Supplementary information

Visible Light Mediated PVA-Tyramine Hydrogels for Covalent Incorporation and Controlled Release of Functional Growth Factors

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Materials and methods:

S1: Hydrogel fabrication, swelling and mass loss study

Dried PVA-Tyr was dissolved in PBS at 80°C. Upon complete dissolution, the polymer solution was cooled to room temperature (RT) and mixed with Ru and SPS stock solutions to a final concentration of 5wt% PVA-Tyr with Ru/SPS concentration in the range of 0.5/5 to 2/20 mM. For composite hydrogels, BSA or growth factor solutions were mixed into the PVA-Tyr solution prior to the addition of Ru and SPS. BSA hydrogels were obtained by mixing a 10wt% BSA solution with Ru and SPS stock solutions to a final concentration of 2/20 mM Ru/SPS. All samples were photo-crosslinked using visible light (OmniCure® S1500, Excelitas Technologies with a Rosco IR/UV filter 400 – 450nm, 3min, 30 mW/cm^2) in cylindrical moulds (h = 1 mm, Ø = 6 mm) in an open environment. After crosslinking, each hydrogel (35 µL) was weighed ($m_{initial,t0}$), out of which three samples per hydrogel composition were directly lyophilised to record their initial dry weights ($m_{dry,t0}$) and determine the actual macromer weight fraction, which is reported as the ratio of the initial dry weight to the initial weight. To determine the initial dry weight of the remaining samples, the factor of the actual macromer fraction and individual initial weight was used (Eq. 1).

$$Actual\ macromer\ fraction = \frac{m_{dry,t0}}{m_{initial}} \quad (1)$$

The remaining samples were allowed to swell in PBS at 37 °C to determine the mass loss. Swollen hydrogel samples were collected to record wet weight ($m_{swollen}$), then lyophilised to obtain the freeze dried weight (m_{dry}) to calculate the mass loss and mass swelling ratio (q) according to equations 2, 3 and 4:

$$m_{dry,t0} = m_{initial} x actual macromer fraction \quad (2)$$

$$Massloss = \frac{m_{dry,t0} - m_{dry}}{m_{dry,t0}} x100 \quad (3)$$

$$q = \frac{m_{swollen}}{m_{dry}} \quad (4)$$

The sol fraction of the hydrogels is defined as percent macromers that are not cross-linked into the hydrogel network, and determined as the mass loss after equilibrium swelling (t = 1 day).

S2: Detection of bi-phenol crosslinks by LC/MS/MS

Dityramine and tyramine-tyrosine crosslinks were detected using liquid chromatography tandem mass spectrometry (LC/MS/MS). Samples used for this study are either 5wt% PVA-7Tyr or 5wt% PVA-Tyr supplemented with 5wt% BSA (PVA-Tyr-BSA). All gels are fabricated as mentioned above, and crosslinked using 0.5/5 mM Ru/SPS and 30 mW/cm^2 of visible light (400-450nm) for 3 minutes. Hydrogel samples were hydrolyzed in 4 M methane sulfonic acid, supplemented with 1 % (w/v) phenol, under nitrogen at 110°C for 18 hours. The hydrolysate was processed by solid phase extraction (SPE) using Strata® C18-E (55 µm, 70 Å) to remove the acid and eluted with 80 % (v/v) methanol. Eluents were dried and reconstituted in 0.1 % (v/v) formic acid for analysis. Reverse-phase HPLC-MS/MS was performed using a Kinetex® 2.6 µm C18 100Å column (150x2.1 mm), and an Agilent 1290 Binary Pump at a flow rate of 200 µl/ minute. The gradient started with 2 % aqueous acetonitrile with 0.1 % (v/v) formic acid for four minutes, increasing to 80 % acetonitrile over three minutes, maintaining this over three minutes then equilibrated with the starting eluent. The analytes were delivered into a Qtrap® 6500 mass spectrometer (Sciex) and detected in multiple reaction monitoring mode using positive ion mode. The ion spray was set to 5.5kV and the temperature was set to 600°C. The collision gas was nitrogen and the collision energy was 25%. The areas of peaks were calculated using Analyst Software v 1.6.2 (Sciex).

