Supplementary Information (SI) for Journal of Materials Chemistry B.

A thermoresponsive PEG-based methacrylate triblock terpolymer as bioink for 3D bioprinting

Kaiwen Zhang[1], Anna P. Constantinou[4], Cathal O'Connell[2,3], Theoni K. Georgiou[4*], Amy Gelmi[1*]

[1] School of Science, RMIT University, VIC 3000, Australia

[2] School of Engineering, RMIT University, Melbourne, VIC 3000, Australia

[3] Aikenhead Centre for Medical Discovery, St Vincent's Hospital Melbourne, Fitzroy, VIC 3065, Australia

[4] Department of Materials, Imperial College London, London, UK

S1 GPC and NMR results of OBD

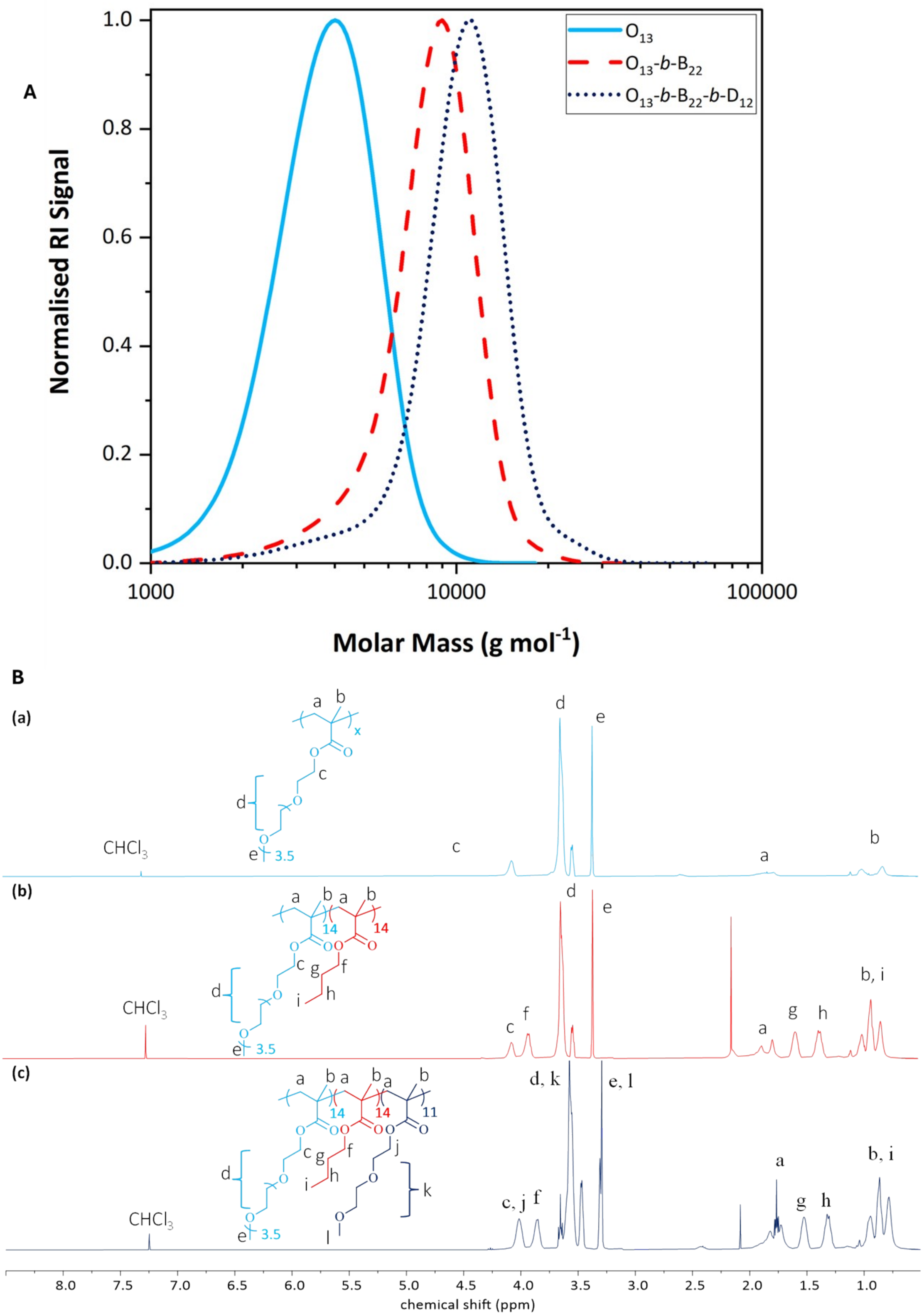


Figure S1. (A) GPC results of OBD in dotted dark blue line, and its linear precursors: homopolymer and diblock copolymer in solid light blue and dashed red lines, respectively. (B) Proton NMR results of OBD (c) and its linear precursors (a) homopolymer and (b) diblock copolymers, in deuterated chloroform.

