RSC Advances

Supporting Information (SI)

Antimicrobial Properties and Biocompatibility of semi-synthetic Carbohydrate-based Ionic Hydrogels

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1. Synthetic procedures and analytical data

Synthetic procedure for the synthesis of Methyl-6-iodo-α-D-glucopyranoside (1). Methyl-α-D-glucopyranoside (10 mmol), triphenylphosphine (15.5 mmol), iodine (14.5 mmol), and imidazole (20 mmol) were refluxed in THF (60 mL) for 4 h. The resulting solid was filtered off, the solvent was removed and the product (91 % yield) was obtained after column chromatography ($CHCl_3$/MeOH 12:1). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.87−2.95 (m, 1H); 3.16−3.27 (m, 3H); 3.31 (s, 3H, CH_3); 3.34−3.42 (m, 1H); 3.50−3.57 (m, 1H); 4.54 (d, 1H, ^{3}J = 3.65 Hz, H-1); 4.78 (d, 1H, ^{3}J = 6.43 Hz, OH); 4.86 (d, 1H, ^{3}J = 4.99 Hz, OH); 5.17 (d, 1H, ^{3}J = 5.83 Hz, OH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 9.5 (C-6); 54.6 (CH_3); 70.9, 71.9, 72.7, 74.1 (C-2, C-3, C-4, C-5); 99.8 (C-1).

Synthetic procedure for the quaternization with imidazoles to 1-(Methyl-α-D-glucopyranosid-6-yl)-3-vinylimidazolium iodide (GVIM-I) (2). Methyl-6-iodo-α-D-glucopyranoside (3 mmol) and the *N*-vinylimidazole (5 mmol) were dissolved in DMF (5 mL) and stirred at 95 °C for 24 hours. After cooling down, ethyl acetate (40 mL) was added and the flask was stored in a fridge overnight. The solvent was decanted and the precipitated solid was repeatedly washed with ethyl acetate and dried under a high vacuum to achieve the product, yielding in a light-brown solid (73 % yield). ^{1}H-NMR (250 MHz, D_2O): δ = 3.25 (s, 3 H, OCH_3); 3.24–3.28 (m, 1H, H-4); 3.58 (dd, 1H, ^{3}J = 9.77 Hz, ^{3}J = 3.77 Hz, H-2); 3.66–3.75 (m, 1H, H-3); 3.95 (ddd, 1H, ^{3}J = 9.96 Hz, ^{3}J = 7.47 Hz, ^{3}J = 2.46 Hz, H-5); 4.50 (dd, 1H, ^{2}J = 14.55 Hz, ^{3}J = 7.38 Hz, H-6a); 4.70 (dd, 1H, ^{2}J = 14.55 Hz, ^{3}J = 2.55, H-6b); 4.85 (d, 1H, ^{3}J = 3.77 Hz, H-1); 5.49 (dd, 1H, ^{3}J = 8.68 Hz, ^{2}J = 2.84 Hz, Vinyl-CH_2); 5.86 (dd, 1H, ^{3}J = 15.58 Hz, ^{2}J = , Vinyl-CH_2); 7.2 (dd, 1H, ^{3}J = 15.58 Hz, ^{3}J = 8.70 Hz, Vinyl-CH); 7.70 (d, 1H, ^{3}J = 2.09 Hz, H_{Ar}); 7.86 (d, 1H, ^{3}J = 2.11 Hz, H_{Ar}); 9.16 (s, 1H, H_{Ar}). ^{13}C-NMR (125 MHz, D_2O): δ = 50.2 (C-6); 55.1 (OCH_3); 69.2 (C-5); 70.5 (C-4); 71.0 (C-2); 72.8 (C-3); 99.3 (C-1); 109.8 (Vinyl-CH_2); 119.4, 123.8 (CH_{Ar}); 123.8 (Vinyl-CH); 135.0 (CH_{Ar}).

1.1 NMR Spectra

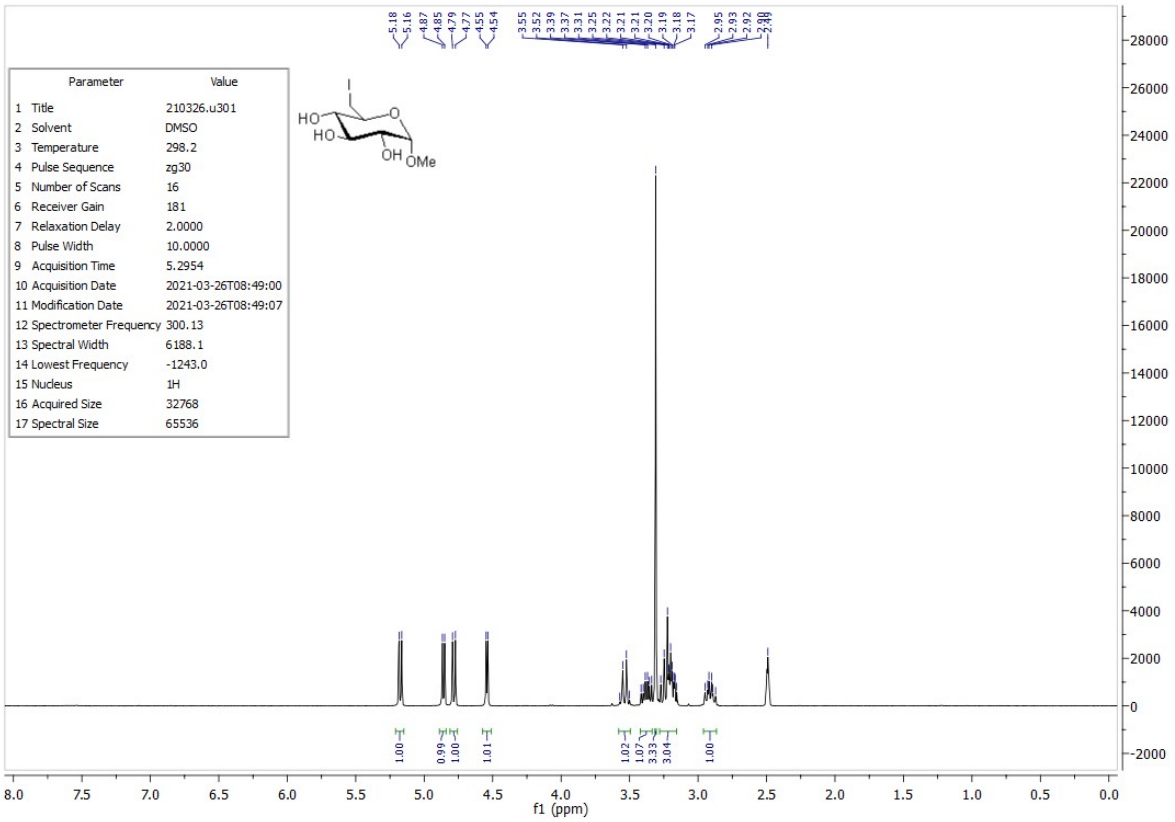

Figure S1. ^{1}H-NMR (DMSO) spectrum of compound Methyl-6-iodo-α-D-glucopyranoside **1**.

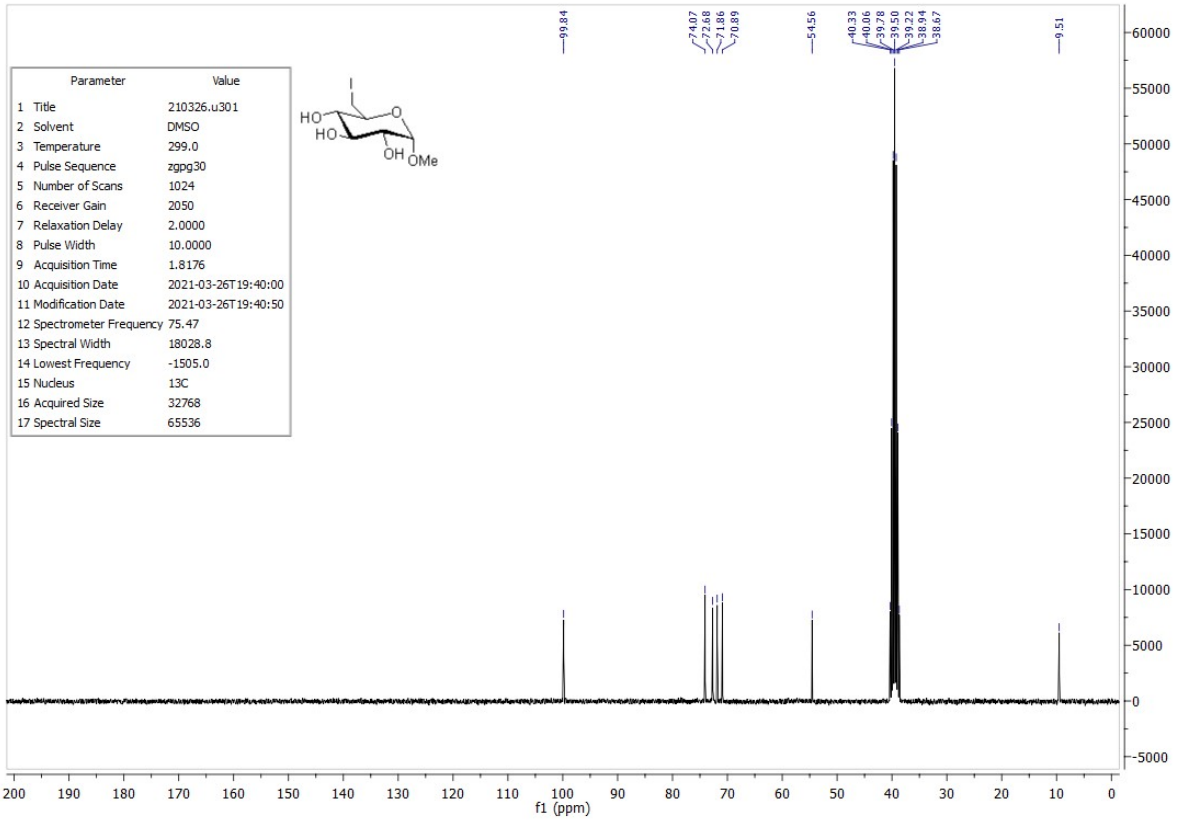

Figure S2. ^{13}C-NMR (DMSO) spectrum of compound Methyl-6-iodo-α-D-glucopyranoside **1.**

