Aqueous two-phase systems (ATPS) based on natural and synthetic poly(aminoacid)s

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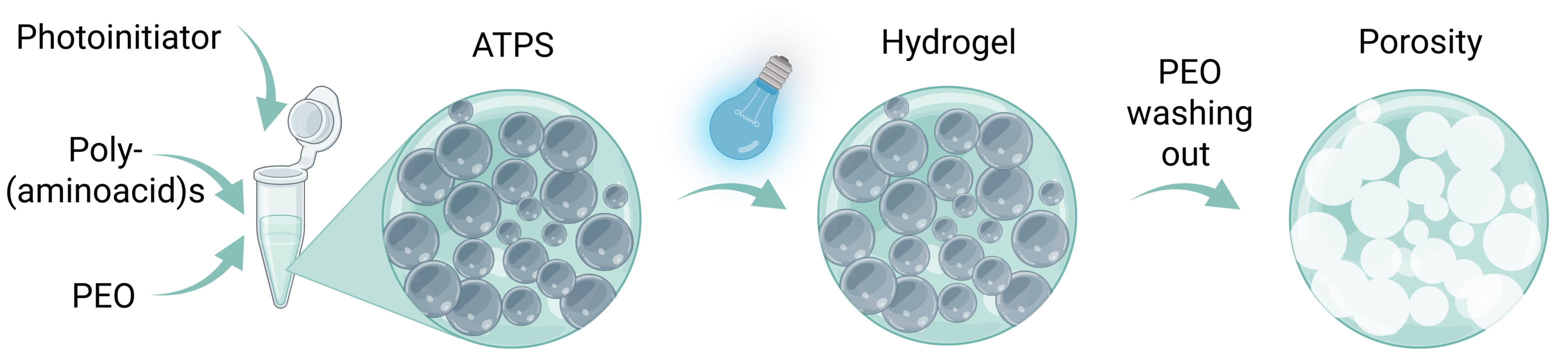
INTRODUCTION

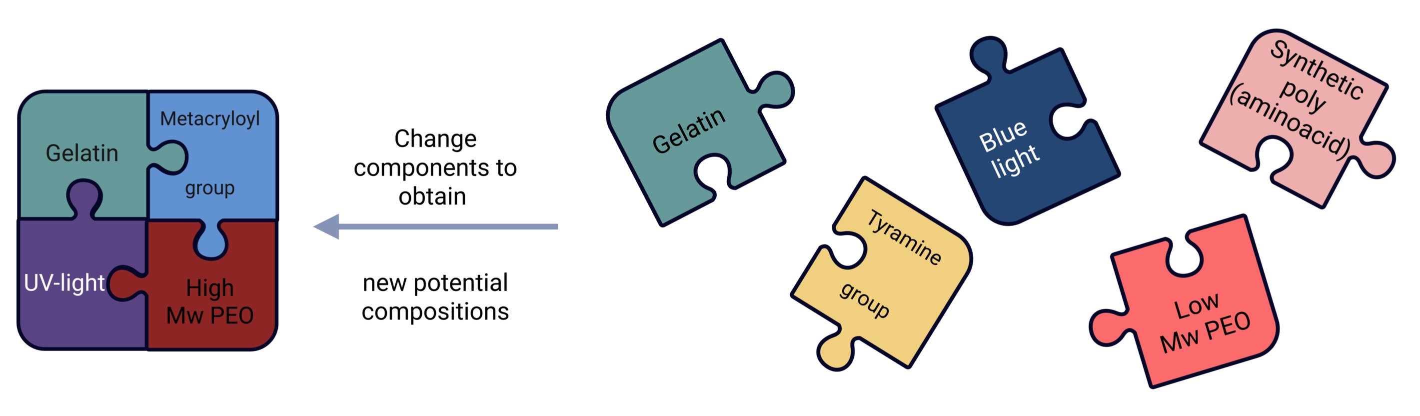
Aqueous two-phase systems (ATPS) are water solutions of limited miscible hydrophilic materials that can form an emulsion upon mixture and used for several bio applications.[1] Lately, these systems have also emerged as promising materials for bioinks for 3D bioprinting.[2] These systems can provide printed materials with porosity required for cell cultivation and tissue engineering.

