SUPPLEMENTARY INFORMATION

Finding the sweet spot: a library of hydrogels with tunable degradation for tissue model development

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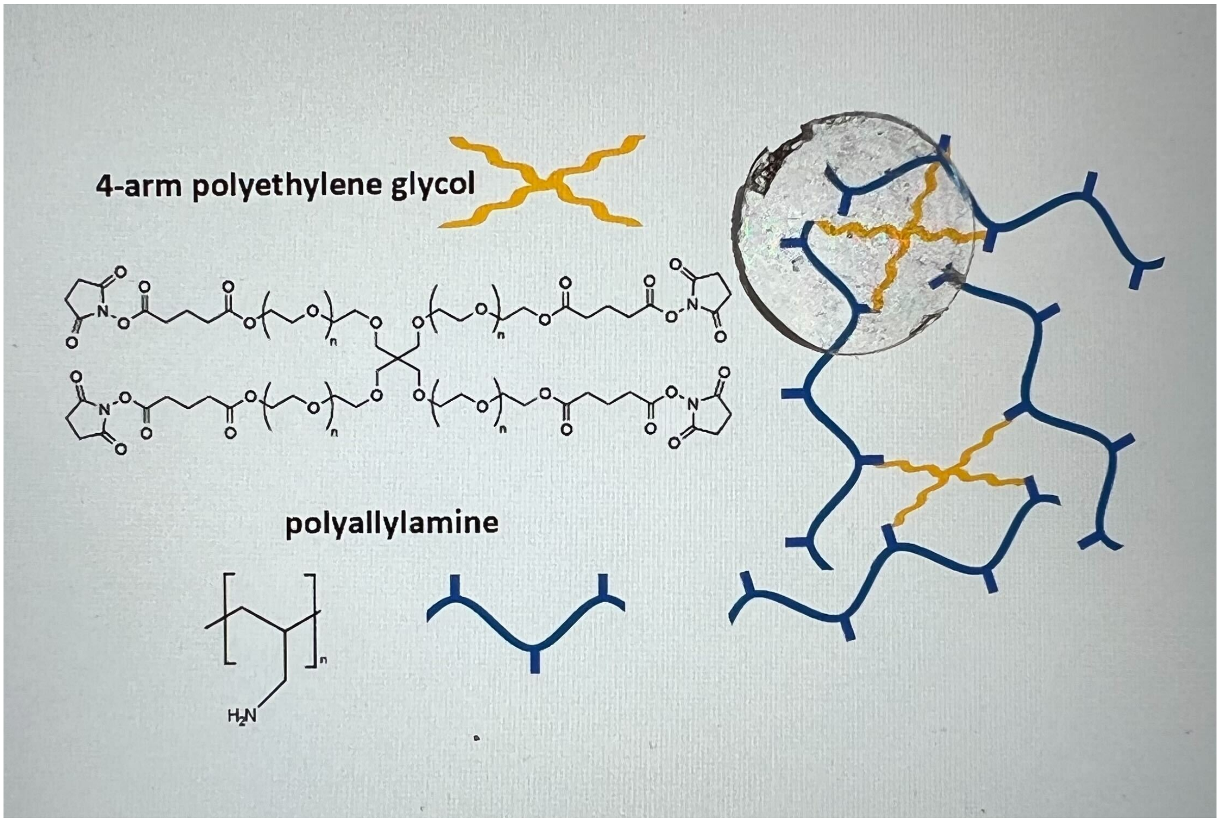

Figure S1. Images of Hydrogel prepared using 10:1 ratio of free amines to succinimides in the PEG-PAA library