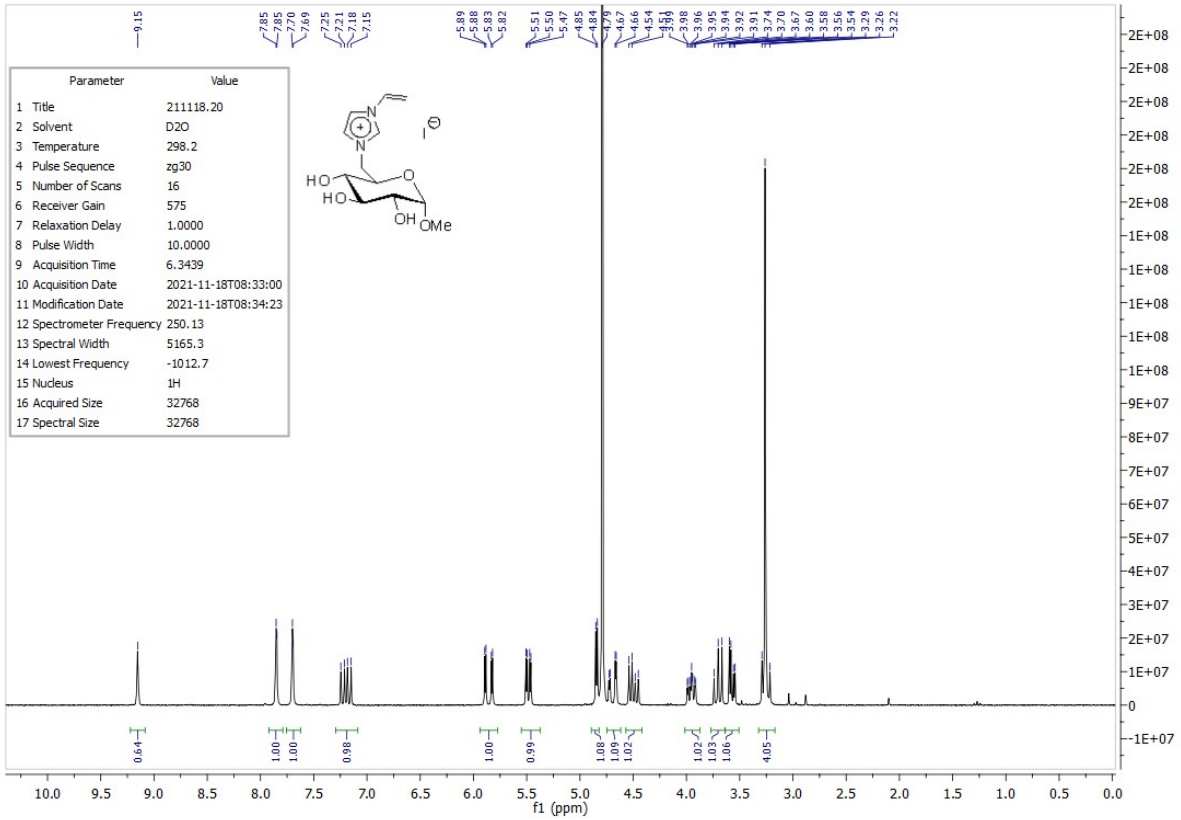

Figure S3. ^{1}H-NMR (D_2O) spectrum of compound 1-(Methyl-α-D-glucopyranosid-6-yl)-3-vinylimidazolium iodide (GVIM-I) **2.**

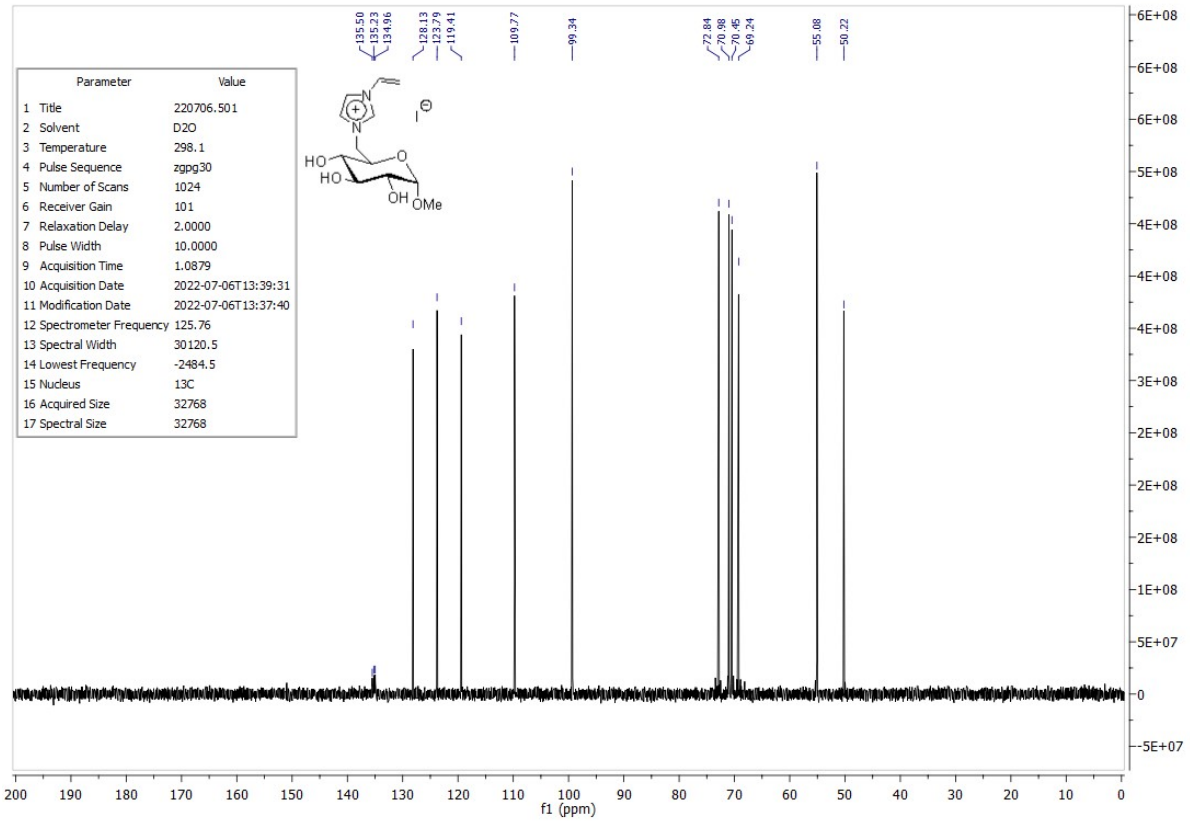

Figure S4. ^{13}C-NMR (D_2O) spectrum of compound 1-(Methyl-α-D-glucopyranosid-6-yl)-3-vinylimidazolium iodide (GVIM-I) **2**.

1.2 ATR-IR spectra

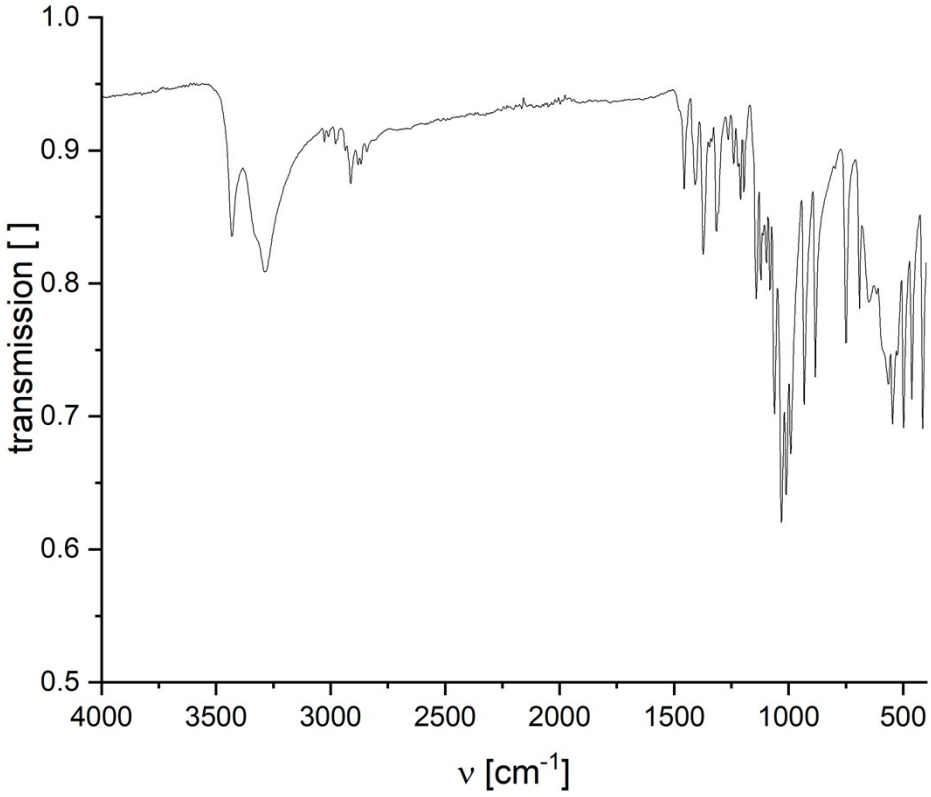

Figure S5. ATR-FTIR spectrum of compound **1**.

