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Supplementary Materials for

3D printing of bacteria into functional complex materials

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Supplementary Materials

section S1. Free-spanning length calculation

With the following equation, we estimated the minimal storage modulus *G'* necessary to print grids containing spanning filaments with acceptable deflection

$$G' \geq 1.4\gamma s^4 D \tag{1}$$

where γ is the specific weight of the ink, *s* is the reduced span distance (=L/D), *L* is the span width and *D* is the nozzle diameter. An acceptable deflection of 0.05D was assumed in this calculation (*36*).

section S2. Printer software and hardware

The printer hardware and software is based on a previously reported similar technique (*48*). The 3D printer was built upon a CNC gantry platform (Heiz S1000T). One I/O port was configured to control the material extrusion. The paste extruder itself was a Viscotec eco-PEN 300, configured to extrude at 28µl / min, while the print-head was traversed at a constant 400mm/min. Extrusions were outputted through a 23 Gauge (0.33mm ID) blunt precision tip of length 6.25mm.

A second I/O on the printer was used to control the laser measurement system. The device (Keyence LKG-5000) was mounted on the print head carriage in-line with the extrusion nozzle. An Arduino Uno microcontroller was programmed to act as an intermediary between the printer's I/O ports and the laser controller's RS-232 interface. On command to make a measurement, the Arduino would request data from the Keyence controller. A full scan was performed by rastering the laser over the desired substrate, making measurements at a user defined resolution, typically every 0.5mm in both X and Y direction.

A bespoke data acquisition program was written in Java, which continually monitored the Arduino microcontroller. Whenever measurement data was received from the laser, it was combined with the corresponding X and Y coordinates, thus creating XYZ point-cloud that fully described the substrate surface. This point-cloud could then be utilized as the basis for NC code rectilinear toolpath waypoints, which defined the deposition path.