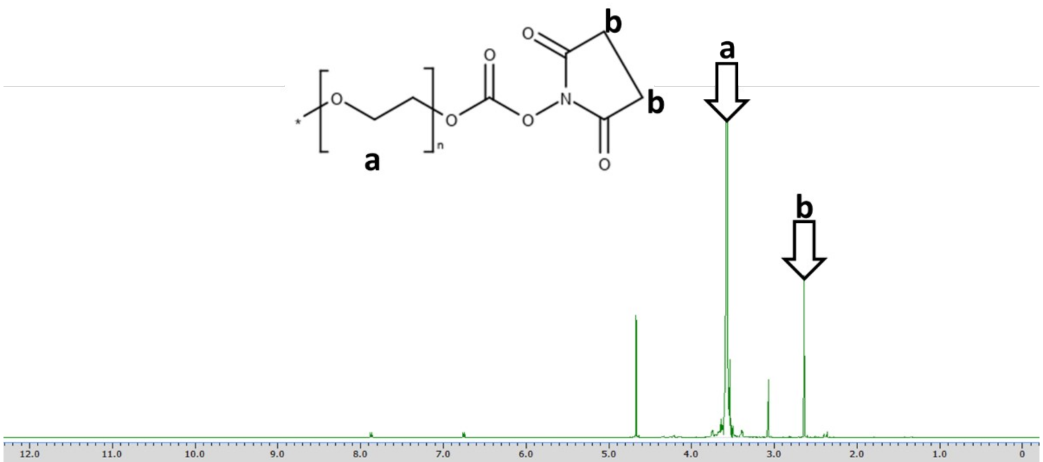

Figure S2. [1]H NMR spectrum of the bifunctional PEG-SG (4.6kDa)

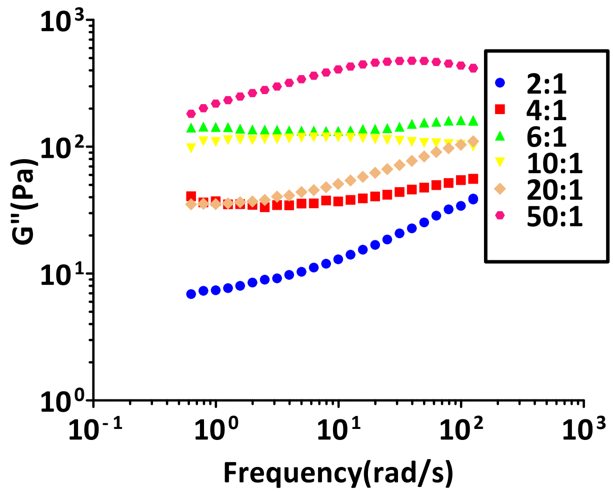

Figure S3. Frequency dependent loss modulus (G'') of PEG-PAA hydrogels with varied ratios of free amines to succinimidyl groups.

Using the relationship between the frequency-independent elastic modulus and the effective crosslinking density (or network junction density) (as presented in Calveat et al.)[1] based on Flory's theory of rubber elasticity:

$$n_e = \frac{G'}{\left(1 - \frac{2}{f}\right)\left(\frac{f}{2}\right)RT} \quad (1)$$

where f is the number of strands linked to a cross linker. For our calculations, the number is close to 3. (The core of the 4 arm PEG has 4 strands attached, and the junction between an arm and polylysine has three arms. Therefore, there is one 4 arm junction for every 4, 3-armed junctions assuming complete reactivity making f equal to 3.2 but it is very unlikely that all the arms reacted. Assuming a 75% coupling rate, then f becomes 3.25.) Using f=3.25 and T=298 K along with the value for the gas constant, R (8.314 $Pa \cdot m^3 \cdot K^{-1} \cdot mol^{-1}$), we find that the effective crosslinking density can be seen in the table, below.

Supplementary Table 1: n_e and M_e based on the Elastic Modulus Results

Ratio Amines:SG	G' (Pa)	f	ne (mol/m^3)	Me (kg/mol)	Me (g/mol)
2	593	3.25	0.38	261.10	261096.08
4	3001	3.25	1.94	51.60	51603.35
6	6169	3.25	3.98	25.10	25102.37
10	5165	3.25	3.34	29.98	29977.46
20	3895	3.25	2.52	39.75	39752.59
50	2555	3.25	1.65	60.61	60605.02

The effective molecular weight between cross links, M_e, is defined by the polymer concentration in kg/m^3 divided by n_e. This assumes the simplest model for crosslinking based on rubber elasticity which is a reasonable approximation since G''<<G'.