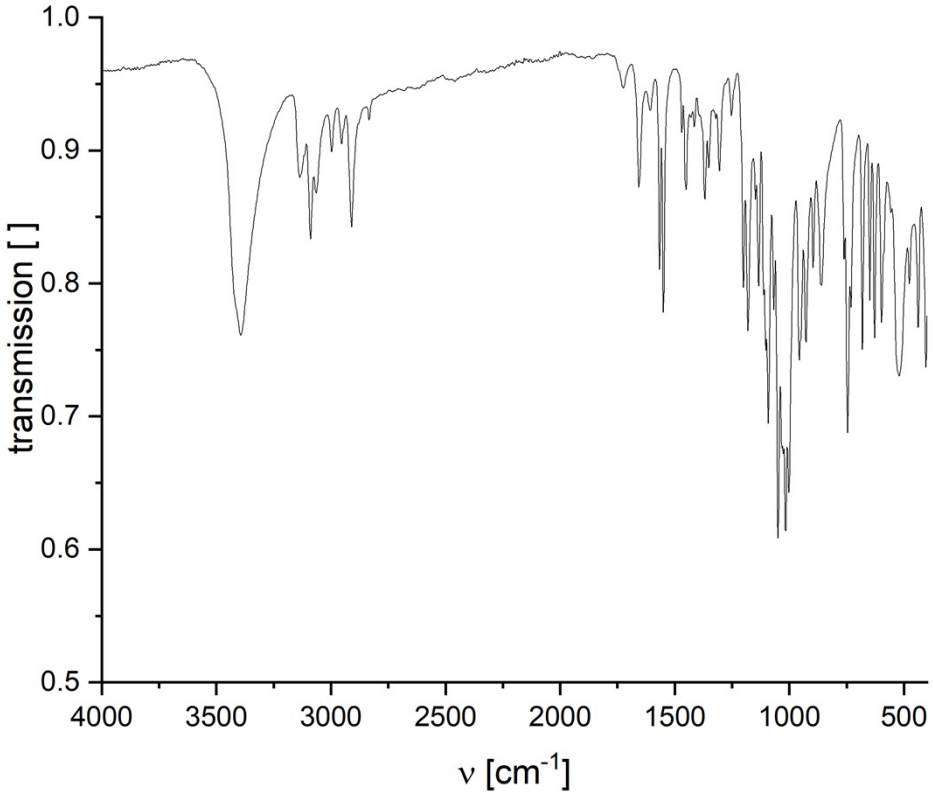

Figure S6. ATR-FTIR spectrum of compound **2**.

1.2 Weighing scales for hydrogel production

Table S1. Weighing-in scale for the production of hydrogels with LAP as initiator, according to i).

	GVIM-I		PBS	Crosslinker		LAP	
	c [mol/l]	m [mg]	V [µl]	c [mol%]	V [µl]	c [w%]	m [mg]
EGDA	1.25	100	200	10	3.9	3.1	9.4
PEGDA 250	1.25	100	200	10	5.7	2.0	6.1
PEGDA 700	1.25	100	200	10	15.7	0.5	1.6
MBAA	1.25	100	200	10	3.9	1.0	3.0
PEGDA 575	1.25	100	200	10	12.9	0.5	1.6

Table S2. Weighing-in scale for the production of hydrogels with APS/TEMED as initiator, according to ii).

	GVIM-I		PBS	Crosslinker		APS/TEMED		
	c [mol/l]	m [mg]	V [µl]	c [mol%]	V [µl]	c_{total} [w%]	V_{APS} [µl]	V_{TEMED} [µl]
MBAA	1.25	100	172.1	13.0	5.0	10.7	27.9[a]	27.9
PEGDA 575	1.25	100	162.5	10.4	13.4	6.8	37.5[b]	18.0

Note to APS solution: a) 400 mg in 1 ml PBS and b) 200 mg in 1 ml PBS.

2. Antimicrobial Activity – Disk Diffusion Method

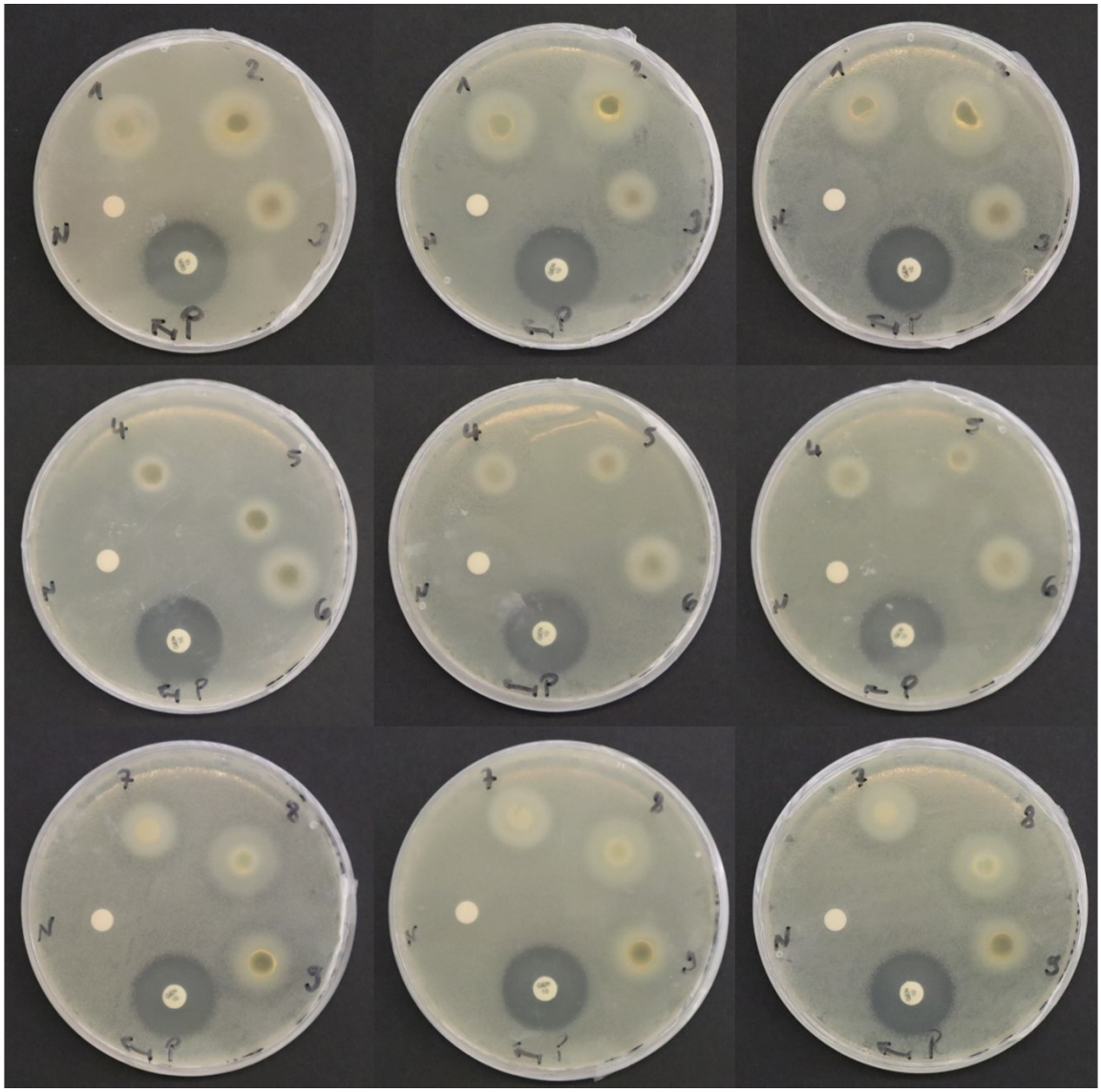

Figure S7. Overview of the disk diffusion tests against *B. subtilis* (P positive control Gentamicin; N negative control LB medium; 1 EGDA; 2 P250; 3 P575 10%; 4 P575 15%; 5 P575 20%; 6 P700; 7 MBAA LAP; 8 MBAA A/T; 9 P575 A/T).

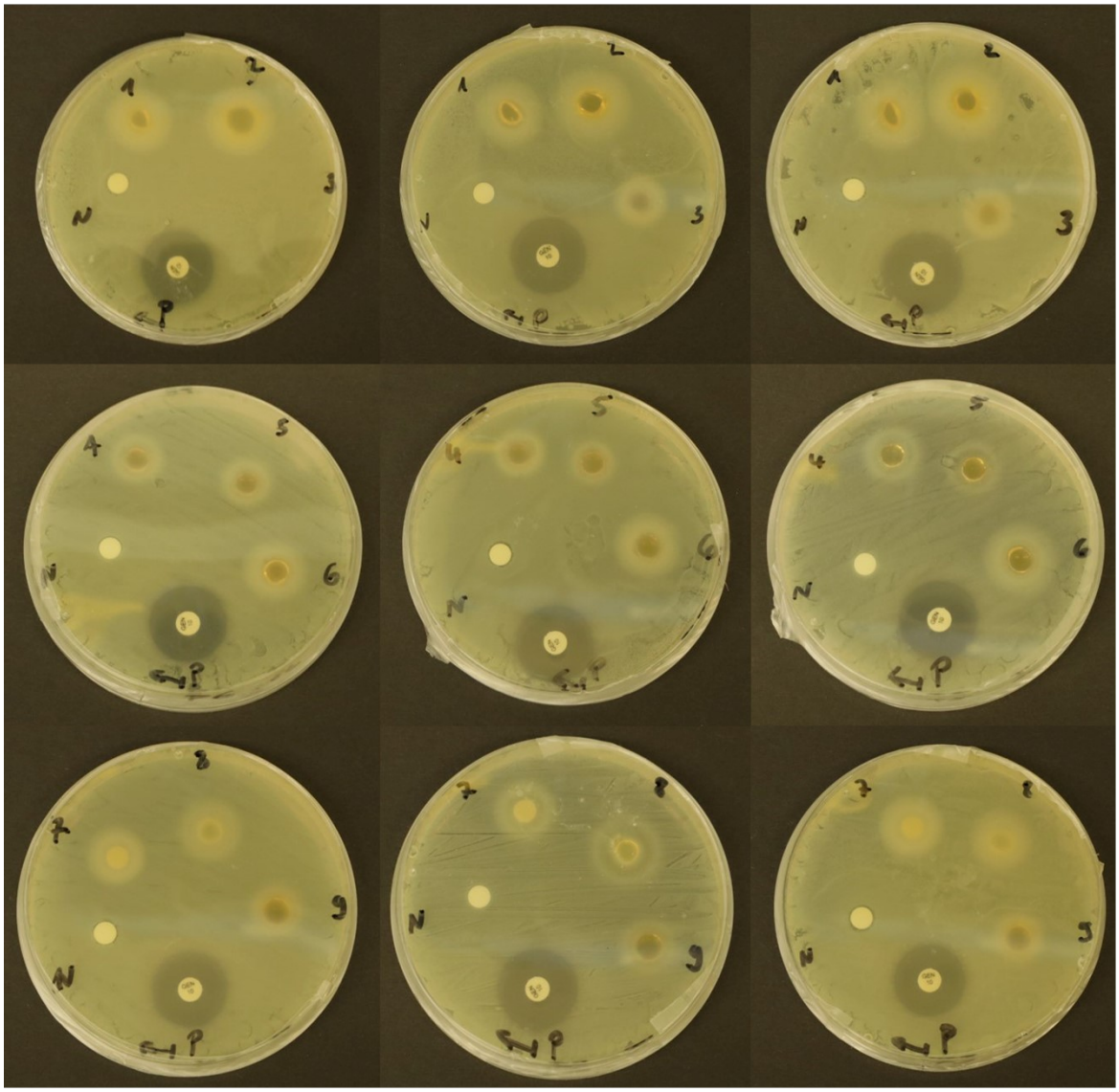

Figure S8. Overview of the disk diffusion tests against *E. coli* (P positive control Gentamicin; N negative control LB medium; 1 EGDA; 2 P250; 3 P575 10%; 4 P575 15%; 5 P575 20%; 6 P700; 7 MBAA LAP; 8 MBAA A/T; 9 P575 A/T).