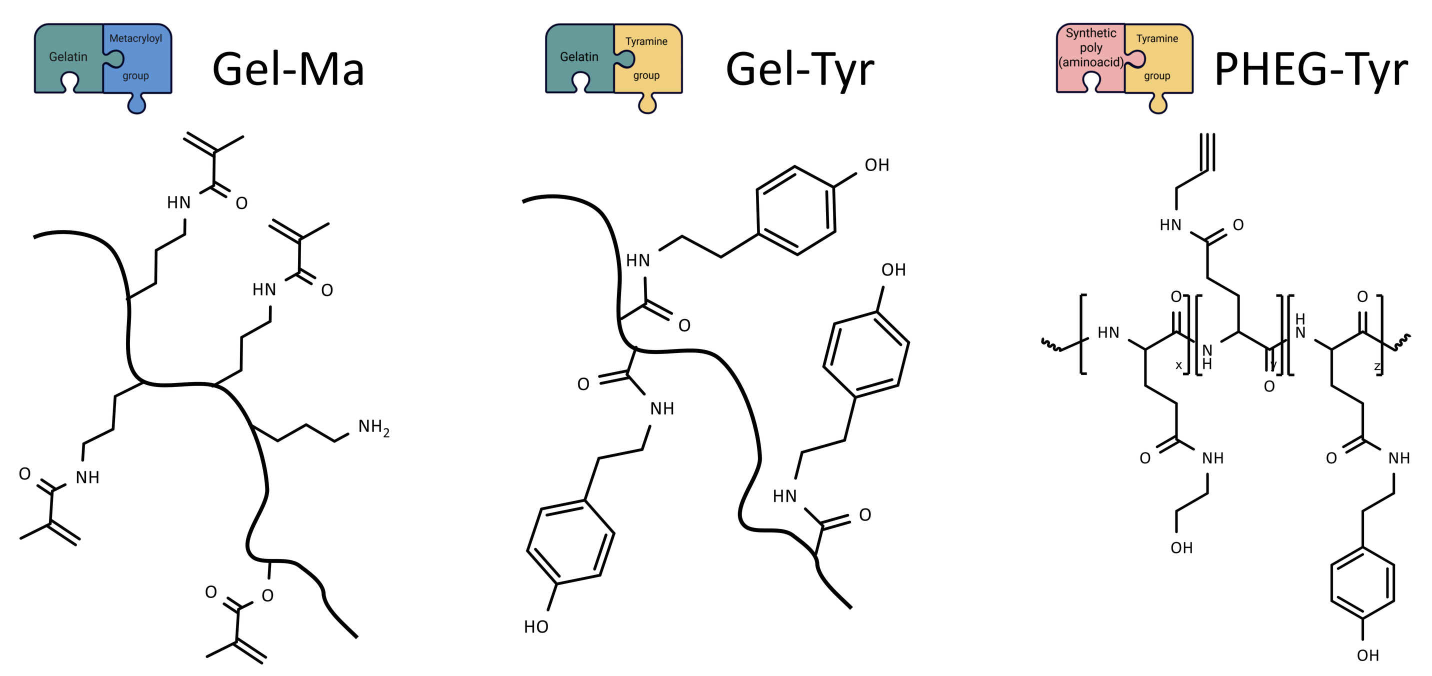


However, the systems known up to date have several drawbacks such as use of UV-light, high molecular weight polyethylene oxide (PEO) as leaving component, and methacryloyl functional group for crosslinking. Moreover, gelatin as natural polymer could induce unwanted immune response.