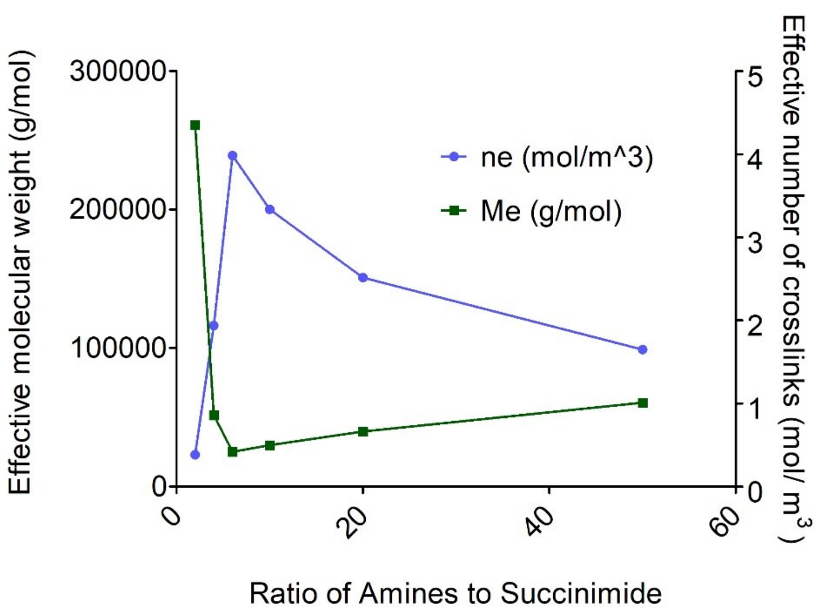

Figure S4. Effective molecular weight and effective number of crosslinks of the PEG-PAA system, this was obtained using the relationship between G' and the network junction density based on Flory's theory of rubber elasticity.

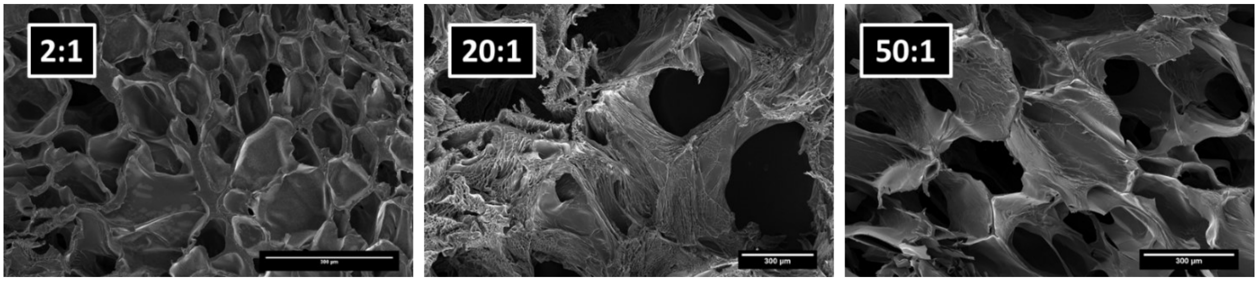

Figure S5. SEM images of the PEG-PAA formed using 2:1, 20:1 and 50:1 ratio of free amines in PAA to succinimidyl groups in PEG-SG showing a larger pore size compared to the other ratios. (Scale bar is 300 µm)