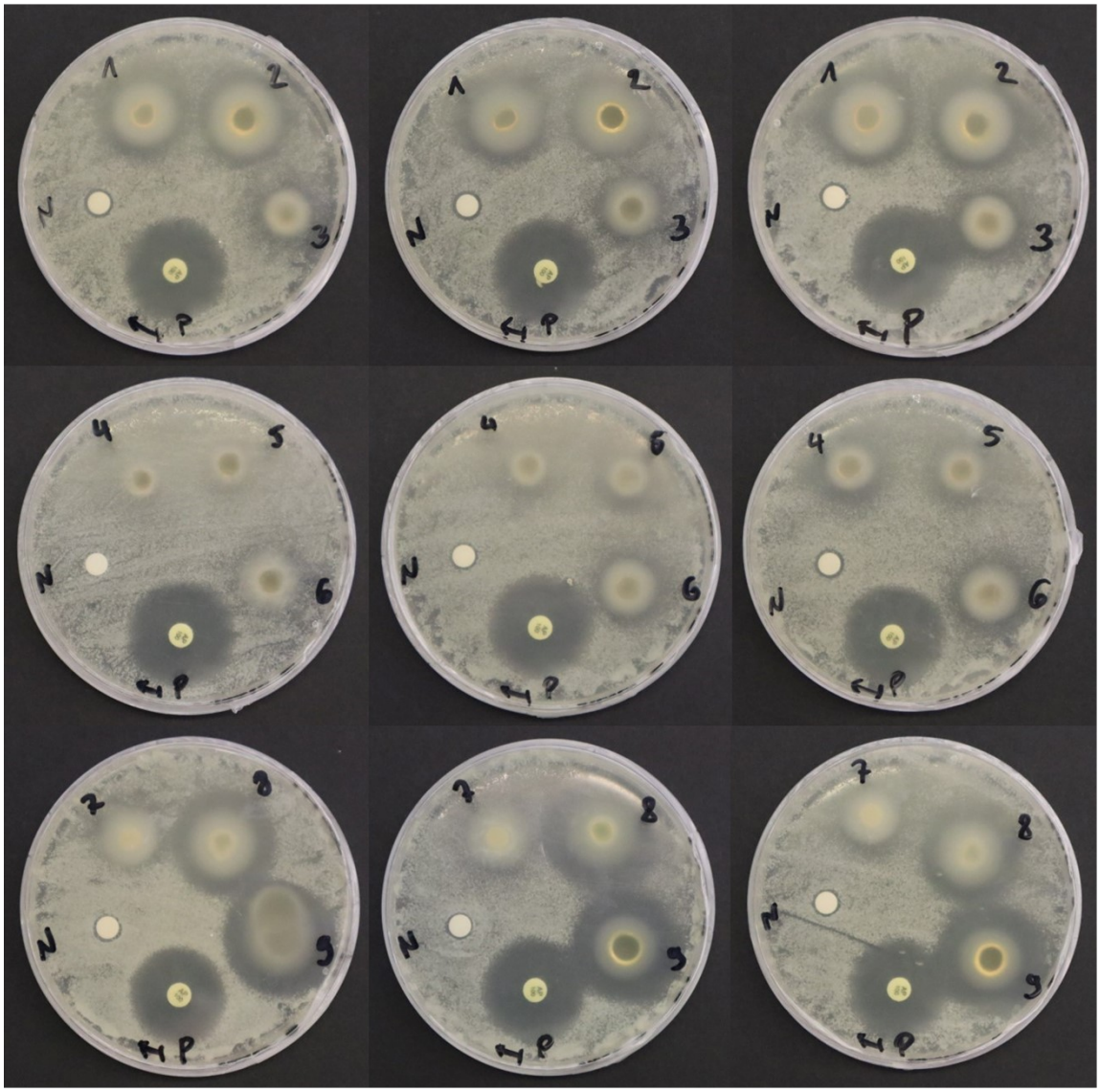

Figure S9. Overview of the disk diffusion tests against *C. aurisi* (P positive control Amphotericin B; N negative control LB medium (+ 2% glucose); 1 EGDA; 2 P250; 3 P575 10%; 4 P575 15%; 5 P575 20%; 6 P700; 7 MBAA LAP; 8 MBAA A/T; 9 P575 A/T).

3. Cell viability of L929 cells - CellTiter blue (CTB) viability assay

3.1. Eluate tests

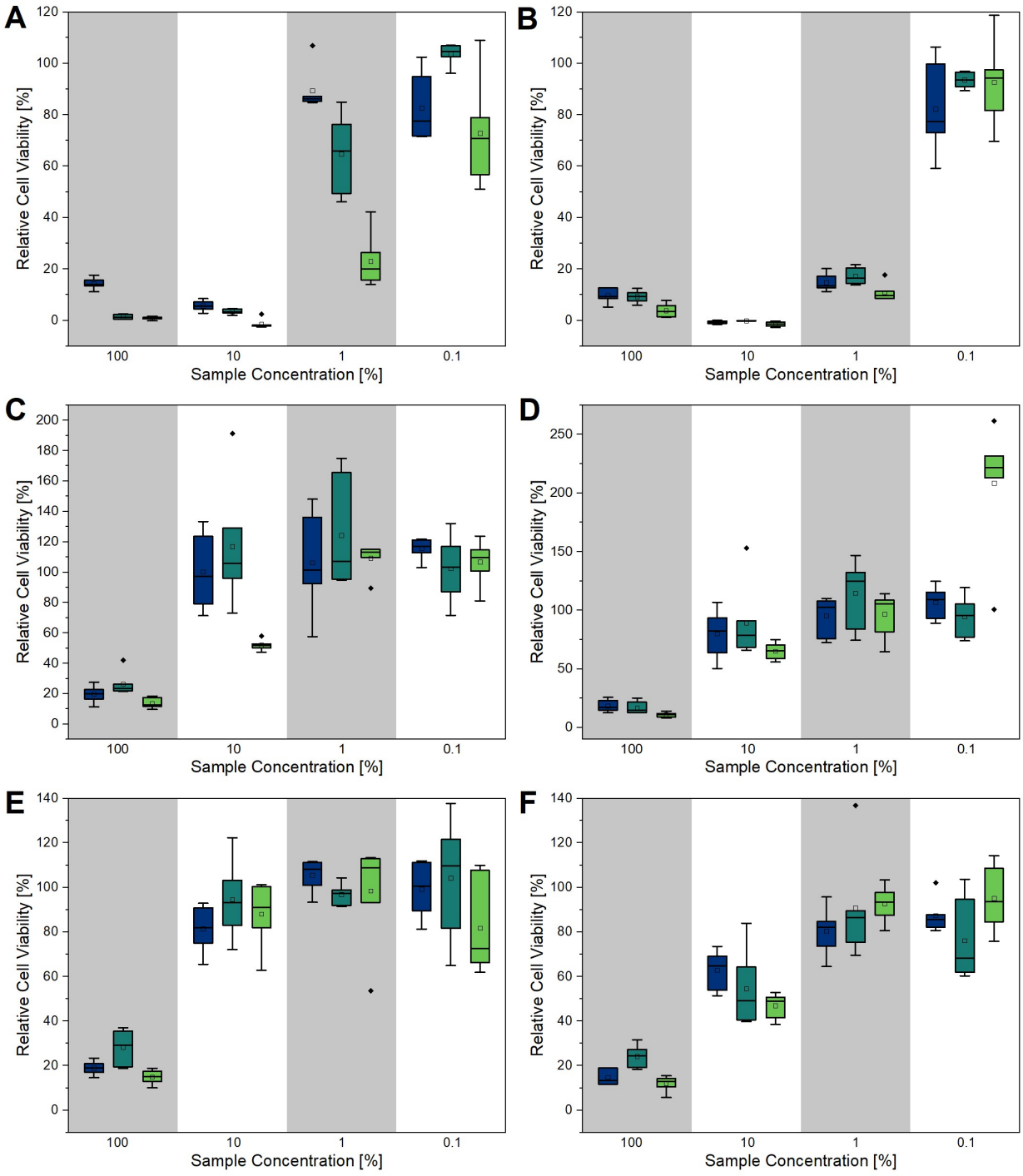

Figure S10. CTB of different hydrogel eluate samples: A EGDA; B P250; C P575 10%; D P575 15%; E P575 20%; F P700.

