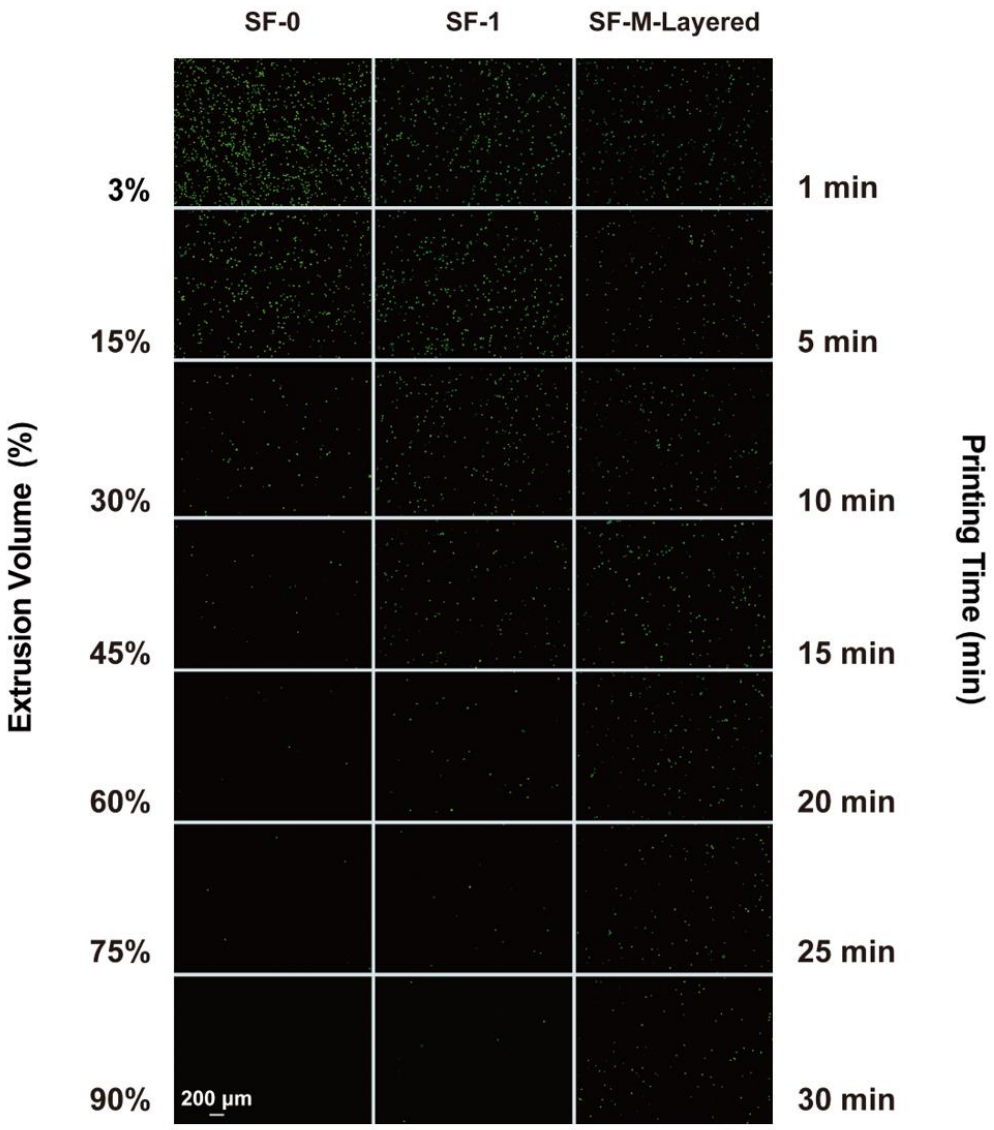


Figure S9. The original images of Figure 4D. The distribution of cells in SF-0-GelMA bioink (left column), SF-1-GelMA bioink (middle column), and SF-M-GelMA bioink (right column). The value of M is 5.

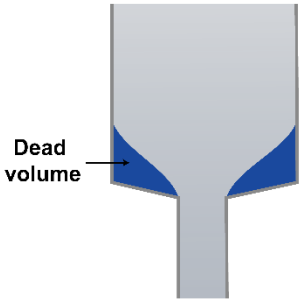


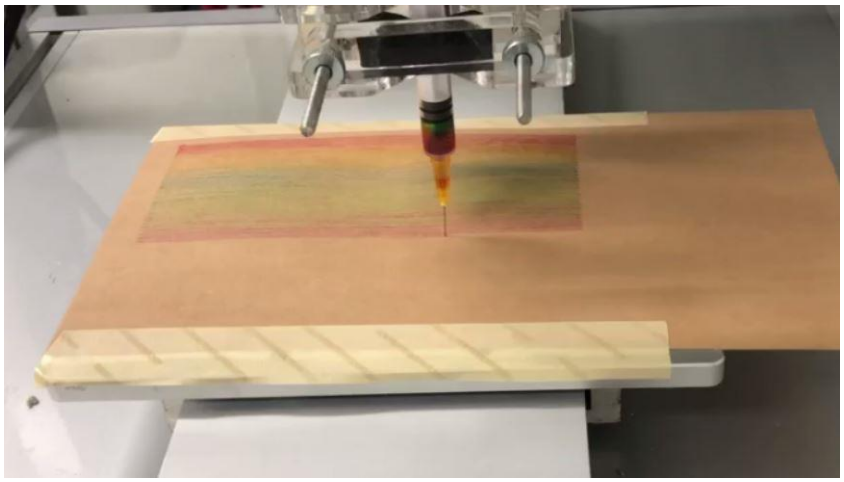
Figure S10. The schematic of the dead volume of ink cartridge. We marked the region of dead volume according to the computational simulations. The dead volume is an inevitable problem in maintaining entirely stability of interfacial tensions in multilayered system. Bioinks loaded in this region was extruded at last.

Table S1. Printing parameters.

Parameters	Value
Feeding speed (μl/min)	30-60
Working temperature (°C)	15-25
XY plotting speed (mm/s)	4-6
Nozzle height (mm)	0.5-2
UV light intensity (mW/cm)	500-1500
UV exposure time (s)	40-60
Nozzle inner diameter (mm)	0.21

Table S2. Parameters of bioinks.

	SF-0	SF-0.5	SF-1	SF-1.5	SF-2
K	11.1902	13.9725	16.1142	18.0131	19.4781
n	0.3095	0.2911	0.2784	0.2587	0.2392
ρ (g/cm^3)	1.0219	1.0231	1.0254	1.0272	1.0293



Video S1. A pre-experiment was performed to detect the stability of the cocktail-like state in the blended bioink. We recorded a video to show the whole printing procedure and observed an ordered output of bioinks during printing that corresponded to the loading order of the different layers.