Supporting Information

Enzyme assisted peptide self-assemblies trigger cell adhesion in high density oxime based host gels

Miryam Criado-Gonzalez[a,b,c‡], *Breyinn Loftin*[d‡], *Jennifer Rodon Fores*[a], *Dominique Vautier*[b,c], *Leyla Kocgozlu*[b,c], *Loïc Jierry*[a], *Pierre Schaaf*[a,b*], *Fouzia Boulmedais*[a], *Eva Harth*[c,d*]

a. Université de Strasbourg, CNRS, Institut Charles Sadron UPR 22, 67034 Strasbourg, France.

b. Institut National de la Santé et de la Recherche Médicale, UMR-S 1121, "Biomatériaux et Bioingénierie", 67087 Strasbourg, France.

c. Université de Strasbourg, Faculté de Chirurgie Dentaire, Fédération de Médecine Translationnelle de Strasbourg and Fédération des Matériaux et Nanoscience d'Alsace, 67000 Strasbourg, France.

d. Department of Chemistry, Center of Excellence in Polymer Chemistry (CEPC), University of Houston, 3585 Cullen Boulevard, Houston, Texas 77030, United States

‡ Both authors contributed equally

MATERIALS AND METHODS

1. Characterization of poly(N,N,-dimethylacrylamide-co-diacetoneacrylamide) [Poly(DMA-co-DAAM)] and bis-Aminooxy PEG

^{1}H-NMR spectra were recorded on JEOL ECX400, and JEOL ECA-600 II spectrometers. Chemical shifts were referenced to solvent resonance signals. Gel permeation chromatography (GPC) was conducted on a ToSOH EcoSEC HLC8320GPC system equipped with a refractive index detector, UV-8320 detector, and TSKgel HHR columns (7.8x300mm G5000HHR, G4000HHR, and G3000HHR) with *N*,*N*-dimethylformamide (HPLC grade, containing 1 g.L^{-1} LiBr) as the eluent at a flow rate of 1 mL.min^{-1}.

1.1. Poly(N,N,-dimethylacrylamide-co-diacetoneacrylamide) [Poly(DMA-co-DAAM)]

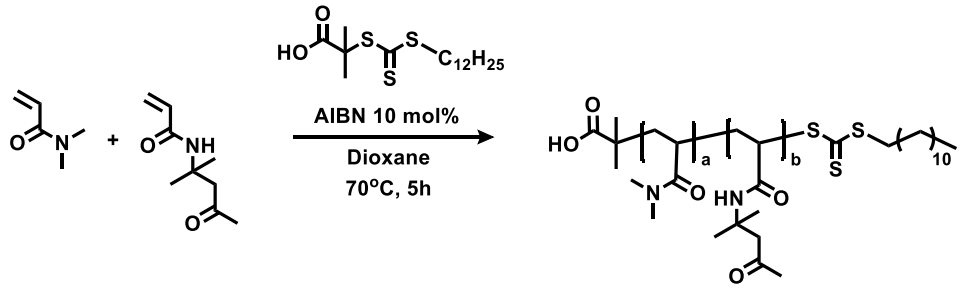

Table S1. Copolymers with *N*,*N*-dimethylacrylamide (DMA) and diacetoneacrylamide (DAAM) of varying comonomer composition.

ENTRY	Feed Ratio [DAAM]/ [DMA]	DAAM[a] mol %	$M_{n,GPC}$[b] (g.mol^{-1})	$M_{w,GPC}$[b] (g.mol^{-1})	M_w/M_n[b]
1	0.11	13	56000	67000	1.19
2	0.18	21	61000	76000	1.24
3	0.25	29	56000	73000	1.23
4	0.33	39	53000	61000	1.16
5	0.43	49	56000	63000	1.13

[a]Determined from ^{1}H-NMR spectroscopy. [b]Determined by GPC with DMF (containing 1 g.L^{-1} LiBr) as the eluent at a flow rate of 1 mL.min^{-1} using PMMA calibration.