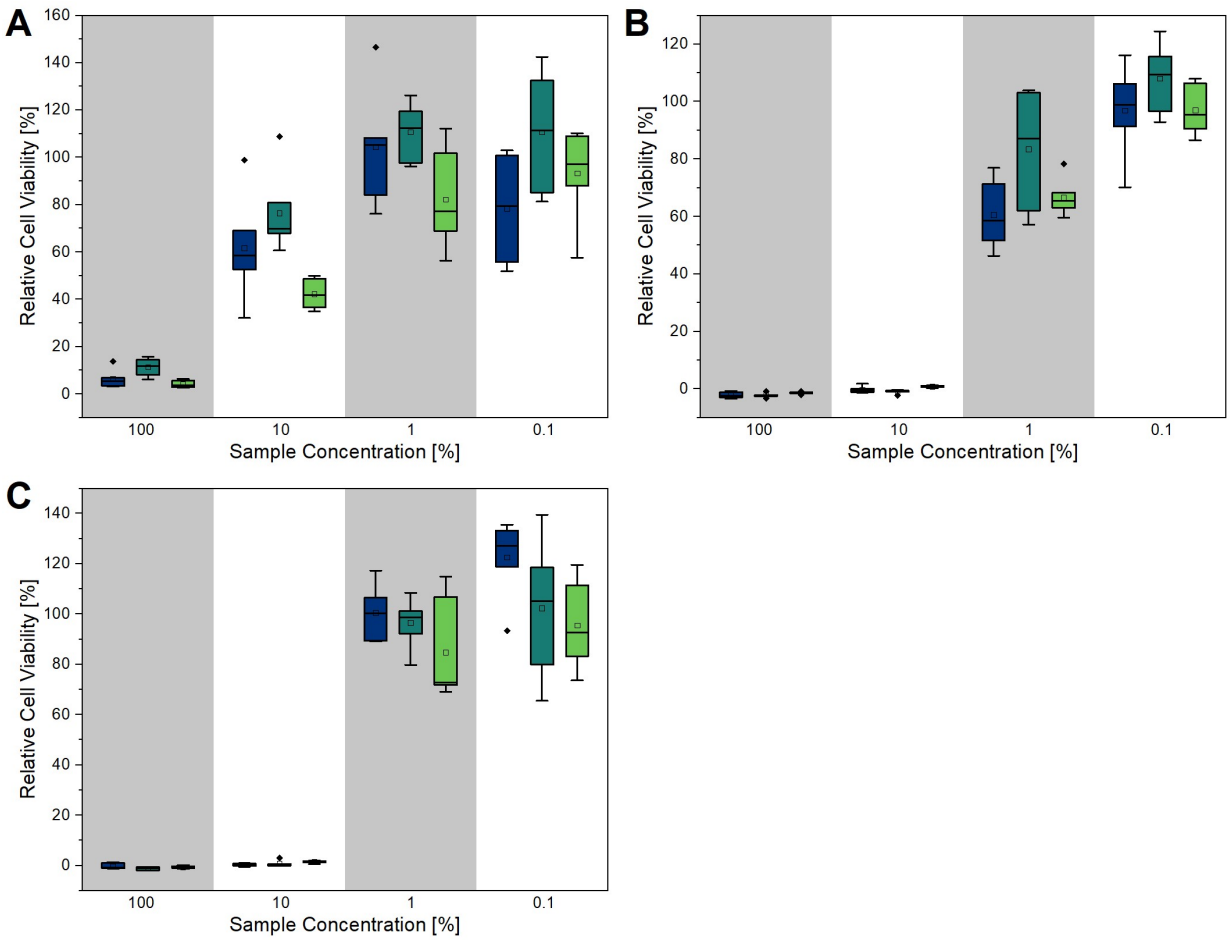

Figure S11. CTB of different hydrogel eluate samples: A MBAA; B MBAA A/T; C P575 AT.

3.2. Direct Contact tests

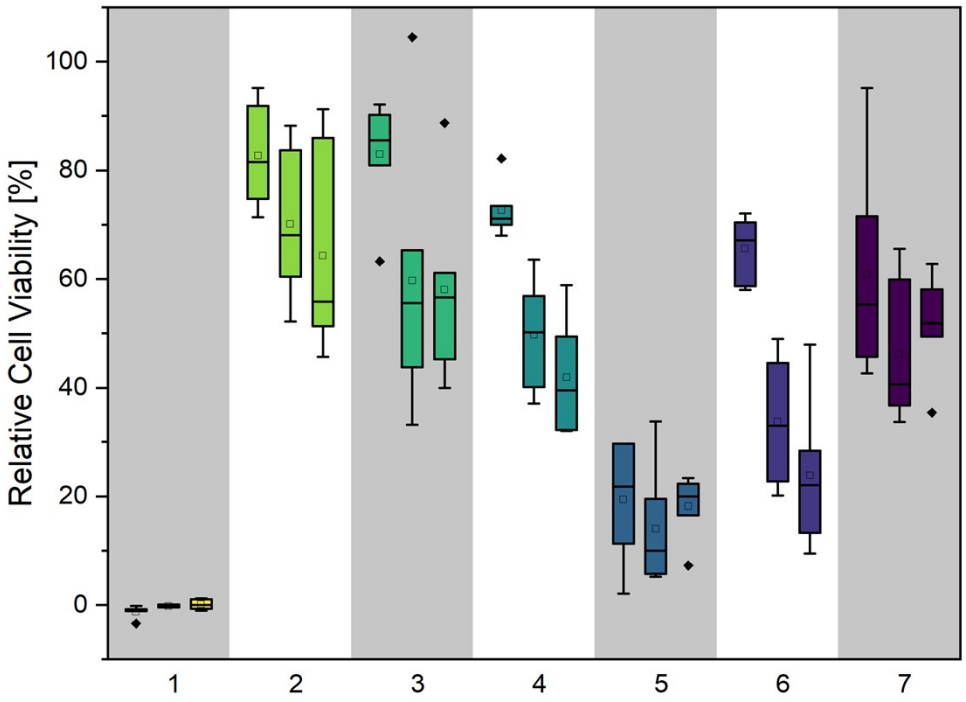

Figure S12. CTB of all 3 biological replicates of different treated hydrogels with **1** no treatment, **2** washing in DMEM 72 h, **3** washing in DMEM 3x24 h, **4** washing in EtOH (70%) 24 h and in DMEM 2x24 h, **5** UV irradiation 1 h, **6** UV irradiation 1 h and washing in DMEM 3x24 h, **7** UV irradiation 1 h, washing in EtOH (70%) and in DMEM 3x24 h.

4. Microscopic images of L929 cells

4.1. Eluate tests

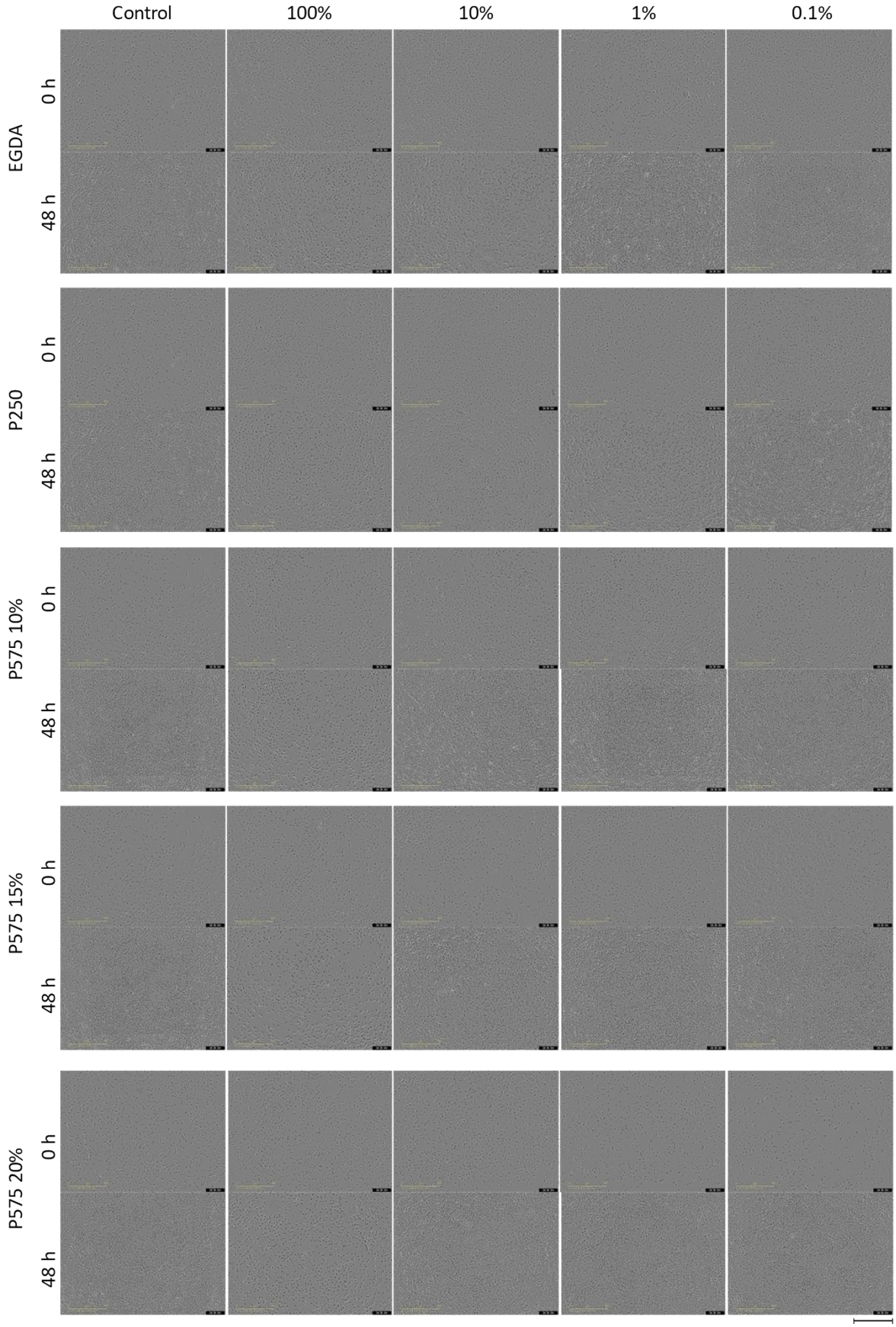

Figure S13. Microscopic brightfield images of L929 cells after 0 h and 48 h in different concentrated (100%, 10%, 1%, and 0.1%) hydrogel eluates (EGDA, PEGDA 250, PEGDA 575 10%, PEGDA 575 15%, and PEGDA 20%). Scale bar 400 µm.