Results:

Table S1. Sol fraction values for PVA-4Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-4Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	34.9 ± 7.3			
1/10 mM Ru/SPS	34.1 ± 11.0	53.6 ± 5.9		
1.5/15 mM Ru/SPS	34.2 ± 4.8	44.8 ± 1.7	73.0 ± 2.9	
2/20 mM Ru/SPS	25.2 ± 9.9	45.3 ± 3.6	63.9 ± 1.4	73.5 ± 5.5

Table S2. Sol fraction values for PVA-7Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-7Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	23.7 ± 3.6			
1/10 mM Ru/SPS	14.0 ± 8.0	18.2 ± 2.7		
1.5/15 mM Ru/SPS	11.8 ± 2.7	18.6 ± 5.2	20.5 ± 2.3	
2/20 mM Ru/SPS	13.9 ± 5.9	14.2 ± 6.9	24.5 ± 5.2	26.4 ± 9.7

Table S3. Sol fraction values for PVA-10Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-10Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	22 ± 4.5			
1/10 mM Ru/SPS	9.2 ± 5.2	19.3 ± 6.2		
1.5/15 mM Ru/SPS	7.8 ± 3.0	14.1 ± 5.4	17.6 ± 2.8	
2/20 mM Ru/SPS	22.6 ± 6.7	17.4 ± 2.6	20.6 ± 6.7	32.6 ± 9.7

Table S4. Swelling ratio values for PVA-4Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-4Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	30.6 ± 9.0			
1/10 mM Ru/SPS	26.4 ± 3.9	31.0 ± 1.0		
1.5/15 mM Ru/SPS	21.6 ± 1.6	18.7 ± 1.5	29.0 ± 5.5	
2/20 mM Ru/SPS	19.5 ± 6.4	16.7 ± 1.3	21.2 ± 2.3	22.25 ± 5.3

Table S5. Swelling ratio values for PVA-7Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-7Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	19.2 ± 1.7			
1/10 mM Ru/SPS	12.9 ± 1.6	12.3 ± 1.1		
1.5/15 mM Ru/SPS	11.8 ± 0.5	10.9 ± 1.0	11.0 ± 0.8	
2/20 mM Ru/SPS	11.9 ± 0.4	10.1 ± 0.8	10.5 ± 0.8	11.0 ± 0.8

Table S6. Swelling ratio values for PVA-10Tyr hydrogels cross-linked at different macromer and Ru/SPS concentration.

PVA-10Tyr	5wt%	10wt%	15wt%	20wt%
0.5/5 mM Ru/SPS	19.7 ± 1.0			
1/10 mM Ru/SPS	15.0 ± 1.8	14.0 ± 1.2		
1.5/15 mM Ru/SPS	13.0 ± 2.0	9.7 ± 0.2	11.3 ± 0.8	
2/20 mM Ru/SPS	12.3 ± 1.6	10.9 ± 1.0	10.5 ± 1.2	10.2 ± 2.0

Table S7. R^2 of the BSA release linear regression for different PVA-Tyr formulations and cross-linking conditions.

	PVA-7Tyr 5 wt% 0.5/5 mM	**PVA-7Tyr 5 wt% 1/10 mM**	**PVA-7Tyr 5 wt% 2/20 mM**	**PVA-10Tyr 5 wt% 0.5/5 mM**	**PVA-7Tyr 10 wt% 1/10 mM**	**PVA-7Tyr 20 wt% 2/20 mM**
R^2	0.9861	0.9560	0.9458	0.9724	0.9541	0.9361

Table S8. Tyrosine residues present in the incorporated growth factors. Available residues were extracted from Protein Data Bank files using Swiss-PDB Viewer at a surface availability threshold of 20%.