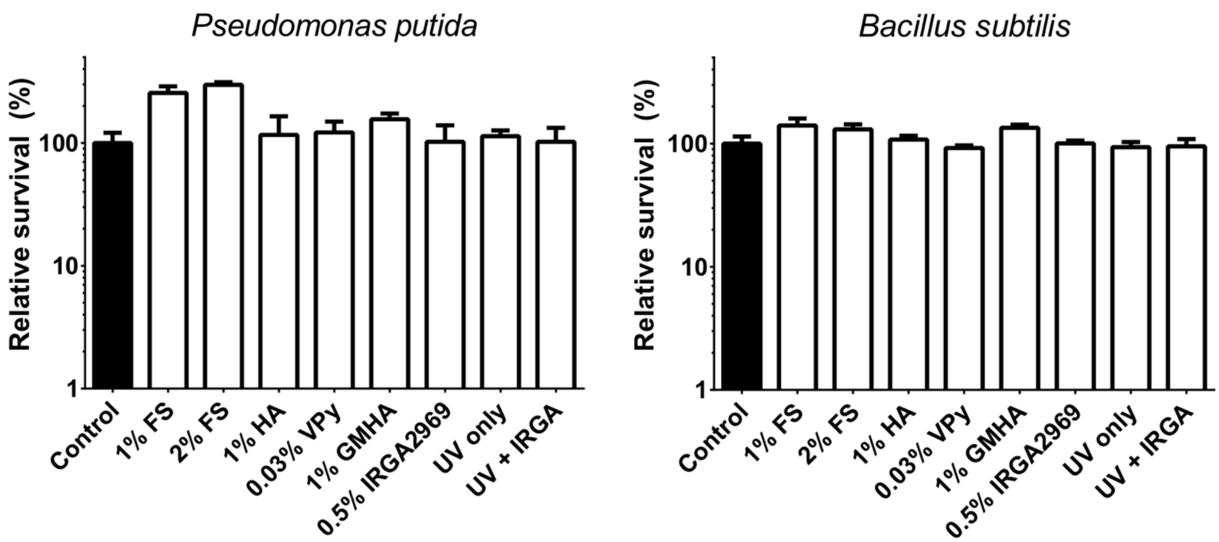

fig. S1. Toxicity assay for *P. putida* and *B. subtilis*. Overnight cultures of *B. subtilis* and *P. putida* were incubated with different ink components (FS: fumed silica, HA: hyaluronic acid, VPy: Vinylpyrrolidone, GMHA: glycidyl-methacrylate modified hyaluronic acid, and Irgacure2969) for 1 h and/or exposed to UV-light (90 mW, 365 nm, 60 s), diluted and subsequently plated on LB-agar and ½ BHI-agar, respectively. Bacterial colonies were quantified after 12 h of growth.

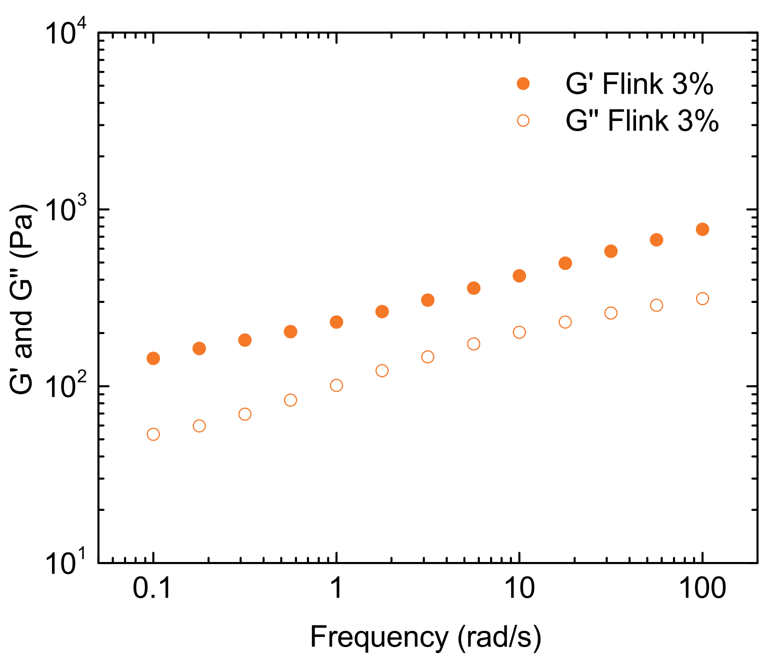

fig. S2. Frequency sweep of a 3 wt % Flink from 0.1 to 100 rad/s at a strain of 1%.

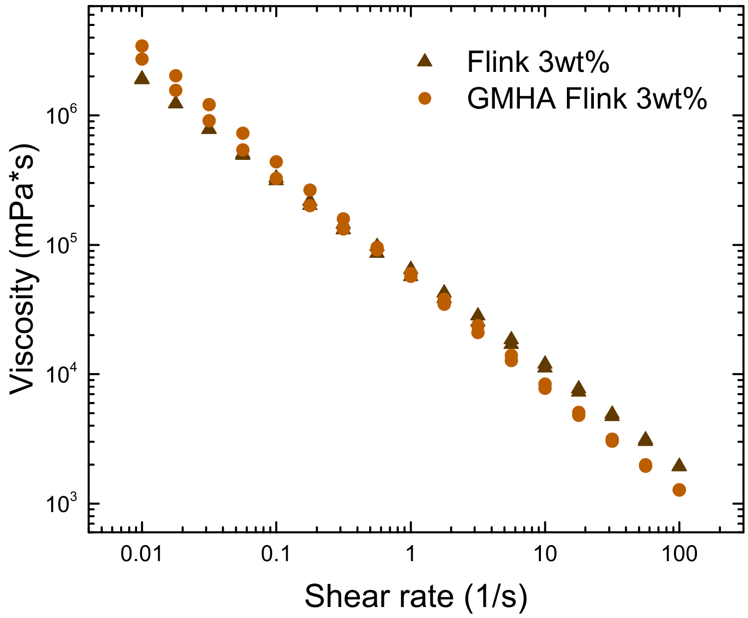

fig. S3. Flow curve of 3 wt % Flink-GMHA and 3 wt % Flink.