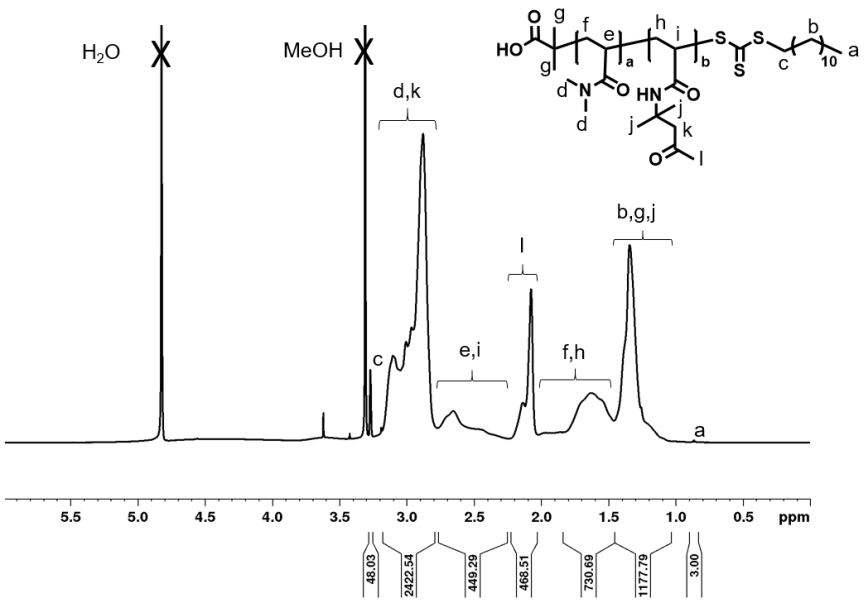

Figure S1. [1]H-NMR of representative poly(DMA-co-DAAM). [1]H NMR (600 MHz, CD_3OD): δ 0.878, 1.04-1.46, 1.46-2.02, 2.02-2.24, 2.24-2.78, 2.78-3.25, 3.25-3.29.