That is why **in this work** we explore formulations of printable bioinks that can form porous hydrogels based on ATPS of poly(aminoacid)s including in house synthesized poly[N^5-(2-hydroxyethyl)-L-glutamine]-based polymer (PHEG-Tyr) and modified gelatin.

What can we change in the system to make it more “cell friendly”?





Gel-Ma	Gel-Tyr	PHEG-Tyr
Substitution Degree 0.38 mol/g	Substitution Degree 0.25 mol/g	Substitution Degree 0.95 mol/g

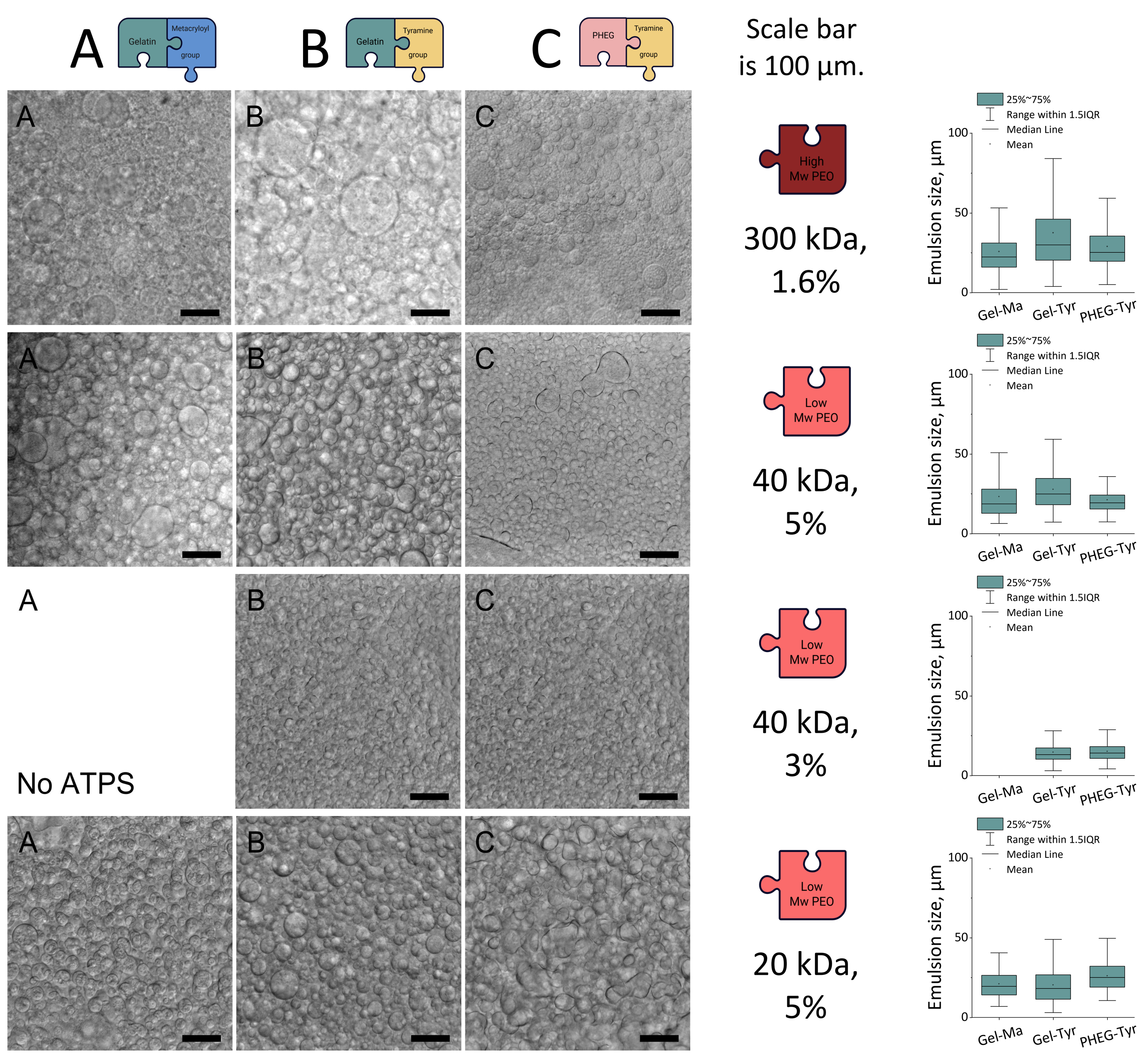
ATPS formation

ATPS appearance while mixing poly(aminoacid)s and PEO 1:1 by volume.

