Water-soluble macromers based on 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (Na-AMPS) for rapid *in situ* hydrogel film formation

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Electronic Supporting Information (ESI)

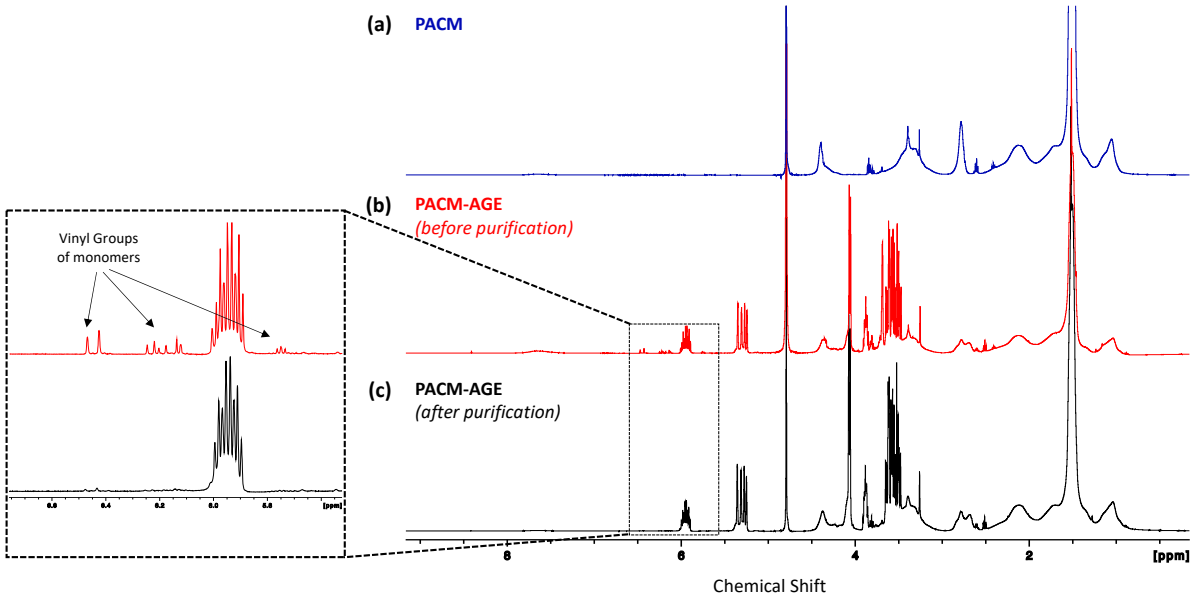

Figure S1. ^{1}H NMR spectra (D_2O) for (a) poly(AMPs-*stat*-CEA-*stat*-MAA) (PACM) before modification; (b) poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE) after modification (without purification); and (c) poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE) after modification and purification.

Comparison of the ^{1}H NMR signals corresponding to the polymer structure before and after modification confirmed that the functionalization reaction was successful: allyl groups from AGE are clearly present following functionalization (Figure S1). The magnified ^{1}H NMR spectra verified the effective elimination of unreacted monomers through purification via dropwise precipitation in acetone, evidenced by the absence of peaks at 5.6 (unreacted of MAA monomer),

6.2 and 6.4 (unreacted of CEA monomer) ppm (Figure S1(c)). Importantly, the allyl protons provided by AGE residues persist after precipitation, thus confirming successful functionalization and polymer chains with pendant allyl functionality.

