Ayres Research Group

at the University of Cincinnati

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Our approach is to use **synthetic polymer chemistry** to look for new opportunities or address problems in **materials science**.

What Questions are we asking?

- How can we use inspiration from nature to design blood-compatible polymers?
- Can the stiffness of a gel control the fate of human cells?
- Can we control the speed of sound by controlling silicone emulsions?

Why is this exciting?

- Currently, all biomaterials in contact with blood cause clotting

- No good models for changes in heart infarction with time (scarring and stiffening)

- Synthesis of new, cheaper, metamaterials

Blood Compatible Polyurethanes and Polyureas

Blood Contact Activation

- The same mechanisms designed to arrest bleeding after injury can create adverse events when artificial surfaces are placed in contact with blood.
- Many examples of surface modification exist to minimize these responses.
- Some of these are based around using or mimicking heparin, our naturally occurring anticoagulant molecule.
- Heparin is a complex linear sulfated polysaccharide

Biomaterials Science, An introduction to materials in medicine eds B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemons, Elsevier Academic Press

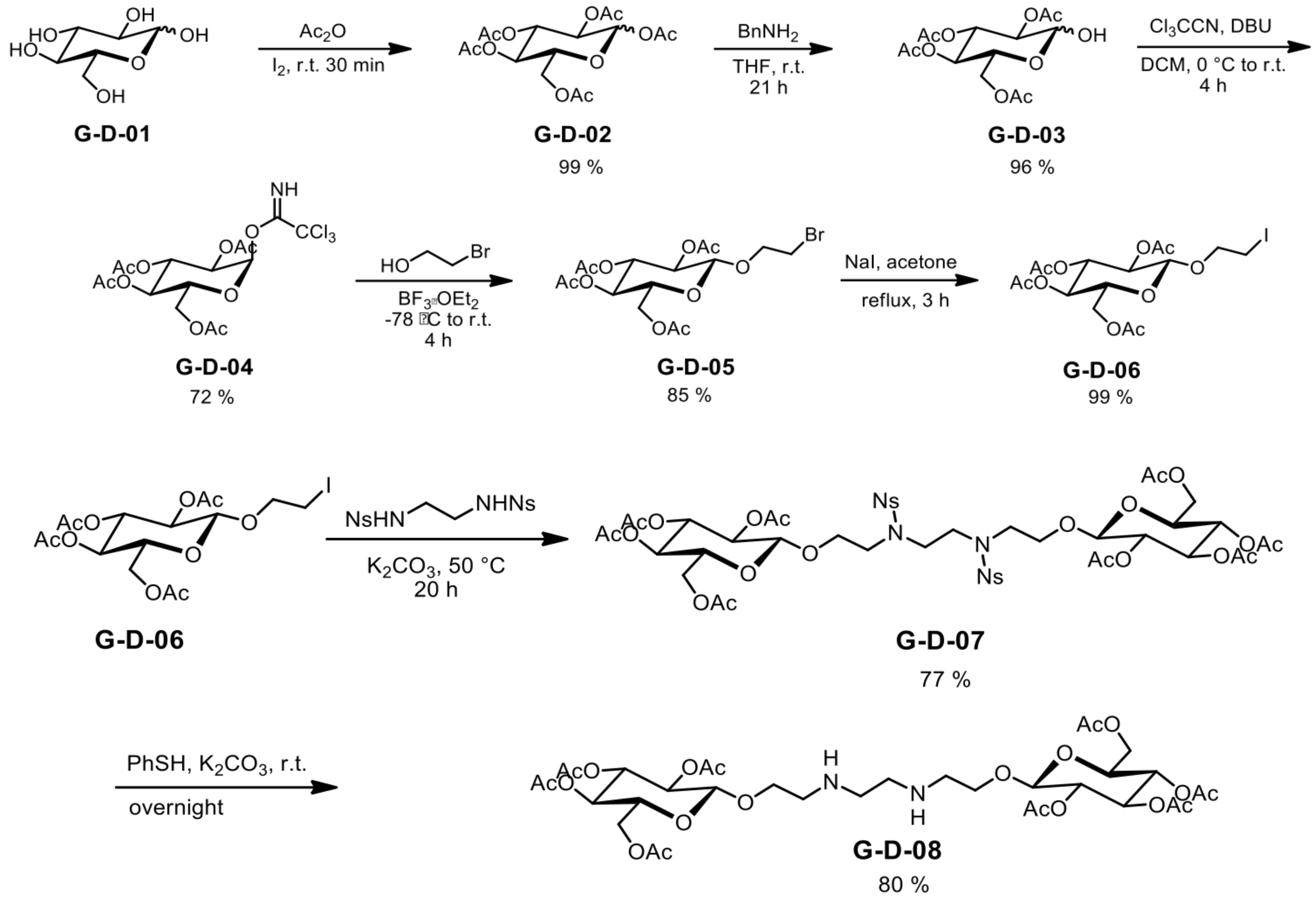
Liu, H. Y.; Zhang, Z. Q.; Linhardt, R. J., Natural Product Reports 2009, *26 (3)*, 313-321.