PEO, g/ml	Gel-Ma (0.1 g/ml)	Gel-Tyr (0.1 g/ml)	PHEG-Tyr (0.15 g/ml)	Gel-Ma (0.1 g/ml)	Gel-Tyr (0.1 g/ml)	PHEG-Tyr (0.15 g/ml)	Gel-Ma (0.1 g/ml)	Gel-Tyr (0.1 g/ml)	PHEG-Tyr (0.15 g/ml)
	300 kDa			40 kDa			20 kDa		
0.016	ATPS	ATPS	ATPS	no ATPS	no ATPS	no ATPS	no ATPS	-	-
0.03	ATPS	-	-	no ATPS	ATPS	ATPS	no ATPS	-	-
0.05	no ATPS	-	-	ATPS	ATPS	ATPS	ATPS	ATPS	ATPS

Optical micrographs of ATPS with different compositions and sizes of corresponding emulsions (box chart).

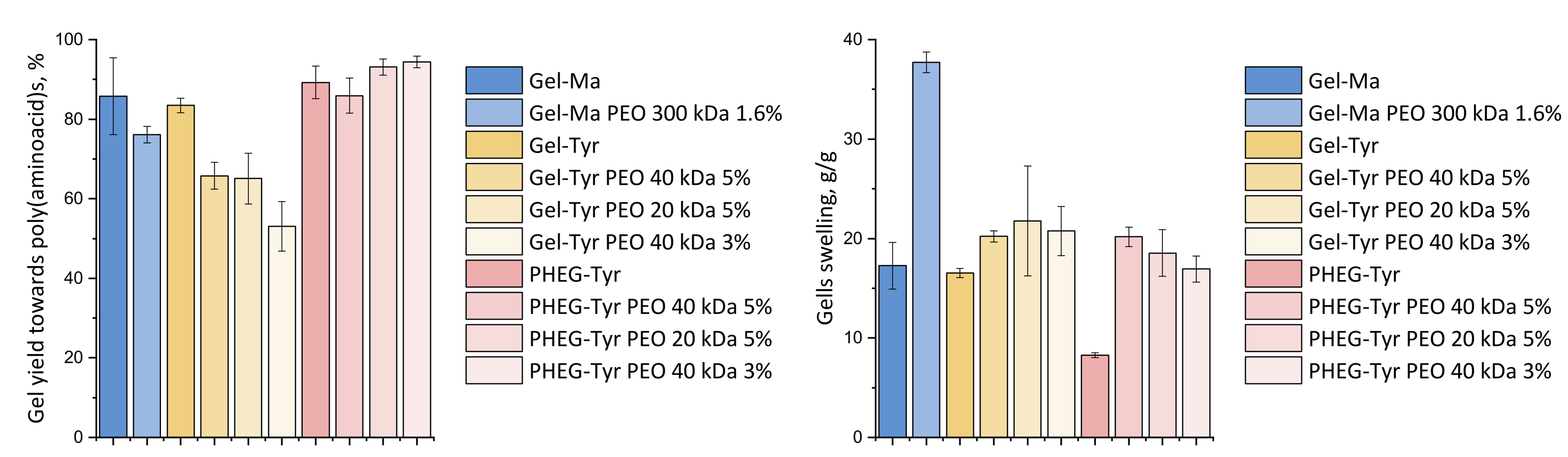
Scale bar is 100 µm.