1.2. Bis-Aminooxy PEG (6k)

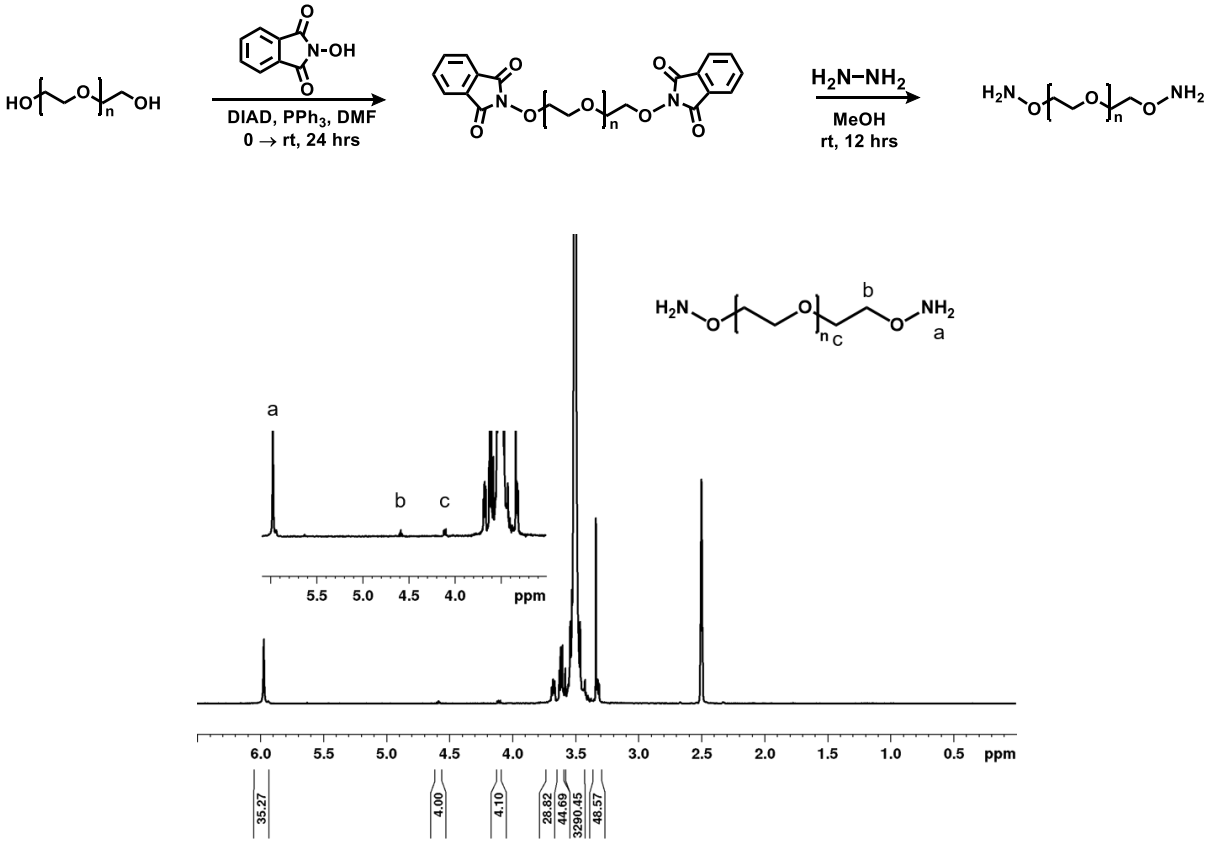

Figure S2. [1]H-NMR of representative bis-amino PEG polymer. The labeled a, b, c peaks are the end group of the polymer. [1]H-NMR (400 MHz, $(CD_3)_2SO$): δ 3.50, 4.11, 4.59, 5.97.

2. Formation of poly(DMA-co-DAAM)/AO-PEG hydrogel

As an example, we show the calculations for the preparation of PDD-AOP hydrogels at 15%wt:

$$5\ mg\ P(DMA-DAAM) \times \frac{mmol}{56000\ mg} \times \frac{206.8\ mmol\ DAAM}{1\ mmol\ P(DMA-DAAM)} = 0.0185\ mmol\ P(DMA-DAAM)$$

$$0.0185\ mmol\ P(DMA-DAMM) \times \frac{0.5\ mmol\ AO-PEG}{1 mmol\ P(DMA-DAMM)} \times \frac{6000\ mg}{mmol} = 55\ mg\ AO-PEG$$

Total polymer mass = 5 mg P(DMA-co-DAAM) + 55 mg AO-PEG = 60 mg total polymer.
For a 15 %wt solution, 60 mg total polymer should be in 400 µL of polymer solution (60 mg/0.15 mg.µL^{-1}) assuming a density of 1 mg.µL^{-1}.

$$\frac{5\ mg\ P(DMA-DAAM)}{0.12 mg/\mu L} = 42\mu L\ P(DMA-DAMM)$$

$$\frac{55\ mg\ AO-PEG}{0.32\ mg/\mu L} = 172\mu L\ AO-PEG$$

where P(DMA-co-DAAM) is in a 12 wt% solution in water and AO-PEG is in a 32 %wt solution in water. Therefore, 42 µL + 172 µL = 214 µL. 400 µL – 214 µL = 186 µL. Hence, adding 186 µL of PBS buffer to P(DMA-co-DAAM) will give a 15 %wt solution when combined with AO-PEG.

In order to optimize the host hydrogel formation, different weight percentages (% wt) were tested keeping constant the 1:1 mole ratio between ketone and aminooxy derivatives (Table S2) following the previous calculations.

Table S2. Composition of PDD-AOP hydrogels at different weight percentages.