Image: Shutterstock

A synthetic heparin-inspired polymer?

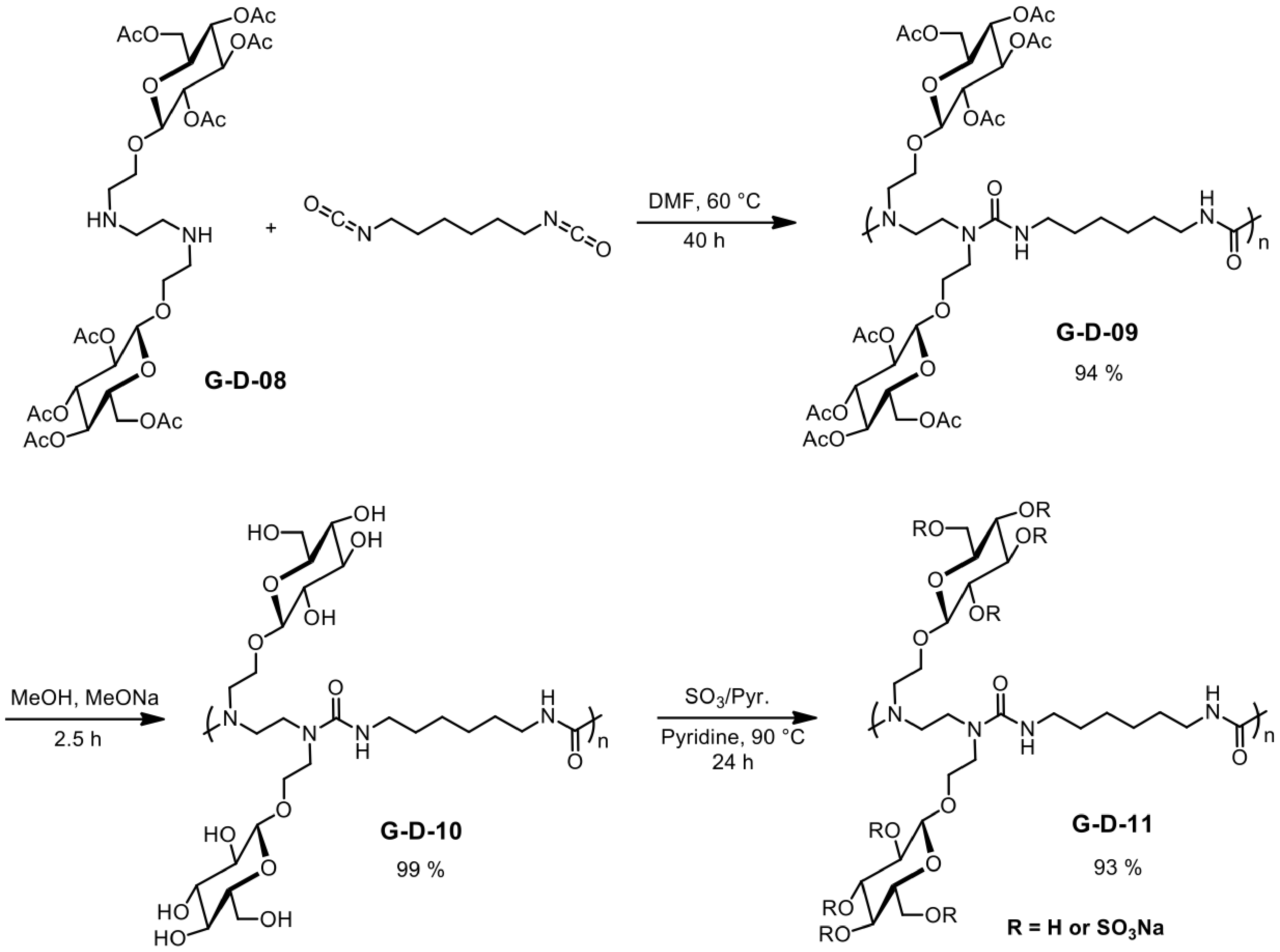
- Our goal was to make a simple polymer that would be similar to many biomaterials currently used (polyurethanes).
- This goal lead us to using step-growth polymerizations, and specifically making polyureas.
- We chose to use commercially available diisocyanates with novel diamines, where we could examine the effects of monomer chemistry on polymer blood compatibility.