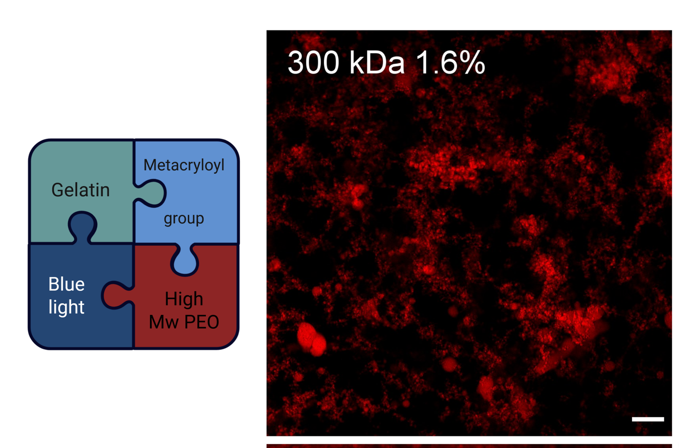


Gelation

Yield and Swelling of gels obtained from ATPS mixtures.

Only compositions of ATPS which were suitable for the gel formation were analyzed further. Gels were made with use of blue light (λ=450 nm) and photoinitiation system of Ru(bpy)$_3$ (0.2 mM) and ammonium persulfate (20 mM), solutions of polymers were mixed 1:1 by volume. Yields and swelling were calculated on the 7th day of incubation in water at 37 °C.