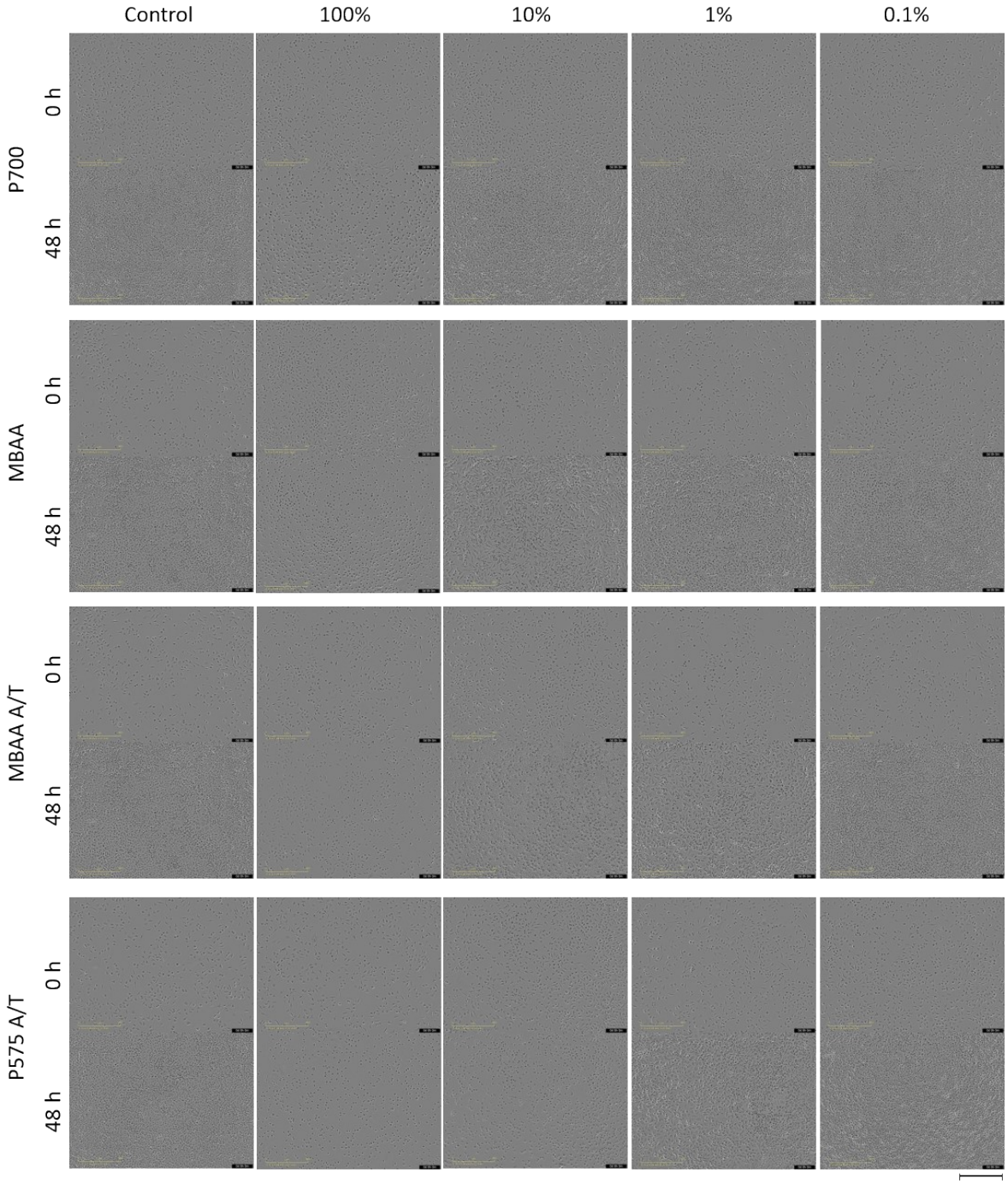

Figure S14.Microscopic brightfield images of L929 cells after 0 h and 48 h in different concentrated (100%, 10%, 1%, and 0.1%) hydrogel eluates (PEGDA 700, MBAA, MBAA A/T, and PEGDA 575 A/T). Scale bar 400 µm.

4.2. Direct contact tests

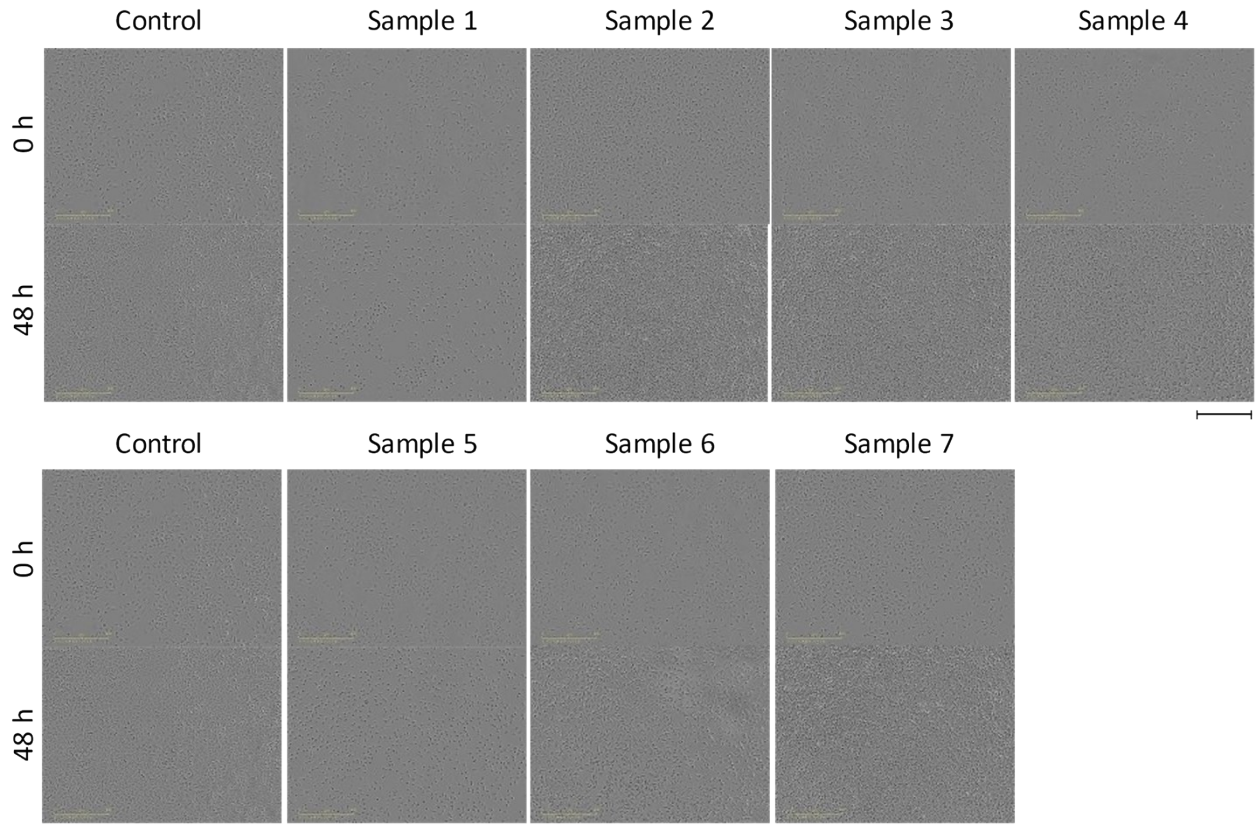

Figure S15. Microscopic brightfield images of L929 cells after 0 h and 48 h contact with different treated hydrogels with **1** no treatment, **2** washing in DMEM 72 h, **3** washing in DMEM 3x24 h, **4** washing in EtOH (70%) 24 h and in DMEM 2x24 h, **5** UV irradiation 1 h, **6** UV irradiation 1 h and washing in DMEM 3x24 h, **7** UV irradiation 1 h, washing in EtOH (70%) and in DMEM 3x24 h.