Preparing a sugar-diamine

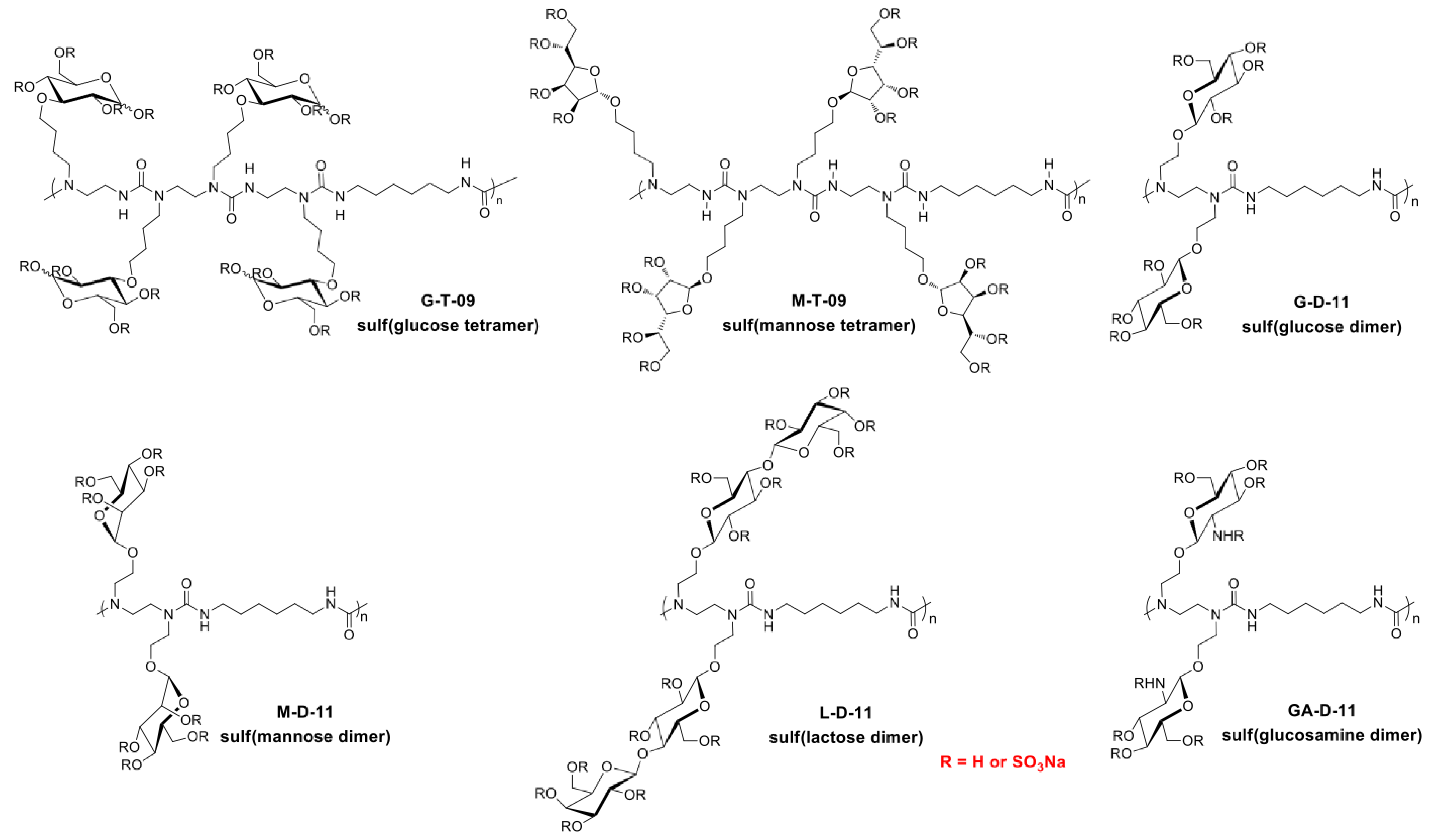


Huang Y., Shaw M.A., Mullins E.S., Kirley T.L., Ayres N. *Biomacromolecules* 2015 *15(12)* 4455-4466