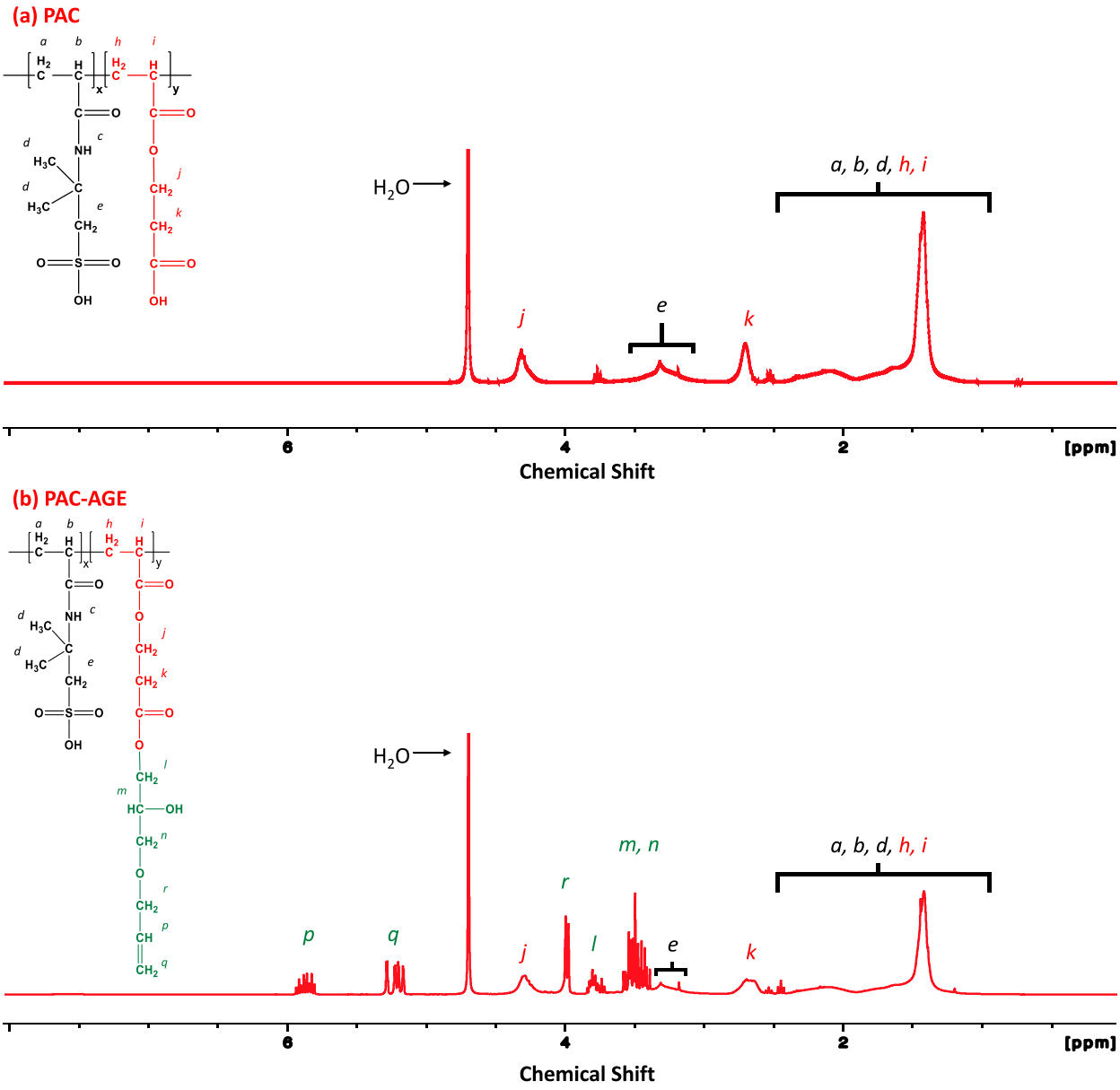

Figure S2. ^{1}H NMR spectra (D_2O) of the polymer (a) before modification; poly(AMPs-*stat*-CEA) (PAC) and (b) after modification and purification; poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE).