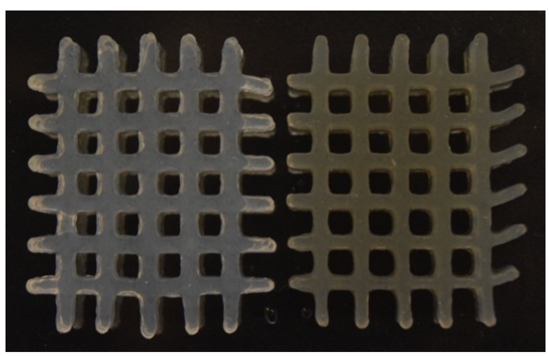

fig. S4. A printed grid structure using a 4.5 wt % Flink-GMHA loaded with *P. putida*, a known phenol degrader, was incubated in an MM with phenol as the only carbon source (left). After growth (right), proliferation is visible through the brown color, an indication of high bacterial densities.

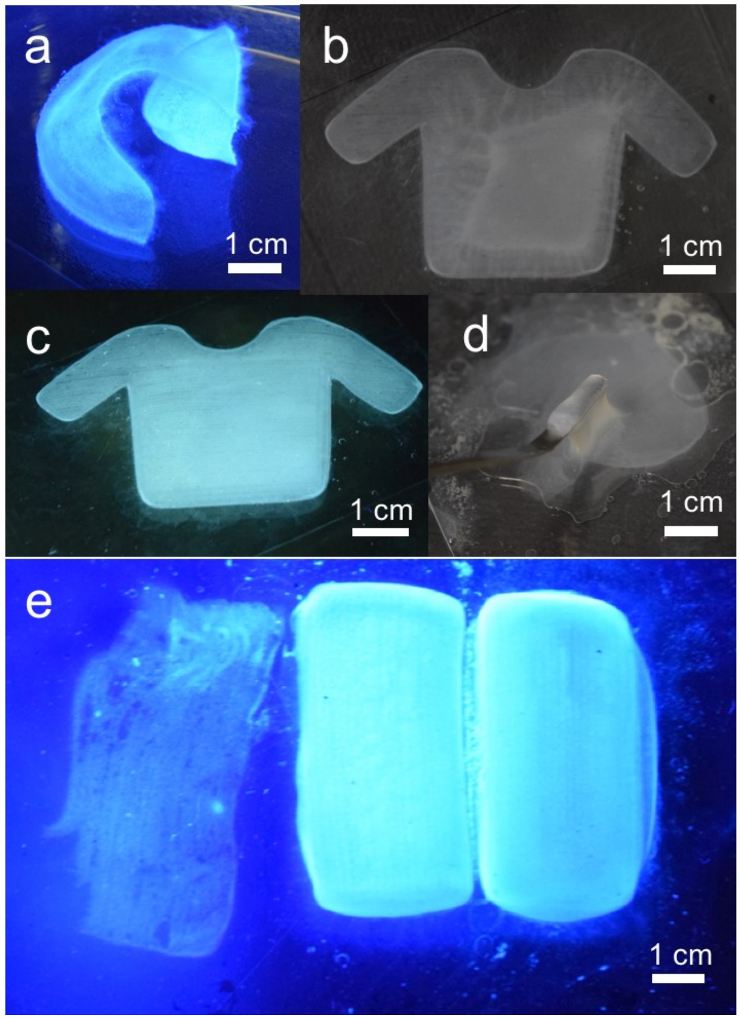

fig. S5. Examples of 3D printed shapes after bacterial cellulose growth and removal of the Flink. (a) Flink 3 wt % half circle dissolved and left in water for several days. (b,c) A T-shirt printed with a 4.5 wt % ink, (b) slowly dissolving it's constituents in water leaving a layer of bacterial cellulose and (c) stained with fluorescent brigthener 28. (d) After removal of constituents, the gel becomes transparent while remaining cohesive. (e) 3D printed rectangles of *A. xylinum* in a Flink of 4.5 wt % with a height of 0.5 mm, 1.5 mm and 2.5 mm.