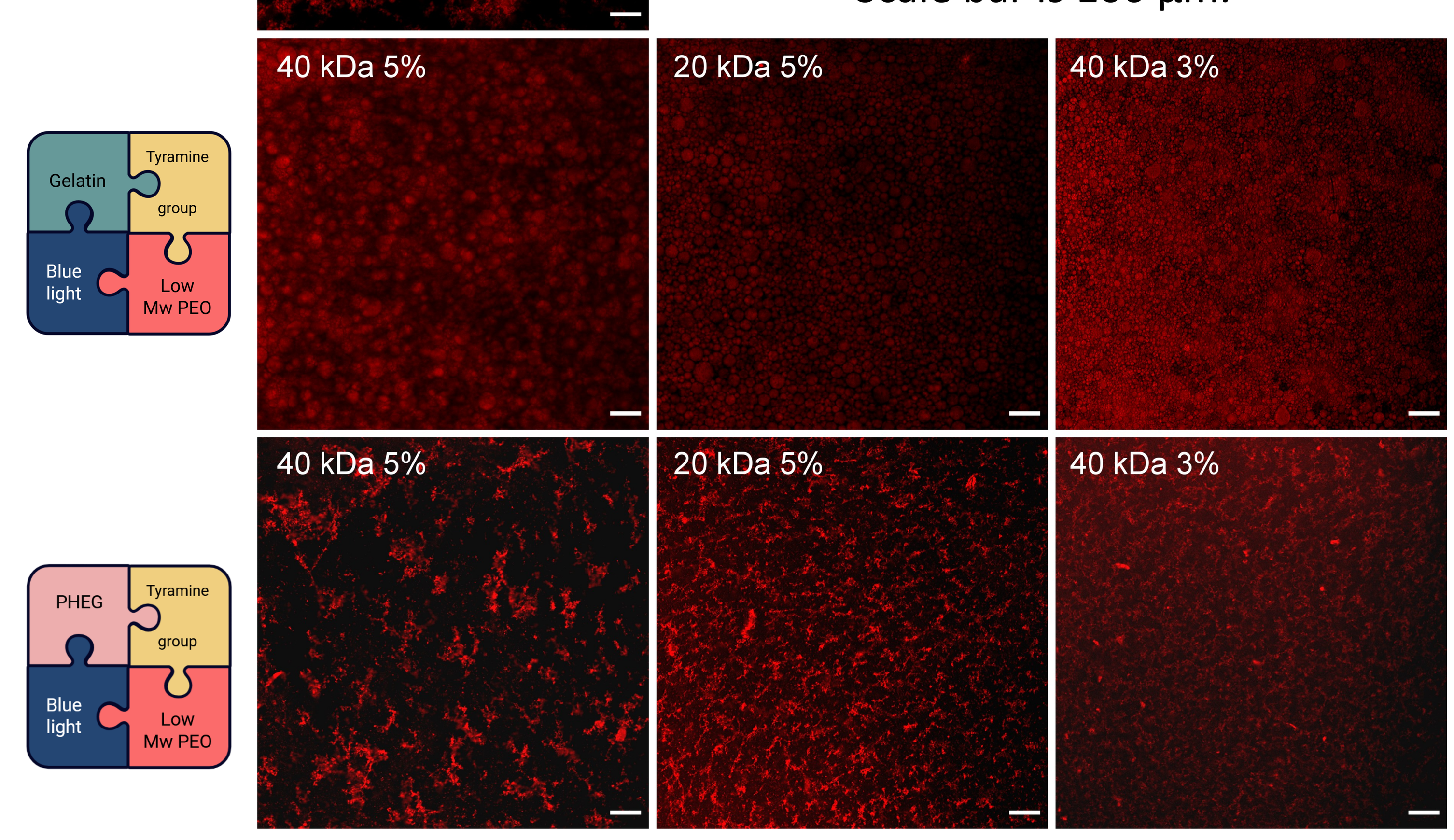




Confocal micrographs of obtained hydrogels.

Gels were dyed with Rhodamine B and measured on the 7th day of incubation in water at 37 °C.

Scale bar is 100 µm.



Printability of the bioinks

Hydrogels printing was performed with use of BIO X 3D bioprinter (CELLINK) with Electromagnetic Droplet (EMD) printhead, which allows to print with low viscous bioinks. Printed constructs were left to incubate in water at 37 °C to check their structural integrity.

Parameters for the printing:

Nozzle **0.3 mm**

Temperature **30 °C**

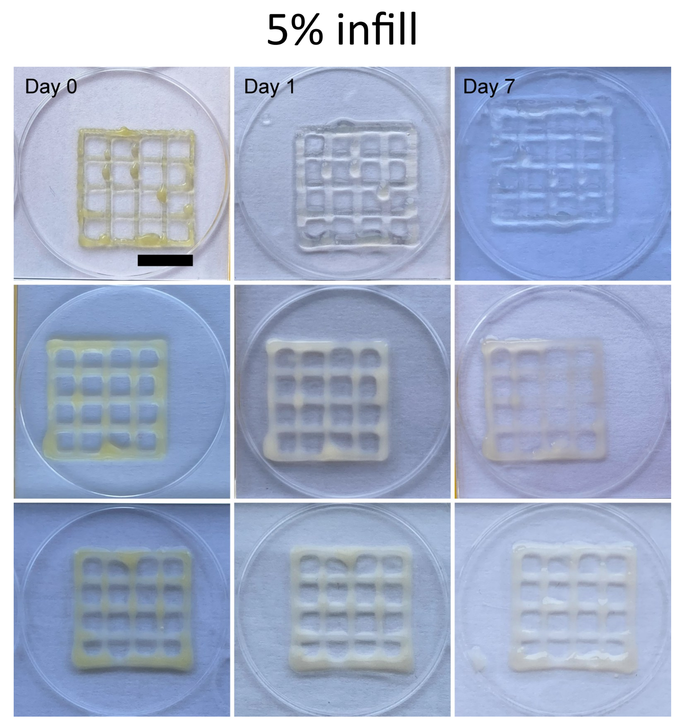
Printing speed **17 mm/s**

Pressure **20 kPa**

Valve open time **1ms** / Time of the cycle **40 ms**

All compositions were printed as 2x2 cm constructs with 5 or 7% infill. Scale bar is 1cm.