PDD-AOP (%wt)	P(DMA-co-DAAM) (µL)	PBS 7.4 (µL)	AO-PEG (µL)
20	42	86	172
15	31	139.5	129
10	21	193	86

SUPPLEMENTARY FIGURES

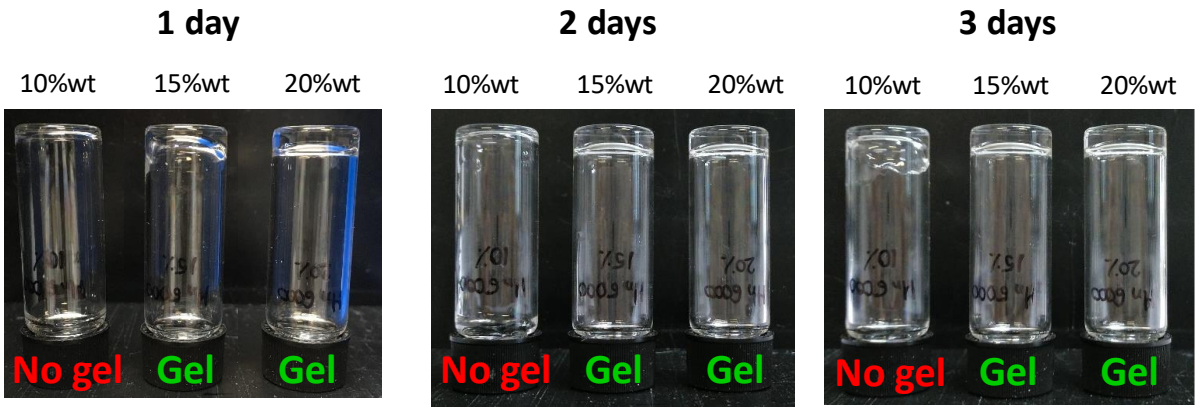

Figure S3. Inverted tube tests for PDD-AOP gel formation over the time.

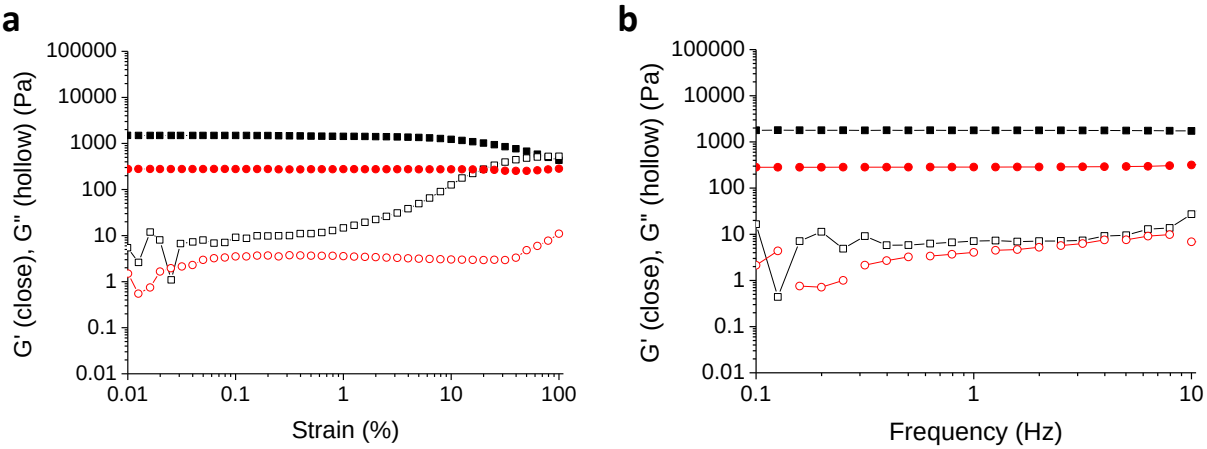

Figure S4. Storage modulus (G' –solid symbols) and loss modulus (G" – hollow symbols) of PDD-AOP gels with 20%wt (black line) and 15%wt (red line) a) as a function of the strain at a fixed frequency of 1Hz and b) as a function of the frequency at a fixed strain of 0.1%.