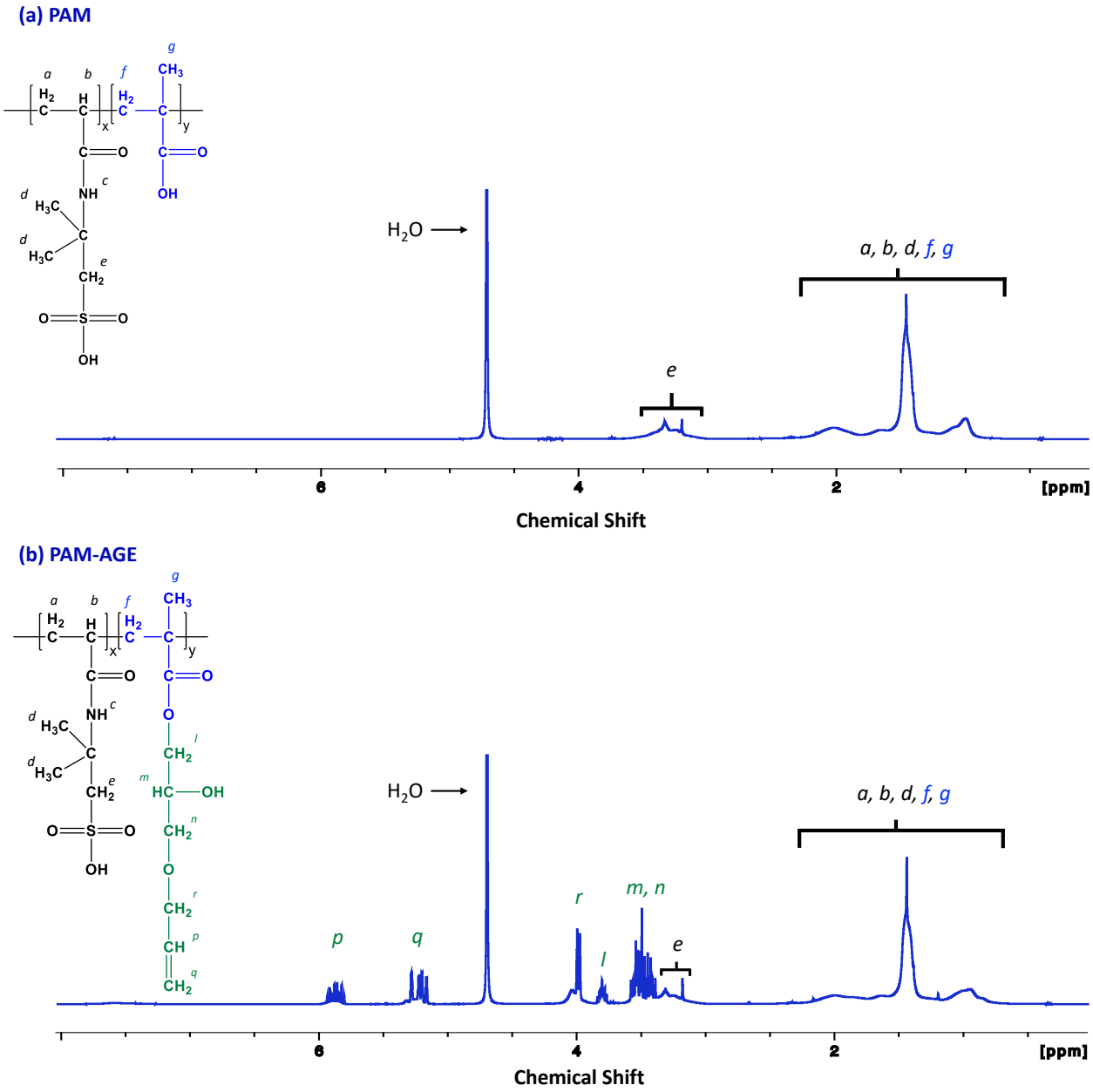

Figure S3. ^{1}H NMR spectra (D_2O) of the polymer (a) before modification; poly(AMPs-*stat*-MAA) (PAM) and (b) after modification and purification; poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE).

