Supplementary Materials

Silk Fibroin-Based Inks for *In Situ* 3D Printing Using a Double Crosslinking Process

## Materials and methods

Viscosity tests

Viscosity of Sil-Ma 15mB and 30mB at 5% (w/V) was measured with HR-2 Rheometer (TA Instrument, Delaware), using 50mm 2,0° cone plate, Peltier plate Aluminium, at 25°C with shear rate ramp from 10 to 1000 s^{-1}. At least 3 replicates per conditions were tested.

Electron Microscopy

Supra 40 Field-Emission Scanning Electron Microscope (Zeiss, Germany) was used to investigate the internal structures of 15 and 30mB double crosslinked (DC) printed samples. All the conditions were printed with a 25G nozzle. After printing, samples were frozen at -80°C for 12 hours, later lyophilized at -50°C and 0 Bar for 24 hours to remove all the water. Lastly, before images observation, all the specimens were sputtered with platinum-palladium (Pt/Pd, 80:20) conductive thin coating (Q150T ES, Quorum Technologies, UK).
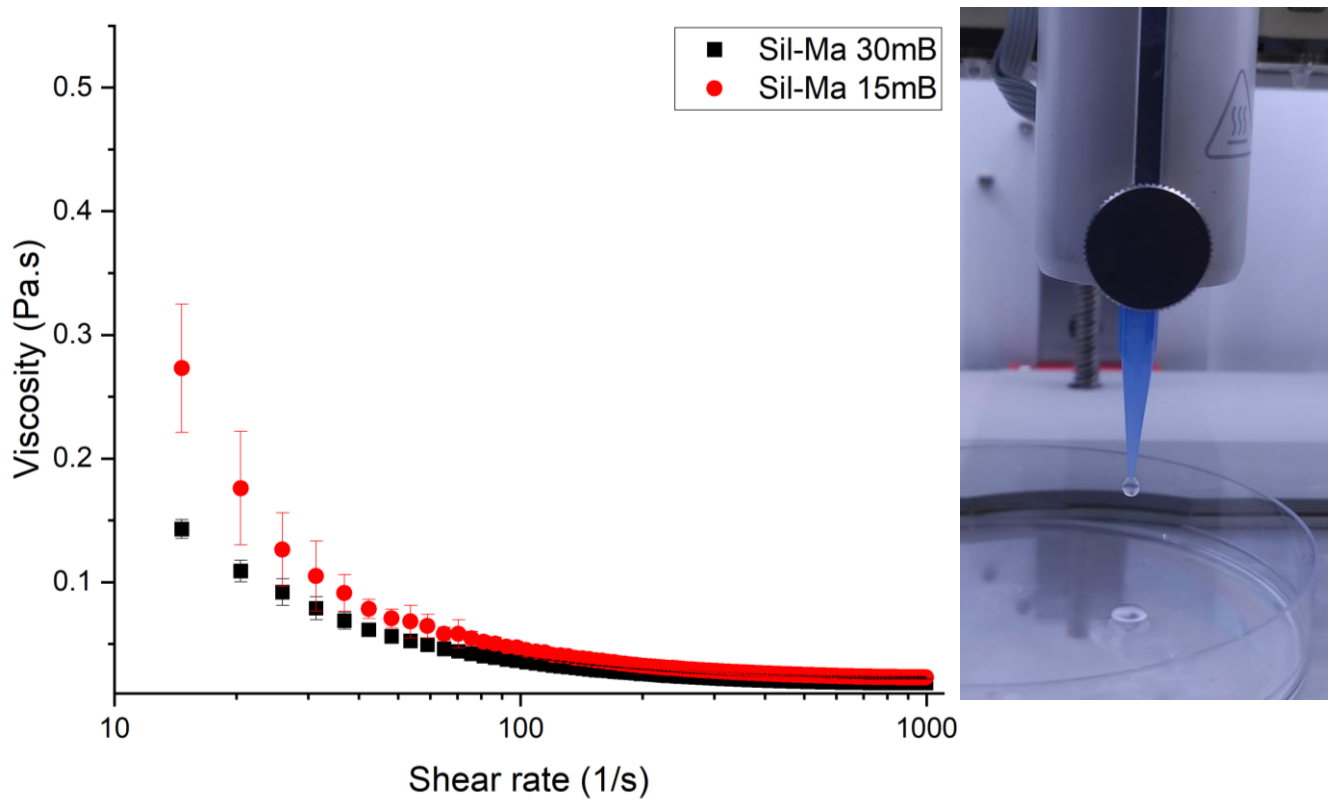
Figure S1. *Viscosity measurement of Sil-ma 15mB and 30mB at different shear rates and 3D printing of the ink without any pre-crosslinking leading to drop formation instead of filament formation, thus lacks any shape fidelity.*
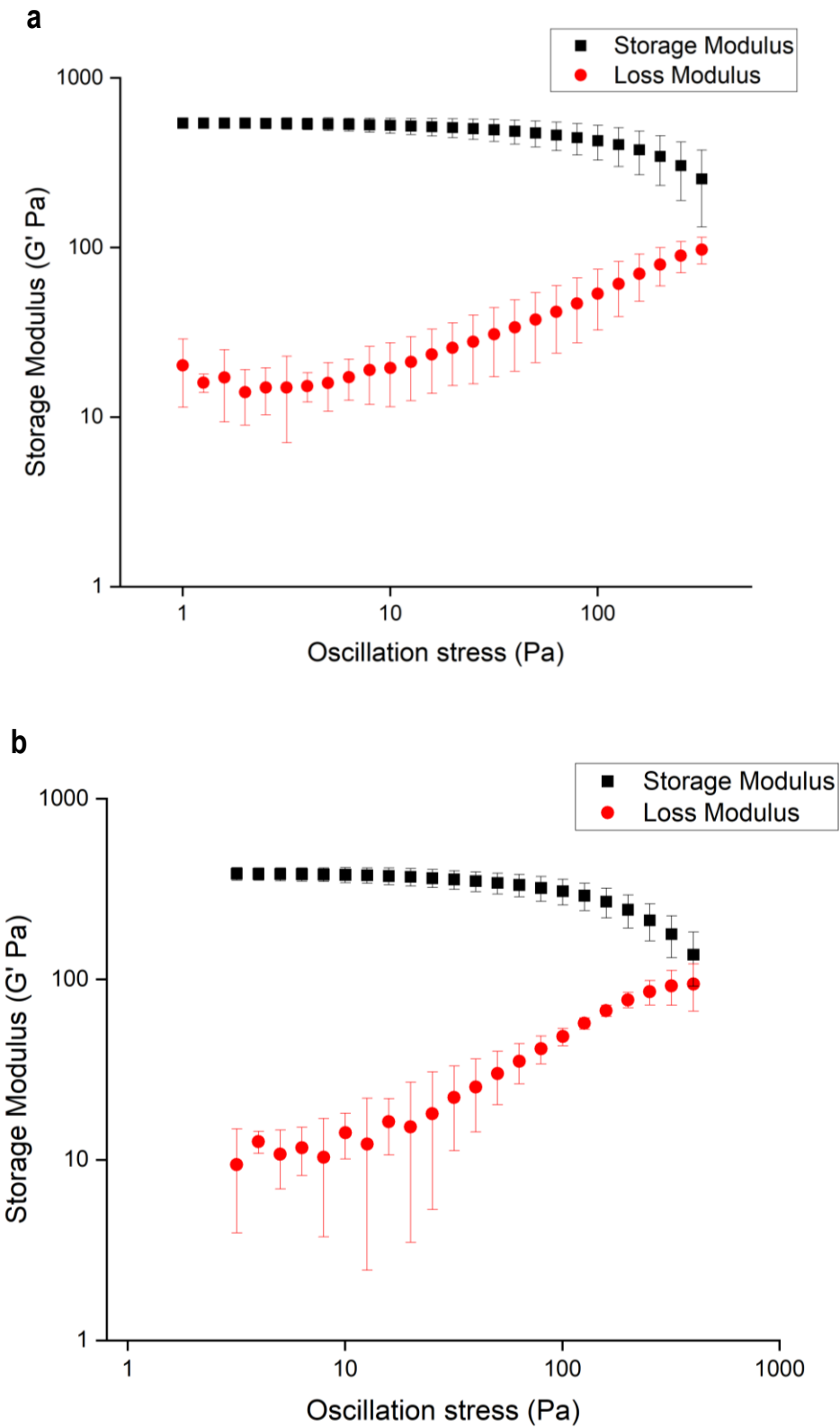
Figure S2. *Viscoelastic analyses to assess ink printability. a) double crosslinked (DC) condition of the 15mB Sil-Ma. No flow points were detected. b) enzymatic crosslinked (EC) condition of 15mB Sil-Ma, no flow point detected. The test was stopped when gels broke.*