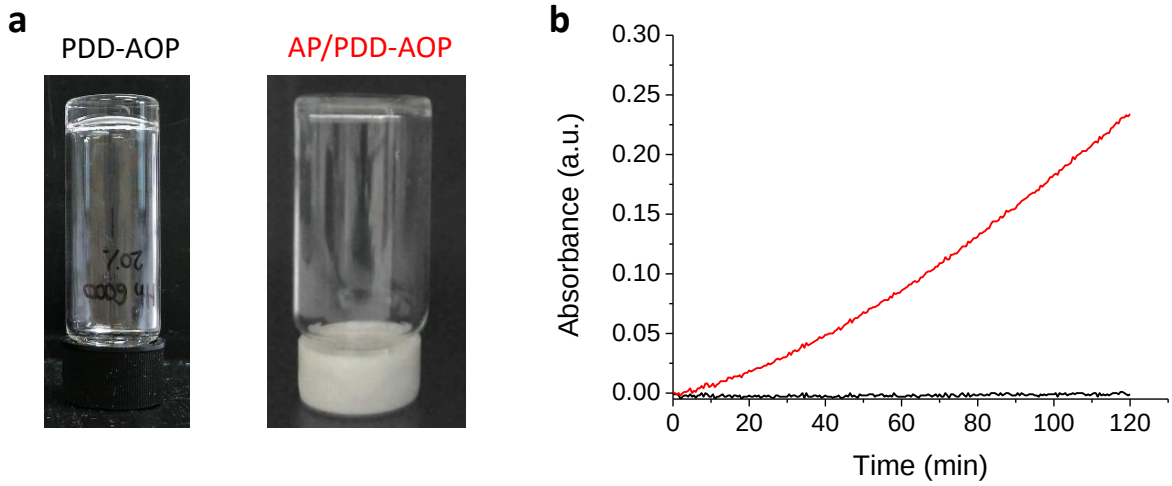

Figure S5. a) Inverted tube tests of PDD-AOP and AP/PDD-AOP gels after 48 h; b) Evolution of the optical density (OD) as a function of time of a *para*-nitrophenyl phosphate (PNP) solution brought in contact with PDD-AOP gel (black curve) and AP/PDD-AOP gel (red curve). The absorbance was measured at 405 nm. PNP is transformed into *para*-nitrophenol (λ_{max} = 405 nm) and phosphate ions by AP embedded into the gel. The values of the slopes are 0 and 2.4 × 10^{-3} min^{-1} for PDD-AOP and AP/PDD-AOP gels, respectively.

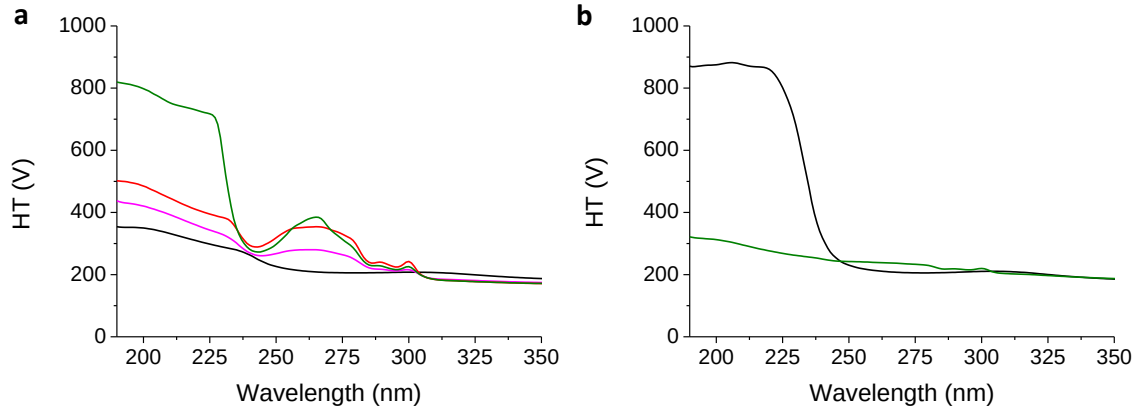

Figure S6. HT voltage curves for a) AP/PDD-AOP gel (black line), 4h (pink line), 24 h (red line) and 48 h (green line) after Fmoc-FF*p*Y diffusion into the host gel and b) PDD-AOP gel (black line) and PDD-AOP gel after 48 hours Fmoc-FF*p*Y (2.5 $mg.mL^{-1}$) diffusion (green line) into the host gel.