5. Cell staining (live/dead assay) - Calcein-AM/PI staining

5.1. Eluate tests

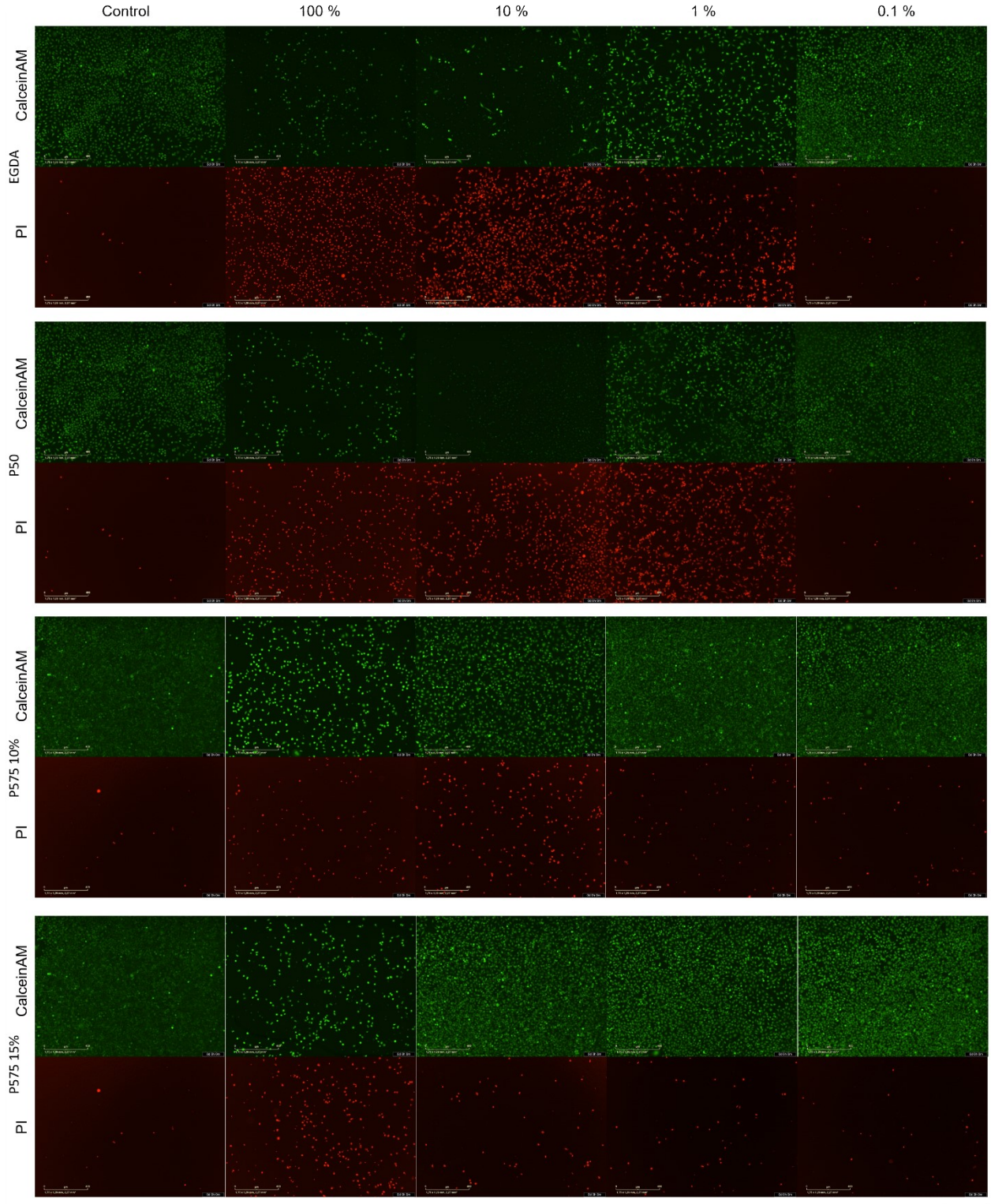

Figure S16. Calcein-AM/PI staining of L929 cell cultivated for 48 h in different concentrated (100%, 10%, 1% and 0.1%) hydrogel eluates from EGDA, PEGDA 250, PEGDA 575 10% and PEGDA 575 15% prepared with LAP (scale bar 400 µm).

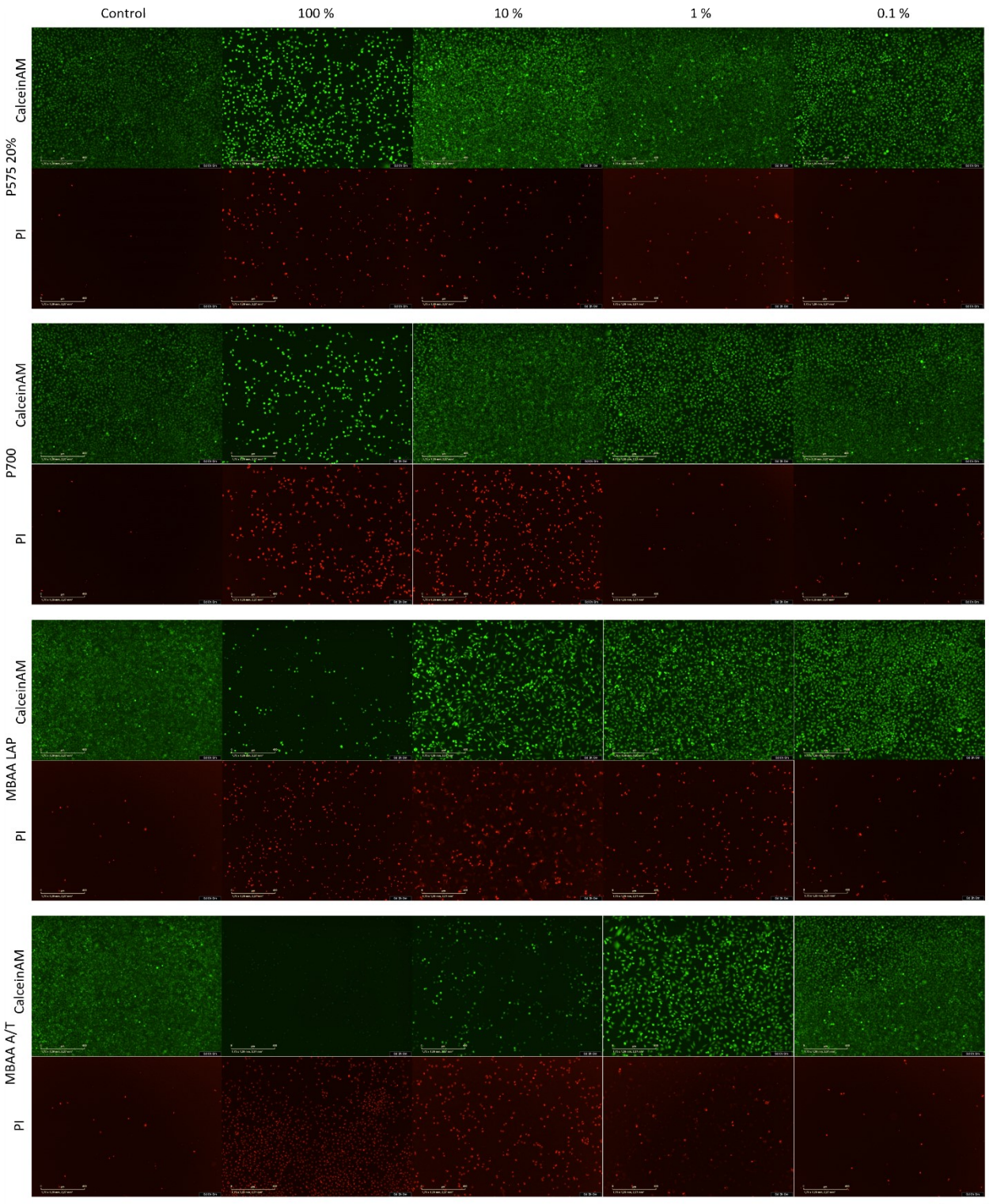

Figure S17. Calcein-AM/PI staining of L929 cell cultivated for 48 h in different concentrated (100%, 10%, 1% and 0.1%) hydrogel eluates from PEGDA 575 20% and PEGDA 700 and MBAA prepared with LAP and MBAA prepared with APT/TEMED (scale bar 400 µm).

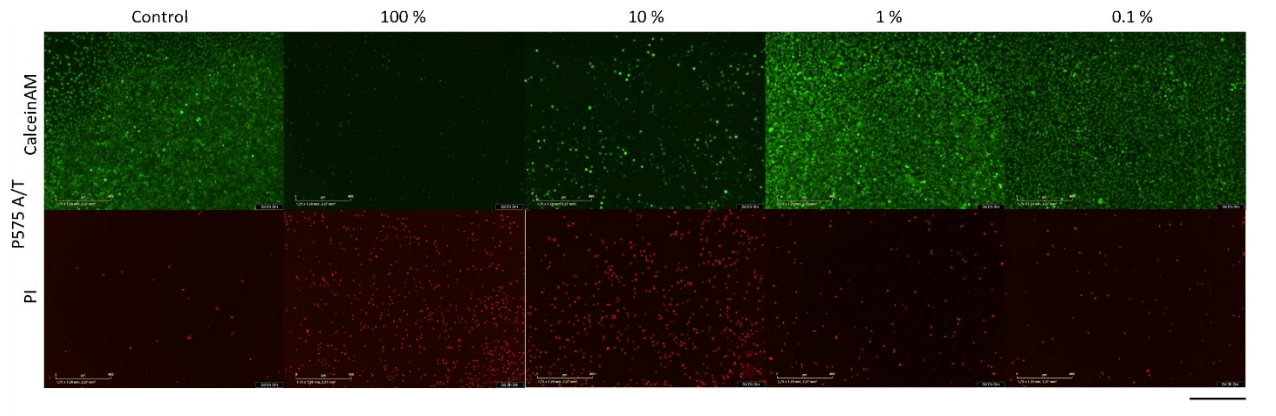

Figure S18 Calcein-AM/PI staining of L929 cell cultivated for 48 h in different concentrated (100%, 10%, 1% and 0.1%) hydrogel eluates from PEGDA 575 prepared with APT/TEMED (scale bar 400 µm).