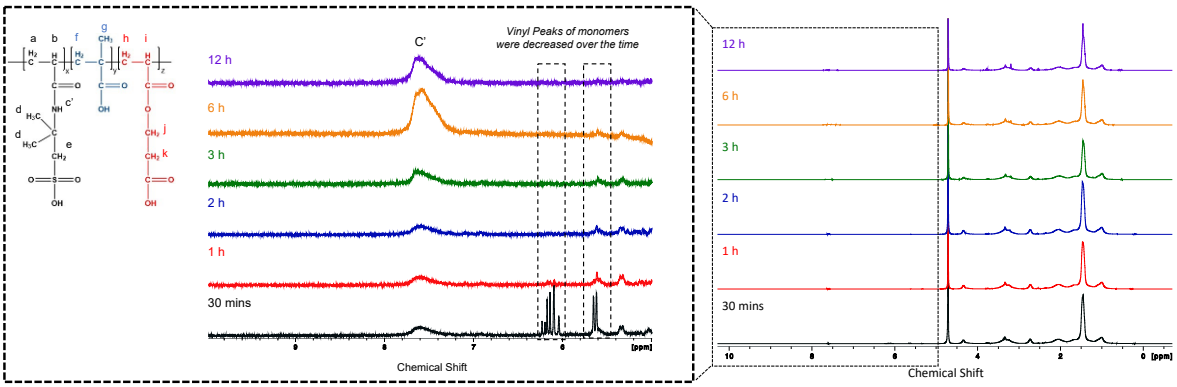

Figure S4. ^{1}H NMR of PACM varied polymerisation time from 30 mins to 12 hours

Investigation of the effect of polymerization time on the synthesis of PACM was studied to observe progress of the reaction over time from 30 mins to 12 h. ^{1}H NMR results indicated the successful reaction of AMPs monomer with complete vinyl peaks disappearance after polymerisation for 6 hours, note 1 hr shows only a small amount of vinyl peaks remaining. These peaks correspond to certain vinyl group of unreacted AMPs monomer, and their disappearance presents successful polymerization.