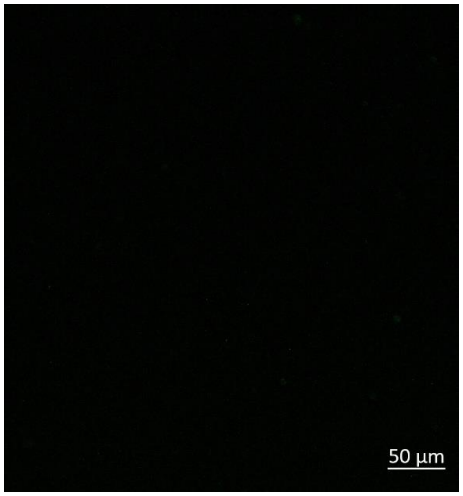

Figure S7. Confocal image of ThT/PDD-AOP hydrogel after 48 hours contact with Fmoc-FF*p*Y (2.5 mg.mL^{-1}).

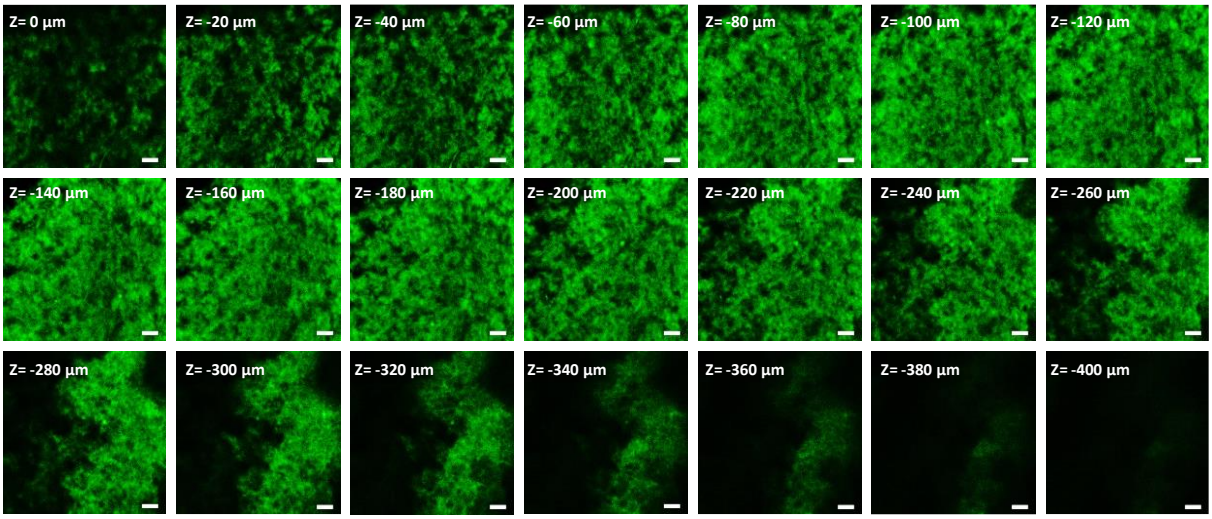

Figure S8. Confocal images taken along the z-axis penetrating inside the PDD-AOP hydrogel, 48 hours after the diffusion of Fmoc-FF*p*Y solution (100 µL, 2.5 mg.mL^{-1}). Scale bar = 100 µm.