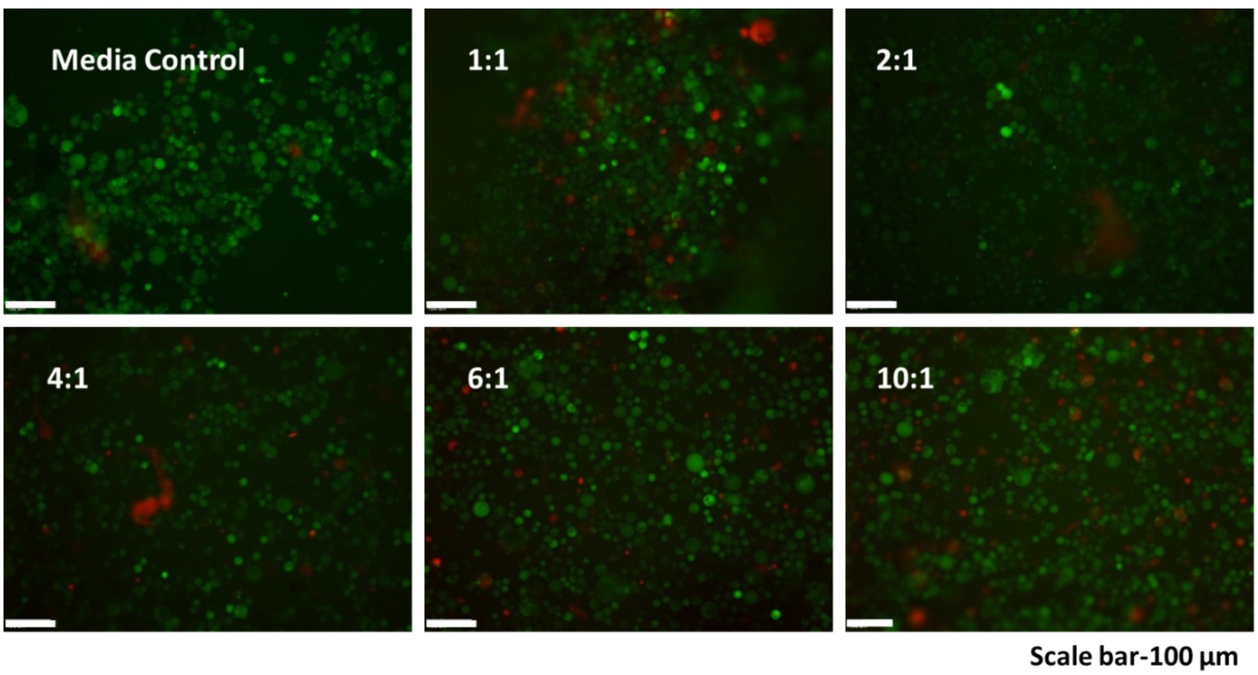

Figure S6. Figure 3. Live/ Dead scans of Caco-2 cells seeded on top of the PEG-PAA hydrogel library (Scale bar is 100 µm)