Table S1. Amide I deconvolution with secondary structure quantification of 15mB and 30mB Sil-Ma, before and after extrusion.

	15mB		30mB	
	Before Extrusion	**After extrusion**	**Before Extrusion**	**After extrusion**
Side Chains	0,4	3,8	0,7	3,8
Antiparallel β-sheets	21,8	41,1	27,3	34,8
Random Coil	54,8	40,5	49,7	44,7
β-turns	23,0	12,1	22,3	14,1
Parallel β-sheets	0,0	2,5	0,0	2,6

Table S2. Amide I deconvolution secondary structure quantification of 15mB Sil-Ma of the pre-photo-crosslinked (PC) and the double crosslinked condition (DC) condition at day 1, 7, and 14 in medium and water
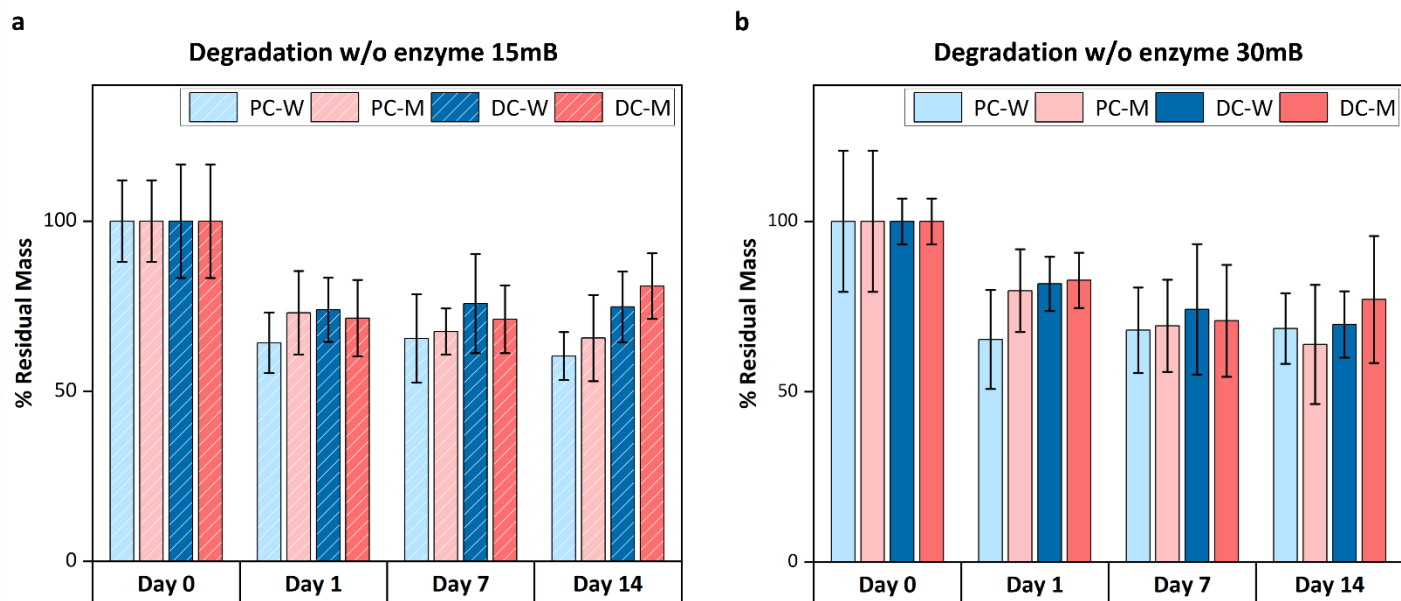
|		**Day 1**	**Day 7**	**Day 14**
PC medium	**Side Chains**	1,2	0,5	0,4
	Antiparallel β-sheets	45,0	48,1	53,0
	Random Coil	34,8	31,5	28,6
	β-turns	13,7	14,3	15,1
	Parallel β-sheets	5,3	5,5	3,0
PC water	**Side Chains**	0,6	0,5	0,3
	Antiparallel β-sheets	47,8	48,8	44,6
	Random Coil	31,1	27,8	30,1
	β-turns	15,0	17,5	19,2