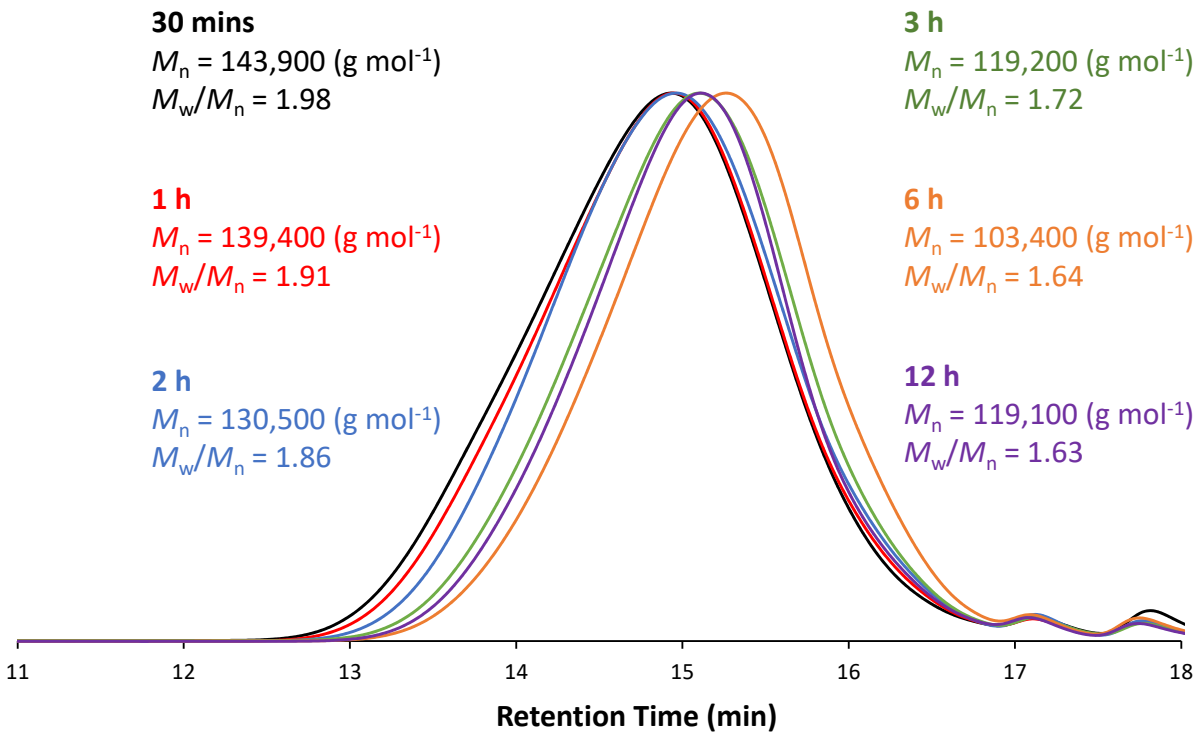

Figure S5. GPC traces of kinetic study varied polymerization of time PACM from 30 mins to 12 hours

Change in molecular weights of copolymer have been identified by GPC. Results of measurement show increasing in molecular weight has been observed with an increase of reaction time. Based on the observed disappearance of these peaks after 6 hours of polymerization, this condition (polymerisation for 6 hrs) as the basis for further experiments.