	MW (Monomer)	**Number of tyrosine groups**	**Number of available tyrosine groups on protein surface**
Albumin	66 kDa	18[1]	3
VEGF	19 kDa	4[2]	3
bFGF	9.9 kDa	10[3]	1
BDNF	13.6 kDa	4[4]	3

Table S9. R^2 of the linear regression of the VEGF, bFGF and BDNF release profiles for different PVA-Tyr cross-linking conditions.

	VEGF 0.5/5 mM	**bFGF 0.5/5 mM**	**BDNF 0.5/5 mM**	**VEGF 1/10 mM**	**bFGF 1/10 mM**	**BDNF 1/10 mM**	**VEGF 2/20 mM**	**bFGF 2/20 mM**	**BDNF 2/20 mM**
R^2	0.696	0.7552	0.6492	0.9148	0.9116	0.8467	0.9084	0.9194	0.9172

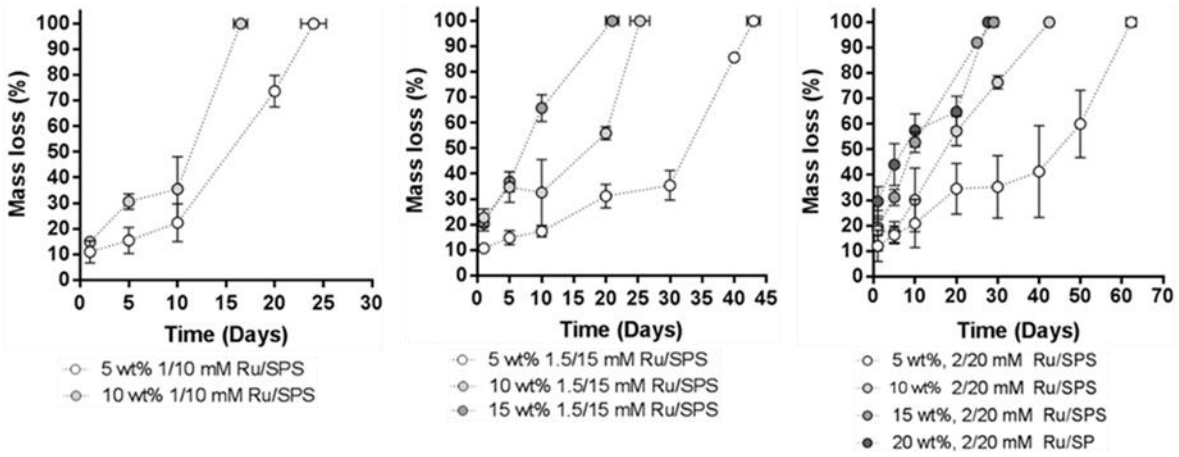

Figure S1. (A-C) Mass loss of PVA-7Tyr hydrogels crosslinked using increasing macromer concentrations (5-20wt%) and a range photoinitiator concentrations (1/10-2/20 mM Ru/SPS).