	Parallel β-sheets	5,6	5,4	5,8
DC medium	**Side Chains**	0,5	0,6	0,0
	Antiparallel β-sheets	44,7	46,1	49,6
	Random Coil	29,8	32,1	32,9
	β-turns	19,6	15,4	14,3
	Parallel β-sheets	5,5	5,8	3,2
DC water	**Side Chains**	0,7	0,6	0,0
	Antiparallel β-sheets	46,4	46,4	51,2
	Random Coil	26,6	34,4	31,9
	β-turns	21,8	12,6	14,2
	Parallel β-sheets	4,6	5,9	2,6
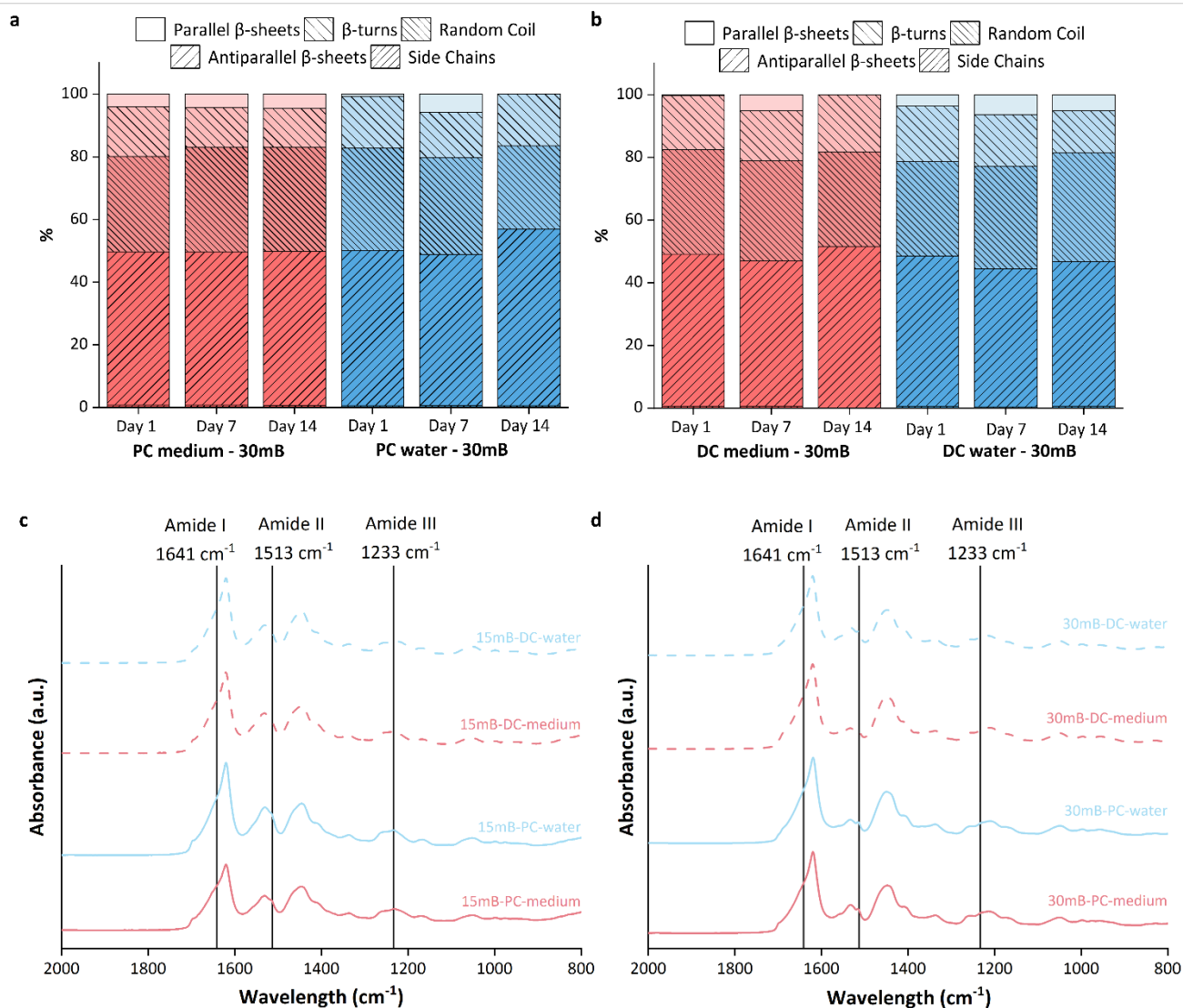
Figure S3. *a) Sil-Ma 30mB amide I deconvolution of the pre-photo-crosslinked (PC) condition at day 1, 7, and 14 both in medium and water; b) Sil-Ma 30mB amide I deconvolution of the double crosslinked (DC) condition at day 1, 7, and 14, both in medium and water. c) Day 1 spectra of 15 mB Sil-Ma pre-crosslinked (PC) and double crosslinked (DC) incubated in medium and water at 37°C for 1 day before the test. d) Day 1 spectra of 30 mB Sil-Ma pre-crosslinked (PC) and double crosslinked (DC) incubated in medium and water at 37°C for 1 day before the test.*