table S1. Data obtained from the oscillatory amplitude sweep measurements for 3, 4.5, 6 and 9 wt % Flinks.

	3 wt % Flink			4.5 wt % Flink			6 wt % Flink			9 wt % Flink		
Shear deformation	G' [Pa]	G" [Pa]	Stress [Pa]	G' [Pa]	G" [Pa]	Stress [Pa]	G' [Pa]	G" [Pa]	Stress [Pa]	G' [Pa]	G" [Pa]	Stress [Pa]
0.01	263.1	112.3	0.0	997.4	327.3	0.1	2325.5	564.4	0.2	4407.4	2176.8	0.5
0.0178	300.8	113.6	0.1	1100.1	288.9	0.2	2434.2	558.4	0.4	4777.3	2074.7	0.9
0.0316	314.3	113.5	0.1	1155.5	268.8	0.4	2446.6	554.3	0.8	4949.4	1996.3	1.7
0.0562	319.6	113.9	0.2	1186.7	261.5	0.7	2462.6	550.9	1.4	5054.0	1935.1	3.0
0.1	319.8	114.7	0.3	1211.3	255.6	1.2	2464.7	551.6	2.5	5115.5	1894.8	5.5
0.178	316.2	115.2	0.6	1218.9	252.8	2.2	2451.8	551.4	4.5	5143.4	1859.9	9.7
0.316	307.2	115.8	1.0	1210.5	252.2	3.9	2411.5	555.0	7.8	5105.6	1832.9	17.2
0.562	292.2	116.7	1.8	1174.5	253.8	6.8	2327.6	562.3	13.5	4974.1	1812.4	29.8
0.999	271.0	117.5	3.0	1103.3	256.8	11.3	2174.1	571.0	22.5	4706.3	1793.5	50.4
1.78	246.1	118.1	4.9	991.9	261.4	18.2	1933.0	581.4	35.8	4223.1	1763.1	81.5
3.16	218.8	117.5	7.9	839.6	267.9	27.8	1614.8	591.0	54.3	3550.4	1720.0	124.7
5.62	182.6	113.0	12.1	660.0	269.4	40.1	1245.6	583.7	77.3	2752.2	1641.8	180.2
10	133.1	102.3	16.8	476.8	254.8	54.1	883.9	544.3	103.8	1946.5	1495.2	245.7
17.8	85.8	85.9	21.6	313.2	221.1	68.1	583.4	471.1	133.5	1278.0	1255.7	318.2
31.6	51.7	67.9	27.0	194.0	180.8	83.8	365.1	380.8	166.6	818.3	998.5	407.7
56.2	31.1	53.5	34.8	115.5	142.8	103.2	224.1	303.0	211.8	515.3	774.1	522.6
99.9	18.7	42.5	46.4	65.9	112.1	129.9	133.1	239.1	273.6	313.7	594.0	671.7
178	11.1	33.9	63.4	36.8	86.8	167.6	73.2	181.0	347.4	180.6	448.8	860.9
316	6.2	25.9	84.4	18.1	60.9	200.8	36.4	126.5	416.0	95.5	322.5	1063.3
562	3.2	18.7	106.6	8.4	40.2	231.2	17.0	82.8	475.3	44.7	213.4	1225.9
999	1.6	12.9	129.4	3.7	25.3	255.9	7.7	52.0	526.4	19.7	132.1	1337.4