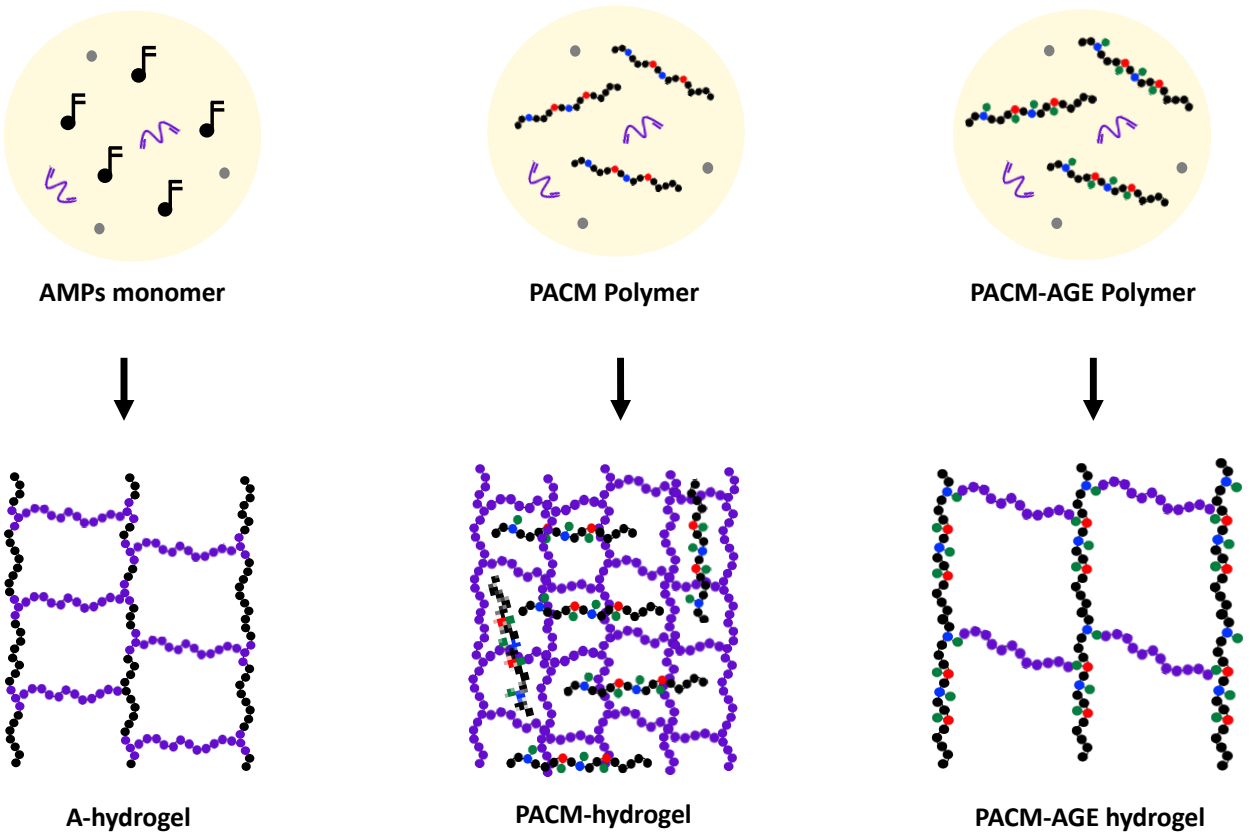

Figure S6 Schematic of the proposed different network structures of fabricated gels from AMPs monomer (A hydrogel), copolymer (PACM Hydrogel), and macromer (PAC-AGE hydrogel)

Calculations

Crosslink density

The crosslink density (v) can be calculated from the Young's modulus using the theory of rubber elasticity. The equation used is:

$$v = E' / 3RT$$

where:

- E' is the elastic modulus obtained from the mechanical testing.
- R is the gas constant (8.314 J/(mol·K)).
- T is the absolute temperature in Kelvin during the test.
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Double Bond Concentration

The double concentration for was calculated by first calculating the number of moles in each hydrogel sample (0.1g PEGDA, 0.04g AGE), then multiplying by avagrados number to give the number of molecules. Then this number was multipled by the number double bonds in each molecule (PEGDA x2 and AGE x1).

$$n = \frac{m}{M}$$

Where:

- n is the number of moles.
- m is the mass of the substance in grams.
- M is the molar mass of the substance, expressed in grams per mole (g/mol).

The equation to calculate the number of molecules (N) is:

$$N = n \times N_A$$

Where:

- N is the number of molecules.
- n is the number of moles.
- N_A is Avogadro's constant (6.022×10^{23} molecules/mol).