Table S3. *Amide I deconvolution, secondary structure quantification of 30mB Sil-Ma of the pre-photo-crosslinked (PC) and the double crosslinked condition (DC) condition at day 1, 7, and 14 in medium and water*

		Day 1	Day 7	Day 14
PC medium	**Side Chains**	0,8	0,8	0,6
	Antiparallel β-sheets	48,9	48,9	49,2
	Random Coil	30,4	33,3	33,2
	β-turns	15,8	12,7	12,3
	Parallel β-sheets	4,1	4,3	4,6
PC water	**Side Chains**	0,5	0,7	0,5
	Antiparallel β-sheets	49,6	48,1	56,5
	Random Coil	32,7	31,0	26,4
	β-turns	16,4	14,3	16,5
	Parallel β-sheets	0,7	5,9	0,0
DC medium	**Side Chains**	0,6	0,5	0,1
	Antiparallel β-sheets	48,5	46,5	51,3
	Random Coil	33,4	31,9	30,2
	β-turns	17,1	16,0	18,3
	Parallel β-sheets	0,4	5,1	0,0
DC water	**Side Chains**	0,7	0,4	0,6
	Antiparallel β-sheets	47,8	44,1	46,2
	Random Coil	30,1	32,8	34,6
	β-turns	17,8	16,4	13,5
	Parallel β-sheets	3,6	6,3	5,1