5% infill

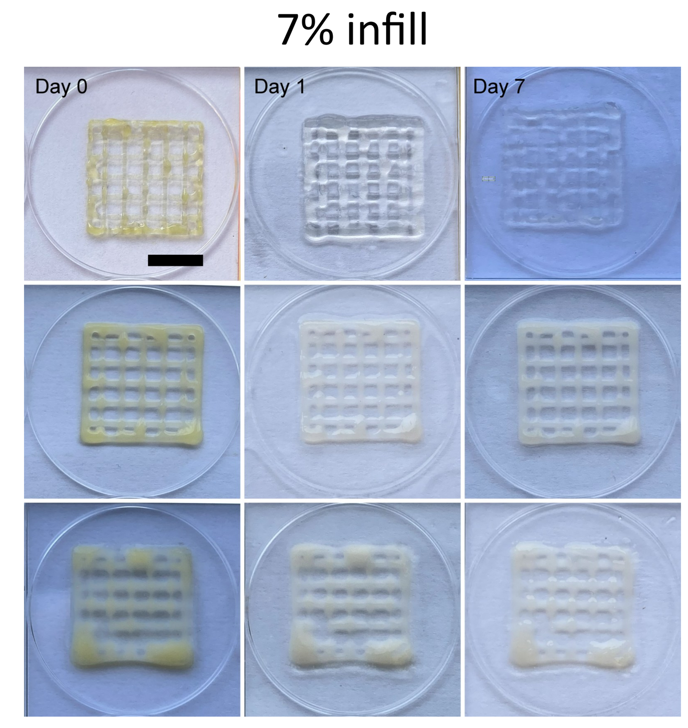


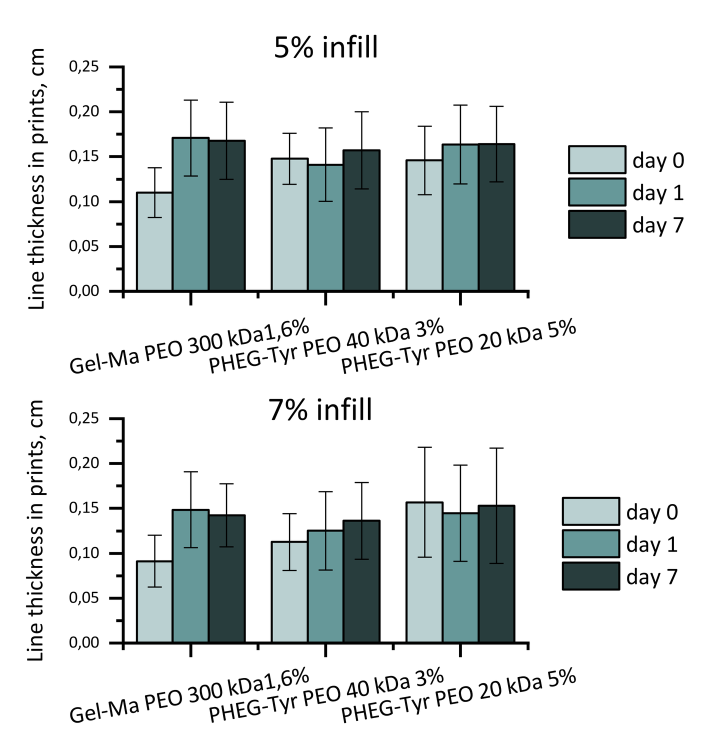
Gel-Ma with PEO 300 kDa 1.6%

PHEG-Tyr with PEO 40 kDa 3%

PHEG-Tyr with PEO 20 kDa 5%

7% infill





References

[1] Wang C, et al. 2023, Aqueous two-phase emulsions toward biologically relevant applications, Trends Chem. 5 61–75

[2] Ying G L, et al, 2018, Aqueous Two-Phase Emulsion Bioink-Enabled 3D Bioprinting of Porous Hydrogels, Adv. Mater. 30 1–9

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