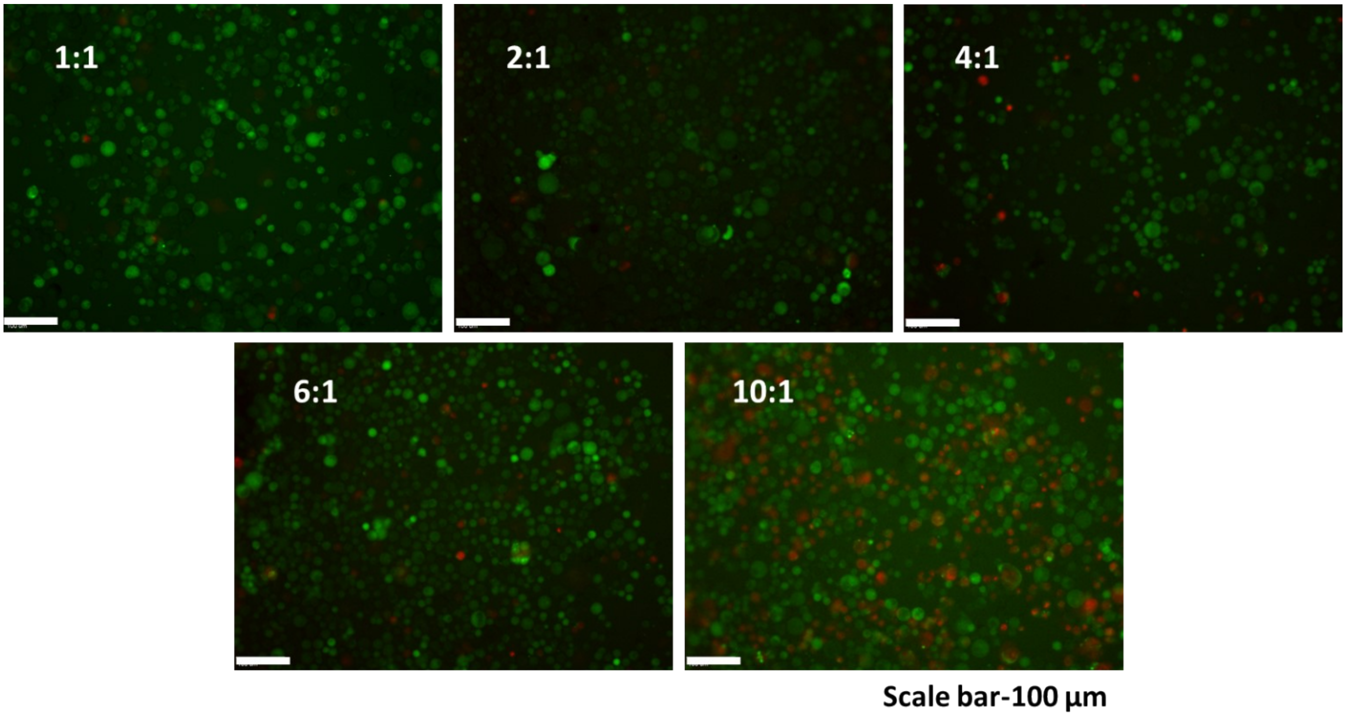

Figure S7. Live/ Dead scans Caco-2 cells seeded on top of the PEG-PAA hydrogel library supplemented with laminin, 1% Laminin is added to the PAA precursor solution and incubated at 37^{o}C for an hour (Scale bar is 100 µm)

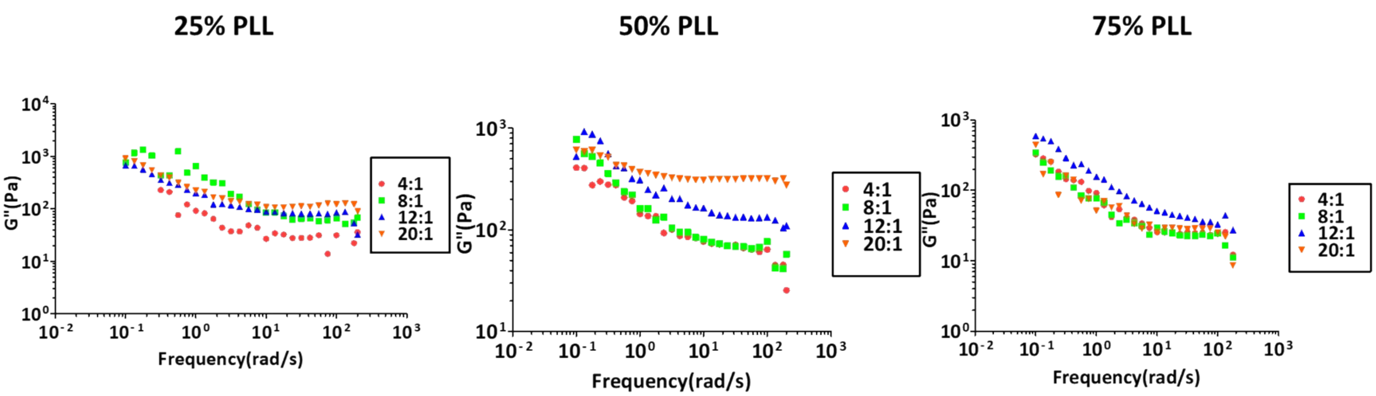

Figure S8. Frequency dependent loss modulus (G'') of PEG-PAA-PLL hydrogels with varied ratios of free amines to succinimidyl groups