a Degradation w/o enzyme 15mB

b Degradation w/o enzyme 30mB

Figure S4. *Degradation kinetics without enzyme performed in medium (M) and water (W) on pre-photo-crosslinked (PC) and double crosslinked (DC) gels. Each condition was tested at days 1, 7, and 14. On the left results with Sil-Ma 15mB (a), on the right with 30mB Sil-Ma (b). no significance difference was detected among the different conditions in each time point.*
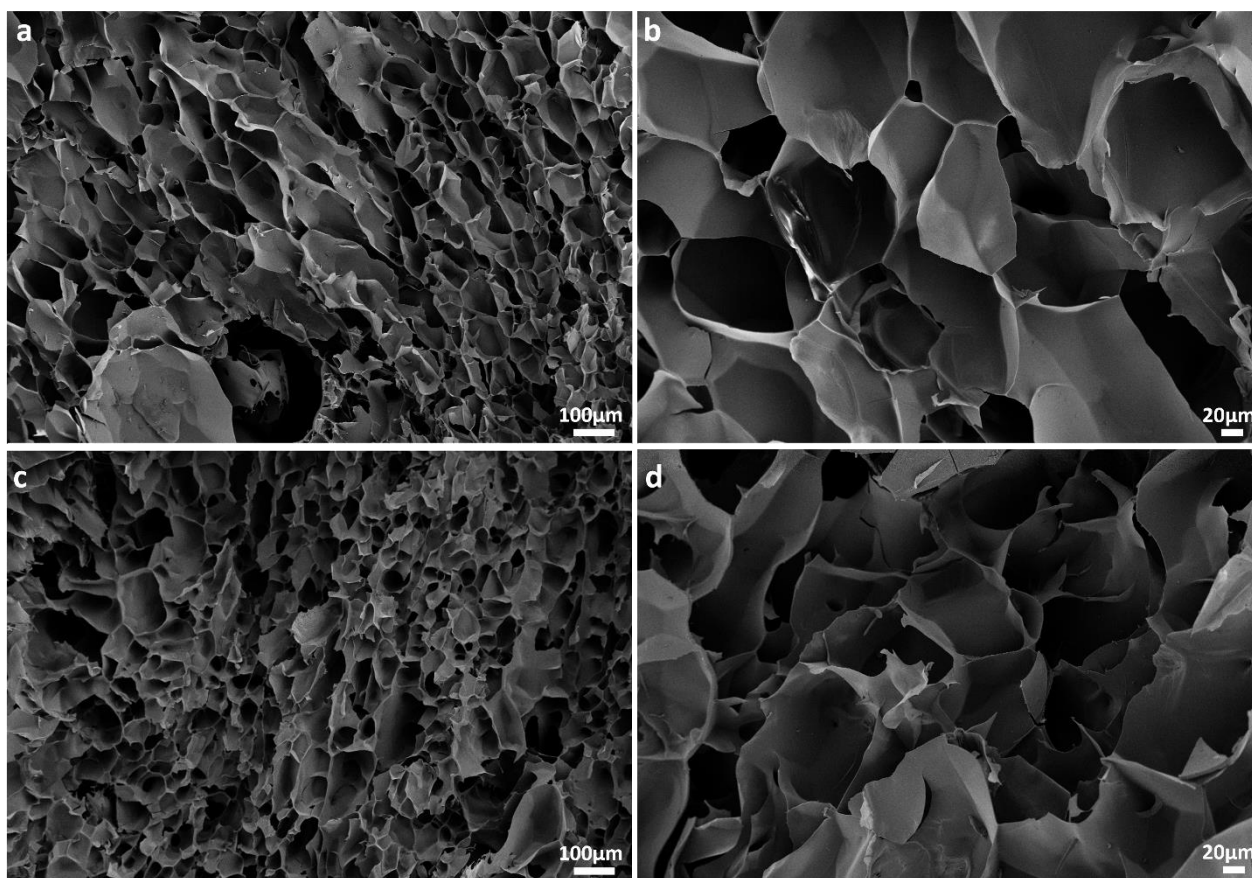
Figure S5. *SEM images of 15mB DC printed gels at 100 μm (a) and 20 scale bars (b). 30mB printed gels at 100 μm (c) and 20 scale bars (d). all the samples were printed with a 25G.*