S2: Preliminary printability test - Filament test

Method

OBD with 10%, 15%, 25%, 35% w/w concentration were tested. The printing pressure was gradually increased from 10 kPa in 5 kPa increments until a steady filament was observed, which indicates the optimal pressure for the specific polymer concentration in 3D printing applications.

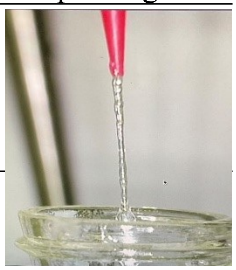
Result

A filament test was conducted to explore the suitable concentration of bioink under appropriate pressure for stable extrusion (Table S1). When the concentration was lower than 35%, OBS showed a droplet morphology, or slow and unstable filament. With increasing concentration, the bioink solution became more viscous, requiring a higher pressure to extrude through the nozzle head. Continuously increasing the pressure can accelerate extrusion and improve printing efficiency. Although high pressure may affect the viability of printed cells, previous studies have shown that cells embedded in 35% polymer content maintained a relatively high survival rate with printing pressures up to 45 kPa.[38]

35% w/w OBD at 75kPa passed the filament test by extruding a filament longer than 30 cm at a reasonable speed and was thus selected for further tests.

Table S1. Observation of polymer filament test with different concentration and pressure. The embedded image showed the extruded filament of 35% w/w OBD at 75kPa.

Concentration	Pressure (kPa)	Morphology	Notes
10% w/w	10	Drop	
	15	Filament	Cloudy gel
15% w/w	10	NA*	
	15	Filament	Slow
	20	Filament	Liquified shortly after printing
25% w/w	10-15	NA	
	20	Filament	Slow
	25	Filament	Slow, curling up
	30	Filament	too fast
35% w/w	10-55	NA	
	60-70	Filament	Slow
	75	Filament	Steady, hangs >5mm



*NA indicates no extrusion of bioink from nozzle.

S3 F127 line printing

Method

Same as 2.5.1

Result

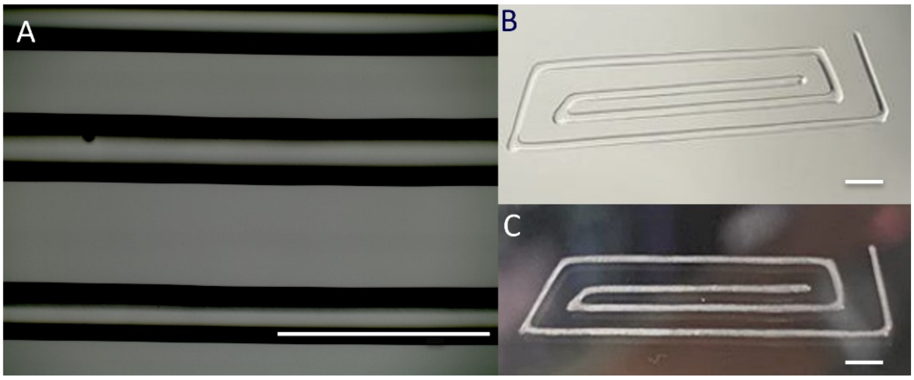


Figure S4. 25% w/w F127 print test with printing speed 300 mm/min, 26 kPa printing pressure at 37°C. (A) Microscopy image of line test pattern with formal patterns. The printed lines were well defined with width 1.08 ± 0.008 mm. (B-C) Photos of printed pattern 0h and 24h after printing, showing good stability of F127 printed pattern at room temperature. Scale bar 2 mm.

S4 35 w/w% OBD 2-layer stacking results

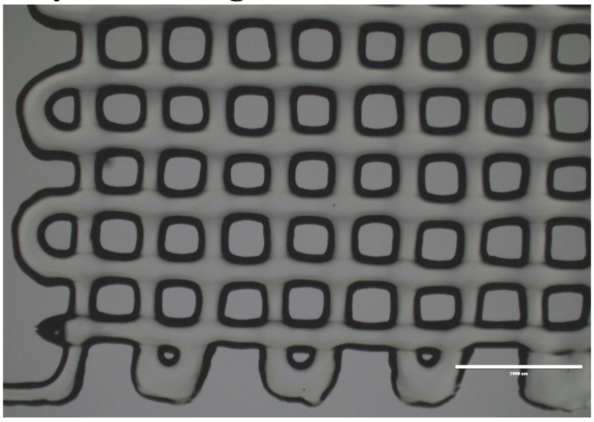


Figure S5. 35% w/w OBD 2-layer print test with printing speed 450 mm/min, 75 kPa printing pressure at 37°C. The average printability calculated with Eq.1 was 0.874. Scale bar 2 mm.