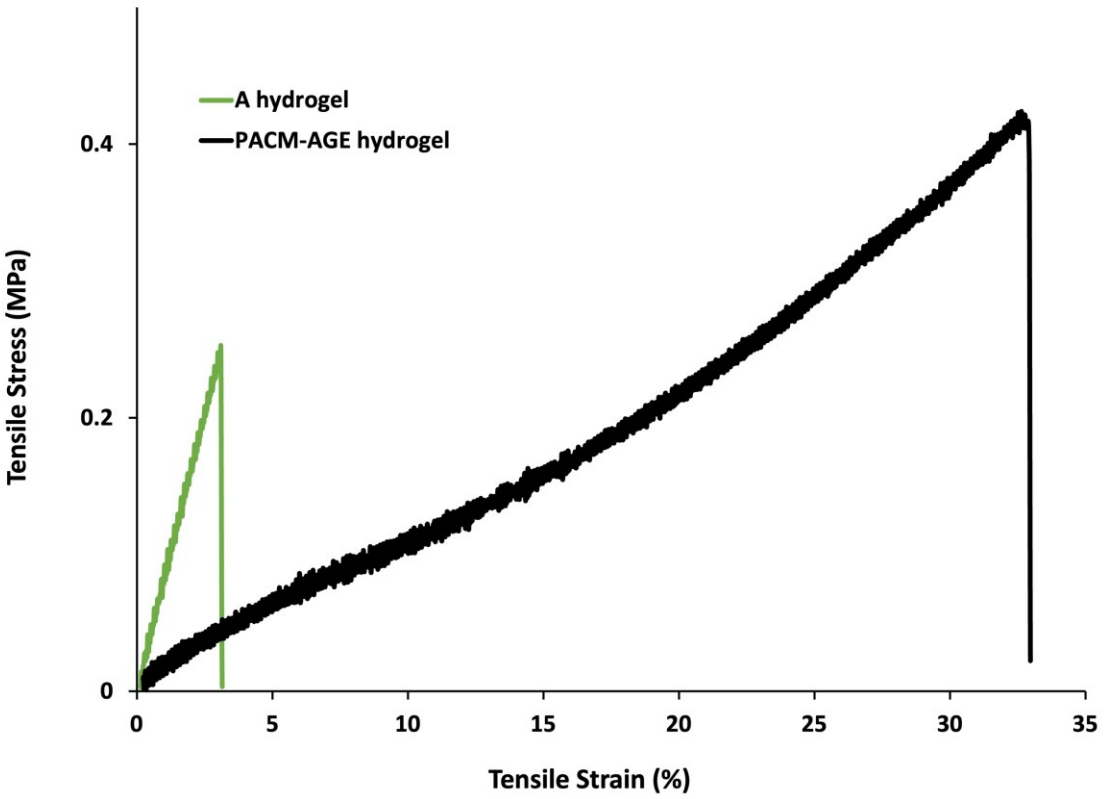


Figure S7. Tensile stress-strain curves of hydrogels; AMPs hydrogel (A) prepared from AMPs monomer (green) and PACM-AGE hydrogel prepared from AMPs-based macromer (black).

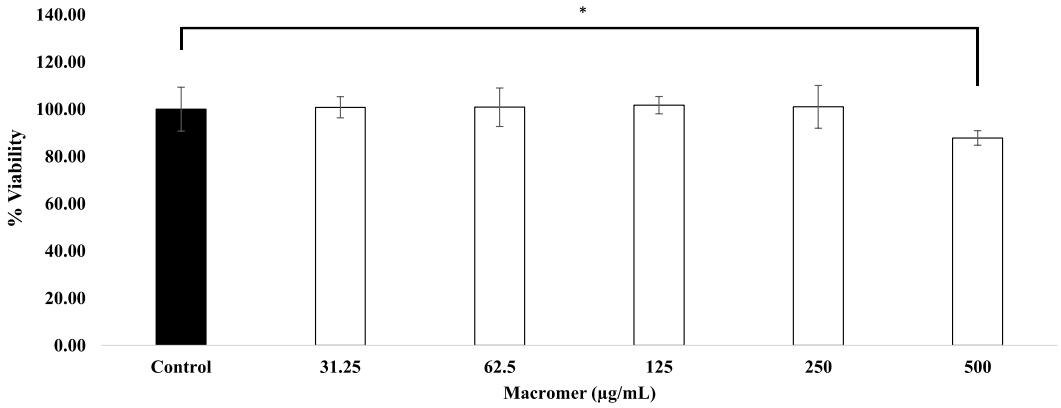
Preliminary toxicity study

The AMPs-based macromer was preliminary tested cytotoxicity. A summary of the details of cytotoxicity follows:

- Normal human dermal fibroblast (passage 11) (Lot no. C-12302, Promocell, Eppelheim, Germany)
- Sample: AMPs based macromer (PACM-AGE)
- Method: Direct Method
- Concentration (µg/ mL): 500, 250, 125, 62.5, 31.25

Macromer

	Control	Macromer (µg/mL)				
		31.25	62.5	125	250	500
1	0.821	0.846	0.903	0.905	0.842	0.727
2	0.907	0.828	0.912	0.832	0.777	0.779
3	0.755	0.915	0.863	0.878	0.859	0.752
4	0.926	0.847	0.759	0.851	0.965	0.735
Average	0.852	0.859	0.859	0.867	0.861	0.748
% Viability	**100.00**	**100.79**	**100.82**	**101.67**	**101.00**	87.80
SD	**9.31**	**4.46**	**8.16**	**3.68**	**9.06**	3.07



***; *P* value 0.01**

Figure S8. Preliminary toxicity study, % Viability of macromer directly exposed to cells at the stated concentrations.

The cytocompatibility of the macromer was initially examined on NHDF cells. According to in vitro ISO standard. The results demonstrated that macromer provided the non-cytotoxicity on cell viability and cell morphology.