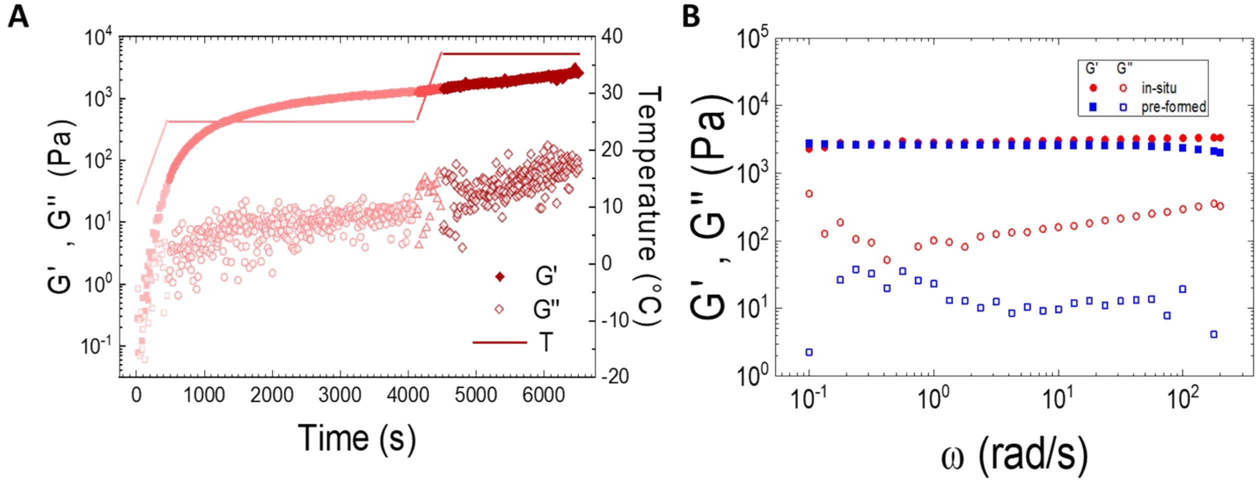

Figure S9. A) Moduli measured during in-situ gelation of 25% PLL 4:1. B) Frequency sweep results for the same gel formed in-situ and pre-formed.