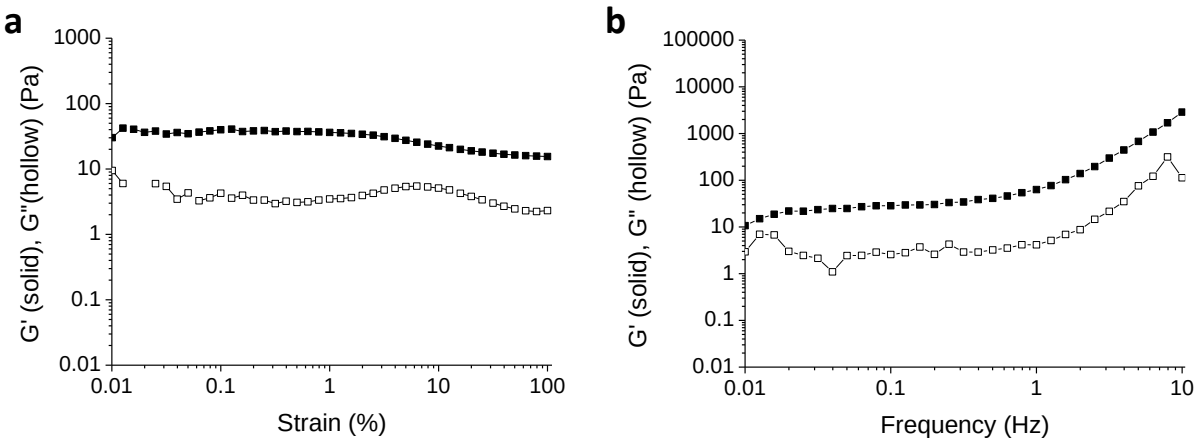

Figure S9. Storage modulus (G' –solid symbols) and loss modulus (G" – hollow symbols) of Fmoc-FF*p*Y (2.5 mg.mL^{-1}) in contact with AP (0.2 mg.mL^{-1}) a) as a function of the strain at a fixed frequency of 1Hz and b) as a function of the frequency at a fixed strain of 0.1%.

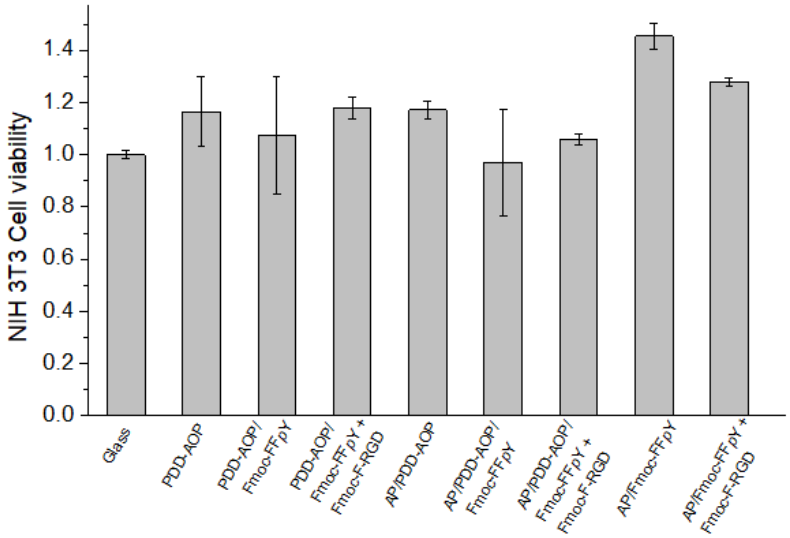

Figure S10. Cytotoxicity assay of hydrogels after 24 hours determined through MTT indirect test using NIH 3T3 mouse embryonic fibroblasts cells.

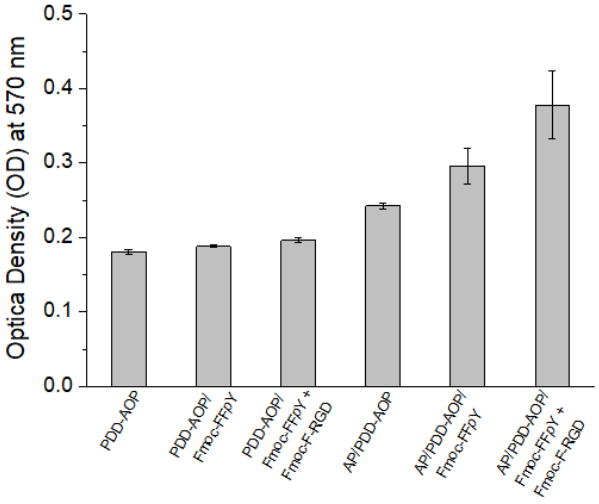

Figure S11. Cytotoxicity assay of hydrogels after 24 hours determined through MTT direct test using NIH 3T3 mouse embryonic fibroblasts cells.