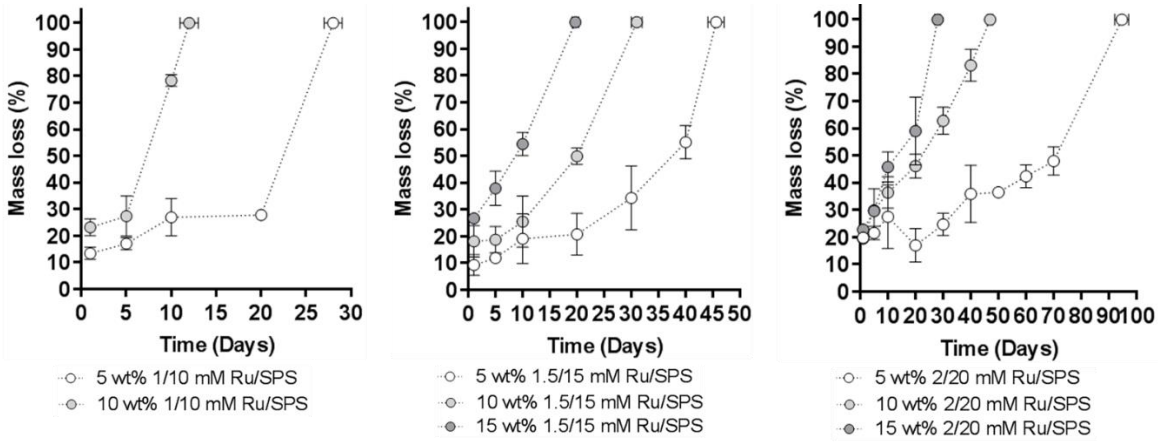

Figure S2. (A-C) Mass loss and (D-F) swelling ratio of PVA-10Tyr hydrogels crosslinked using increasing macromer concentrations (5-15wt%) and a range photoinitiator concentrations (1/10-2/20 mM Ru/SPS).

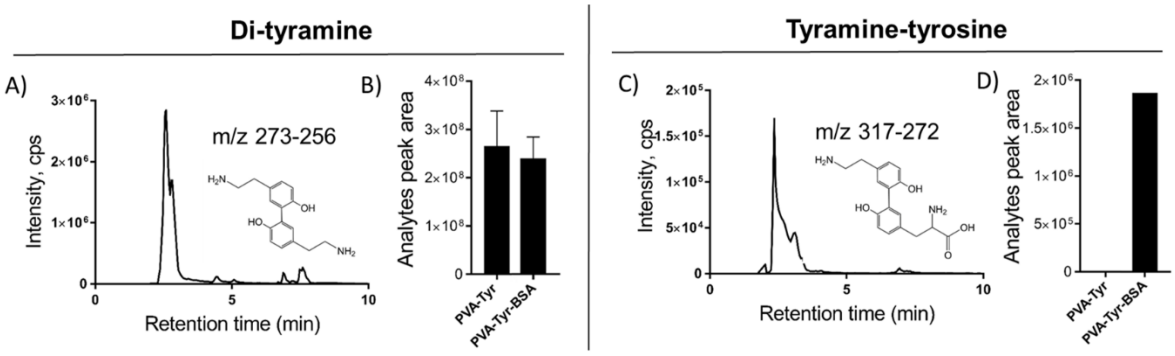

Figure S3. Chromatogram and respective peak area for identification of the bi-phenol bonds present in the PVA-Tyr and PVA-Tyr-BSA hydrogels: Di-tyramine (A, B) and tyramine-tyrosine (C, D).

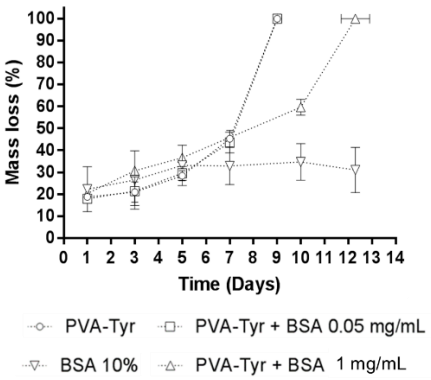

Figure S4. Mass loss of PVA-Tyr hydrogels incorporated with BSA at different concentrations.