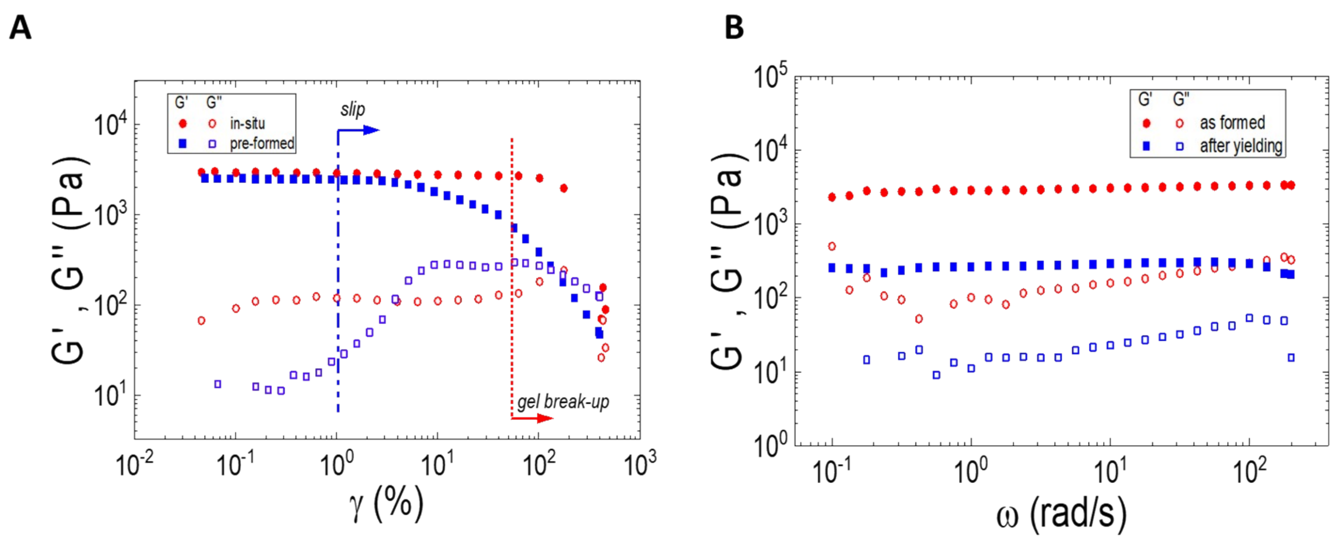

Figure S10. A) Comparison of strain sweep results between 25% PLL 4:1 gelled in-situ and pre-formed. Note the onset of slip in the pre-formed gel and true non-linear deformation and break-up in the in-situ gel. B) Frequency sweeps of the in-situ formed gel before and after performing the strain sweep, showing considerable damage due to break-up.

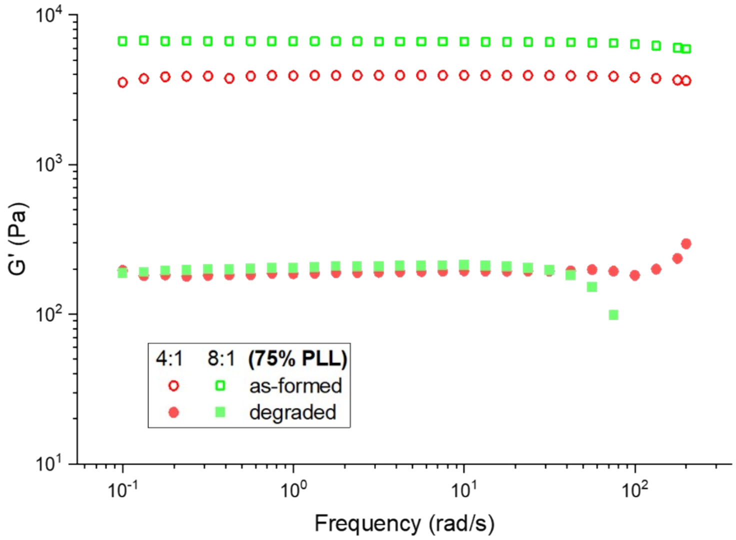

Figure S11. Storage moduli as a function of frequency for as-formed (hollow symbols) and degraded gels (filled symbols) for 4:1 and 8:1, with 75% PLL.