S5 G-code for line test

```
G21 ; set units to millimetres
G90 ; use absolute coordinates
G0 X-20 Y0 F1500
G0 Z0.25
;pre-extrusion
M760
G0 X-20 Y15 F150
G0 X-10.0 Y15 F150
;Line 1
G0 X10.0 Y15 F300 ;Move in a horizontal line at 7.5 mm/s
;Line 2
G0 X10.0 Y13.5 F450
G0 X-10.0 Y13.5 F450
;Line 3
G0 X-10.0 Y12 F600
G0 X10 Y12 F600
;Line 4
G0 X10 Y10.5 F750
G0 X-10 Y10.5 F750
;Line 5
G0 X-10 Y9 F900
G0 X10 Y9 F900
;Line 6
G0 X10 Y7.5 F1050
G0 X-10 Y7.5 F1050

M761
G0 Z20 F450 ;post structure code
M84 ;End GCODE
```

S6 G-code for 2-layer stacking test

```
G21 ; set units to millimetres
G90 ; use absolute coordinates
G0 X-20 Y-10F1500
G0 Z0.30
;pre-extrusion
M760
G0 X-20 Y-10 F150
G0 X-15.0 Y-10 F150
; layer 1
G1 X-10 Y-10 Z0.2 F450
G1 X10 Y-10 Z0.2 F450
G1 X10 Y-8 Z0.2 F450
G1 X-10 Y-8 Z0.2 F450
G1 X-10 Y-6 Z0.2 F450
G1 X10 Y-6 Z0.2 F450
G1 X10 Y-4 Z0.2 F450
G1 X-10 Y-4 Z0.2 F450
G1 X-10 Y-2 Z0.2 F450
G1 X10 Y-2 Z0.2 F450
G1 X10 Y0 Z0.2 F450
G1 X-10 Y0 Z0.2 F450
G1 X-10 Y2 Z0.2 F450
G1 X10 Y2 Z0.2 F450
G1 X10 Y4 Z0.2 F450
G1 X-10 Y4 Z0.2 F450
G1 X-10 Y6 Z0.2 F450
G1 X10 Y6 Z0.2 F450
G1 X10 Y8 Z0.2 F450
G1 X-10 Y8 Z0.2 F450
G1 X-10 Y10 Z0.2 F450
G1 X10 Y10 Z0.2 F450
G1 X10 Y10 Z0.45 F450
; layer 2
G1 X10 Y-10 Z0.45 F450
G1 X8 Y-10 Z0.45 F450
G1 X8 Y10 Z0.45 F450
G1 X6 Y10 Z0.45 F450
G1 X6 Y-10 Z0.45 F450
G1 X4 Y-10 Z0.45 F450
G1 X4 Y10 Z0.45 F450
G1 X2 Y10 Z0.45 F450
G1 X2 Y-10 Z0.45 F450
G1 X0 Y-10 Z0.45 F450
G1 X0 Y10 Z0.45 F450
G1 X-2 Y10 Z0.45 F450
G1 X-2 Y-10 Z0.45 F450
G1 X-4 Y-10 Z0.45 F450
G1 X-4 Y10 Z0.45 F450
G1 X-6 Y10 Z0.45 F450
G1 X-6 Y-10 Z0.45 F450
G1 X-8 Y-10 Z0.45 F450
G1 X-8 Y10 Z0.45 F450
G1 X-10 Y10 Z0.45 F450
G1 X-10 Y-10 Z0.45 F450

M761
G0 Z20  F450 ;post structure code
M84 ;End GCODE
```

S7 G-code F127 printing

```
G28 ; home all axes

G21 ; set units to millimeters

G90 ; use absolute coordinates

M83 ; use relative distances for extrusion

G1 X-20 Y0 F1500

G1 Z0.05


;Line 1

G0 X10.0 Y14 F300   ;Move in a horizontal line at 5 mm/s

;Line 2

G0 X12 Y7.5 E0.17912 F300

G0 X-12.0 Y7.5  E0.17912 F300

;Line 3

G0 X-10.0 Y12.5 E0.17912 F300

G0 X8 Y12 E0.17912 F300

;Line 4

G0 X9 Y9 E0.17912 F300

G0 X-9 Y9 E0.17912 F300

;Line 5

G0 X-8 Y10.5 E0.17912 F300

G0 X7 Y10.5 E0.17912 F300


M761

G0 Z20 F450 ;post structure code

M84 ;End GCODE
```