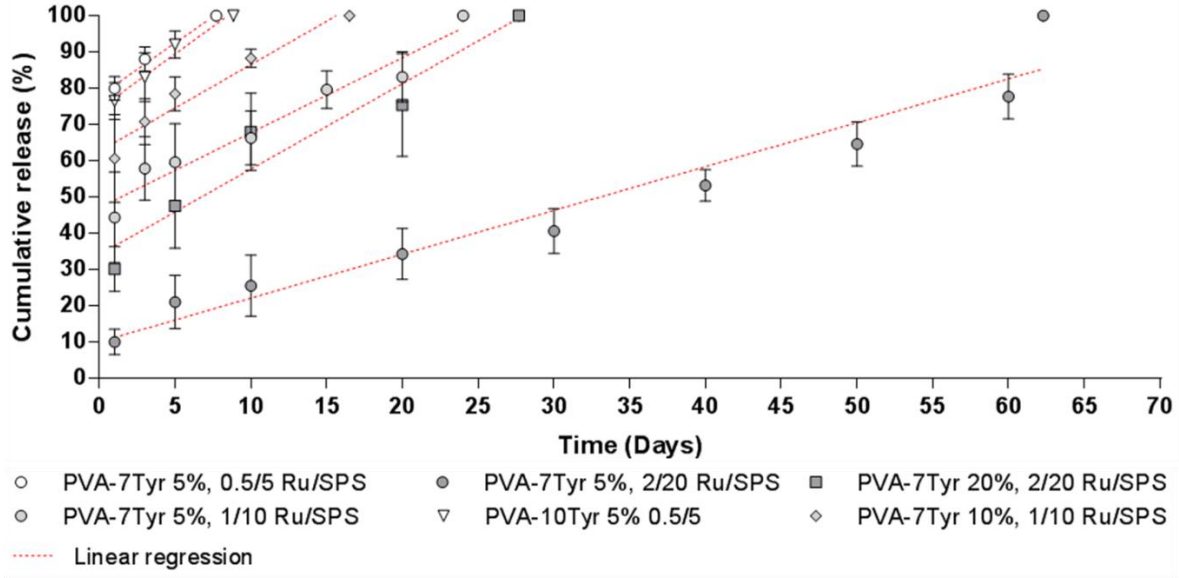

Figure S5. Linear regression of BSA release profiles from PVA-Tyr hydrogels of different formulations and crosslinking conditions.

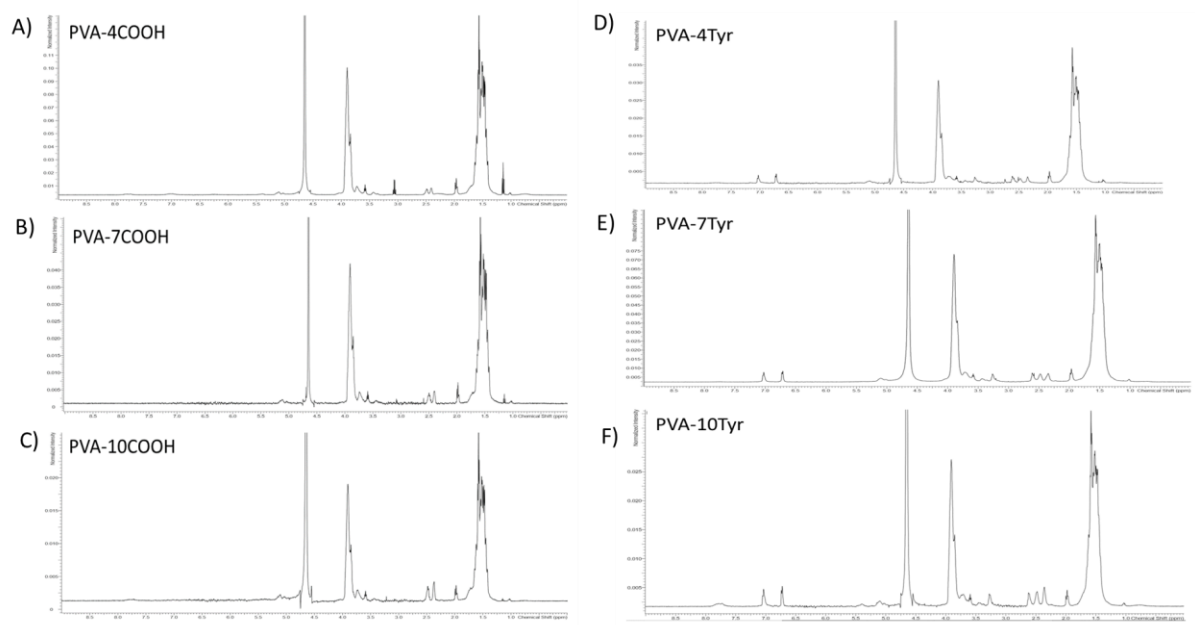

Figure S6. ^{1}H-NMR spectra for PVA-COOH (A, B, C) of different degree of carboxylation, and PVA-Tyr of different degree of tyramination (D, E, F).

References

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