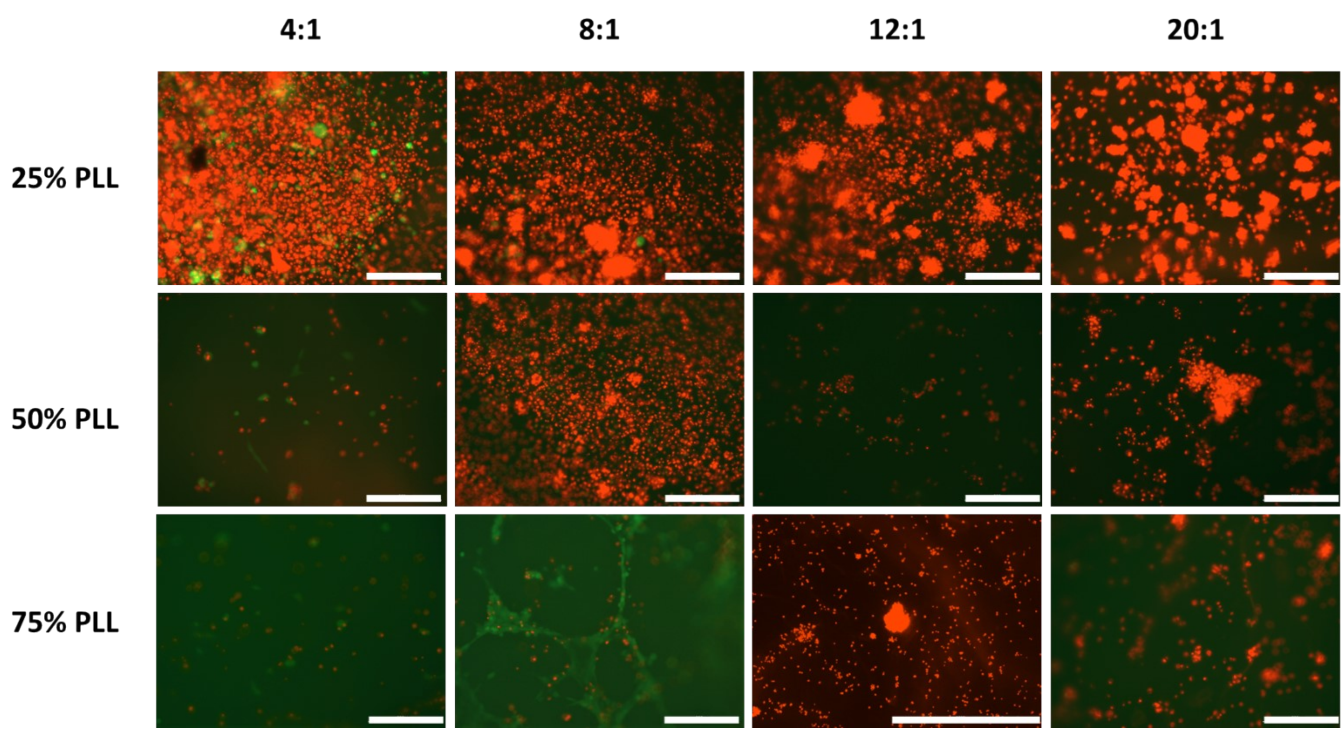

Figure S12. Live/ Dead scans of rat endothelial cells seeded on top of the PEG-PAA-PLL hydrogel library at day-4 (Scale bar is 200 µm, except for the 12:1, 75%PLL gel which has a scale bar of 1000 µm)

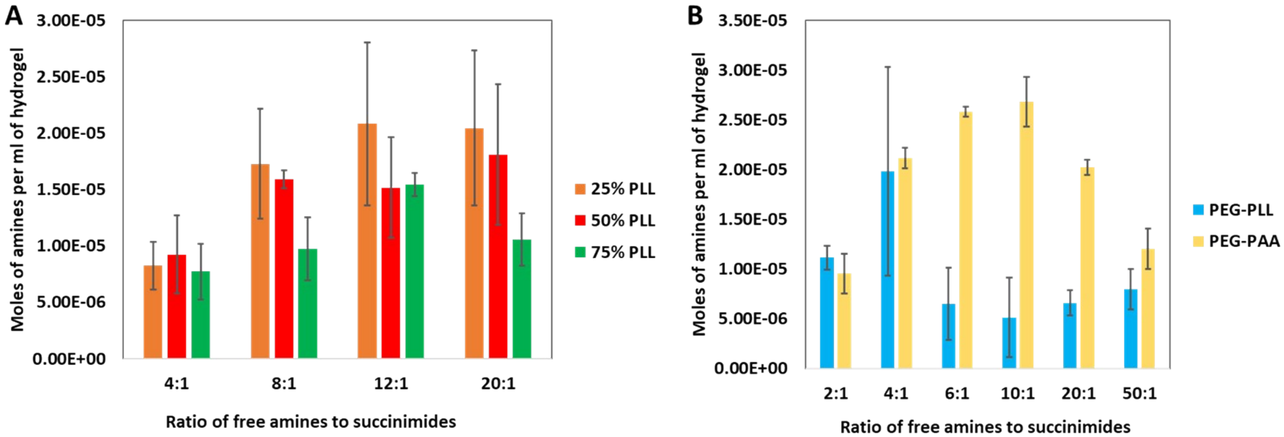

Figure S13. Comparison of the number of moles of amines per ml of the hydrogel obtained by using an OPA assay on the a) PEG-PAA-PLL library and the b) PEG-PAA and PEG-PLL hydrogel libraries.