5.2. Direct contact tests

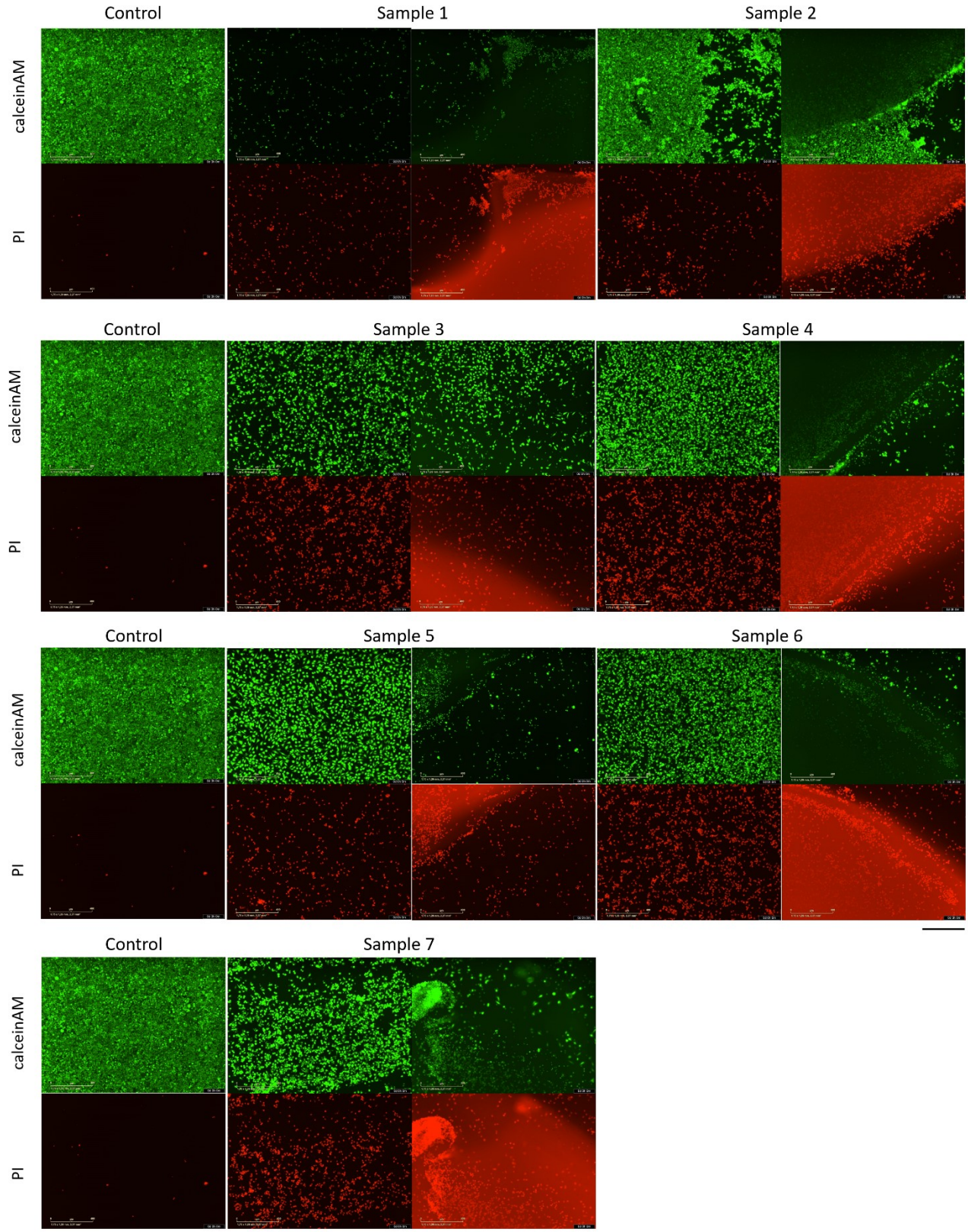

Figure S19. Calcein-AM/PI staining of L929 cell cultivated for 48 h in contact with different treated hydrogels with **1** no treatment, **2** washing in DMEM 72 h, **3** washing in DMEM 3x24 h, **4** washing in EtOH (70%) 24 h and in DMEM 2x24 h, **5** UV irradiation 1 h, **6** UV irradiation 1 h and washing in DMEM 3x24 h, **7** UV irradiation 1 h, washing in EtOH (70%) and in DMEM 3x24 h. For each sample, a picture is shown with and without hydrogel (scale bar 400 µm).

6. LC-MS

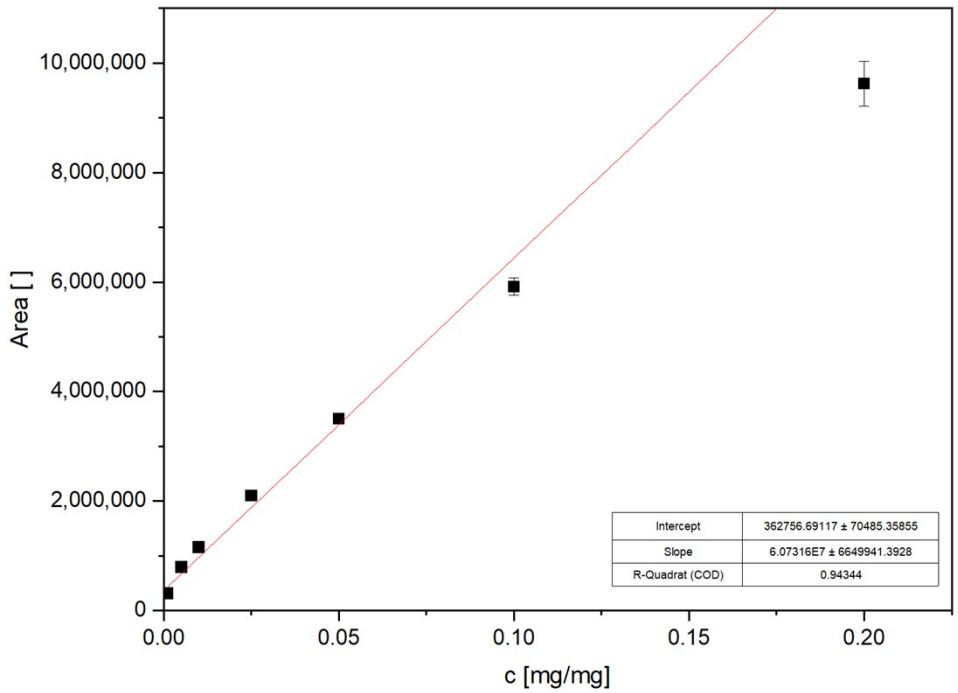

Figure S20. Calibration of GVIM-I via LC-MSMS.

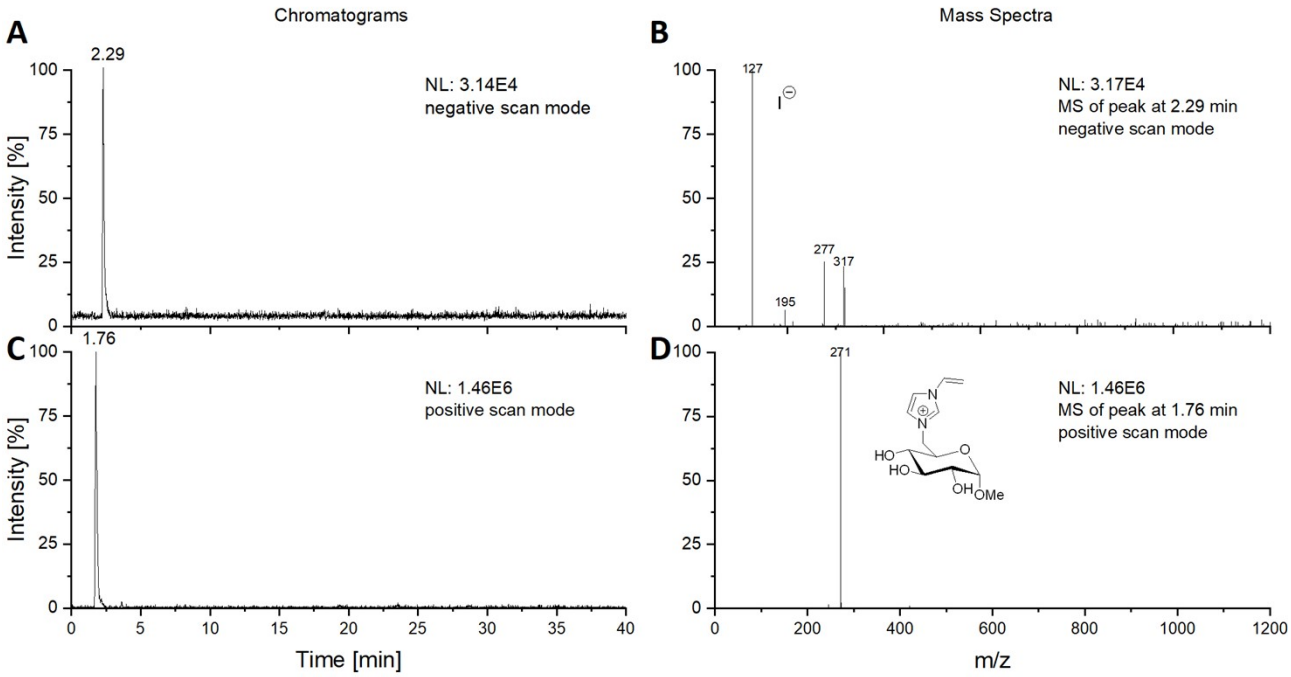

Figure S21. **A** Chromatogram of pure GVIM-I in negative scan mode, **B** Mass spectrum corresponding to A, **C** Chromatogram of pure GVIM-I in positive scan mode and **D** Mass spectrum corresponding to C.

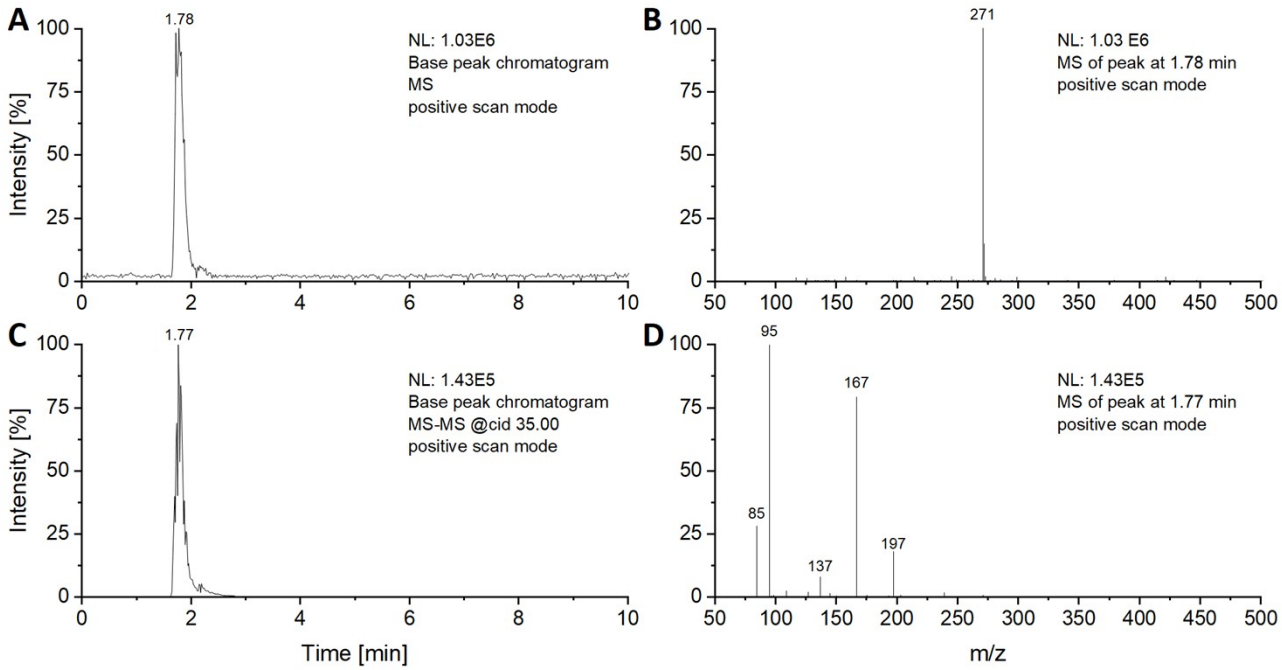

Figure S22. **A** Chromatogram of sample B1 48 h in positive scan mode (MS), **B** mass spectra (MS) of sample B1 48 h corresponding to A, **C** Chromatogram of sample B1 48 h in positive scan mode (MSMS) and **D** mass spectra (MSMS) of sample B1 48 h corresponding to C.