Polymer synthesis and modification



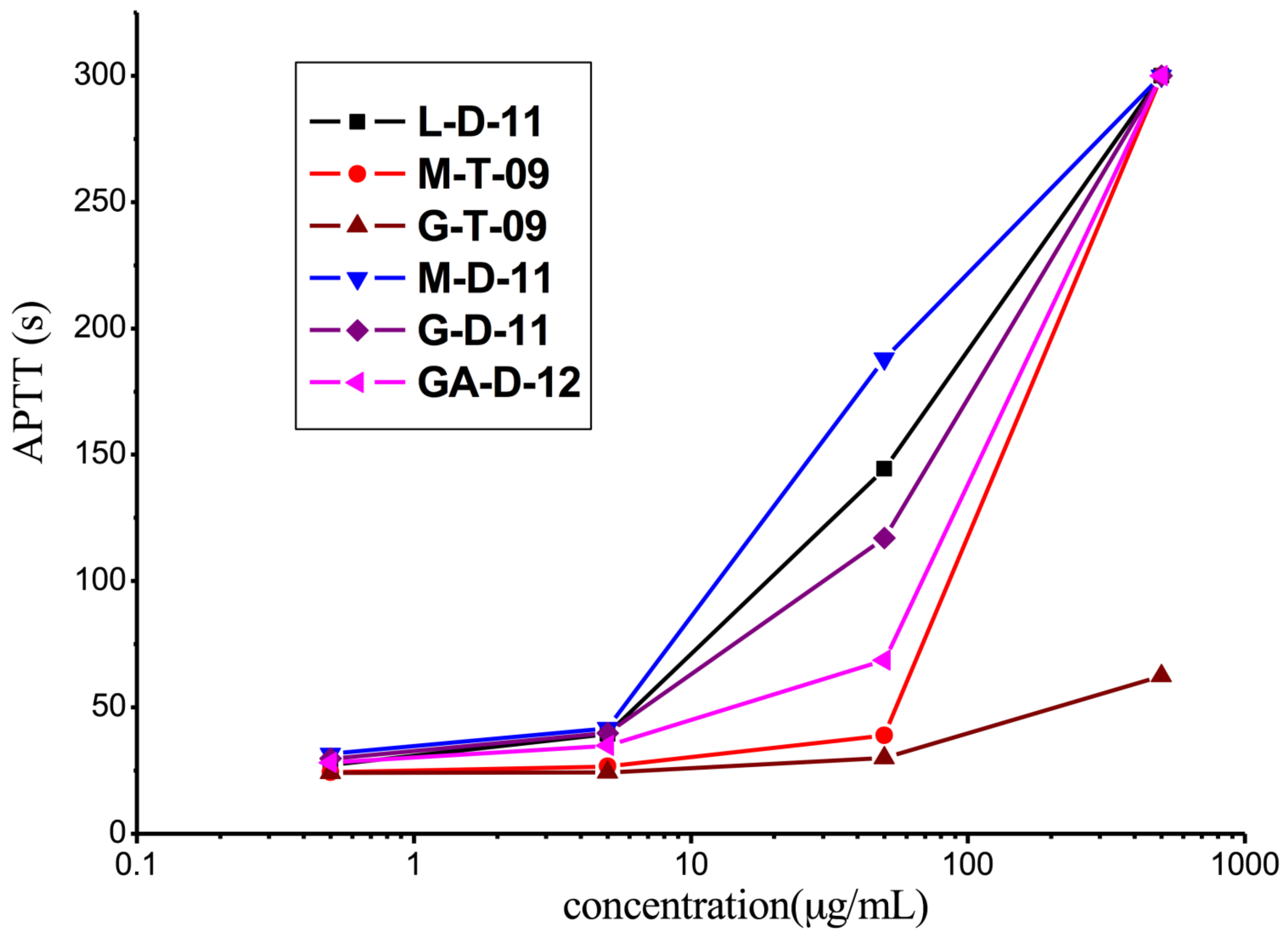
Polymer Summary



Y. Huang, L. Taylor, X. Chen, and N. Ayres *Journal of Polymer Science, Part A: Polymer Chemistry* 2013 51(24) 5230-5238
Y. Huang, M.A. Shaw, E.S. Mullins, T.L. Kirley, and N. Ayres *Biomacromolecules* 2015 *15(12)* 4455-4466

Blood Compatibility

aPTT times (s)



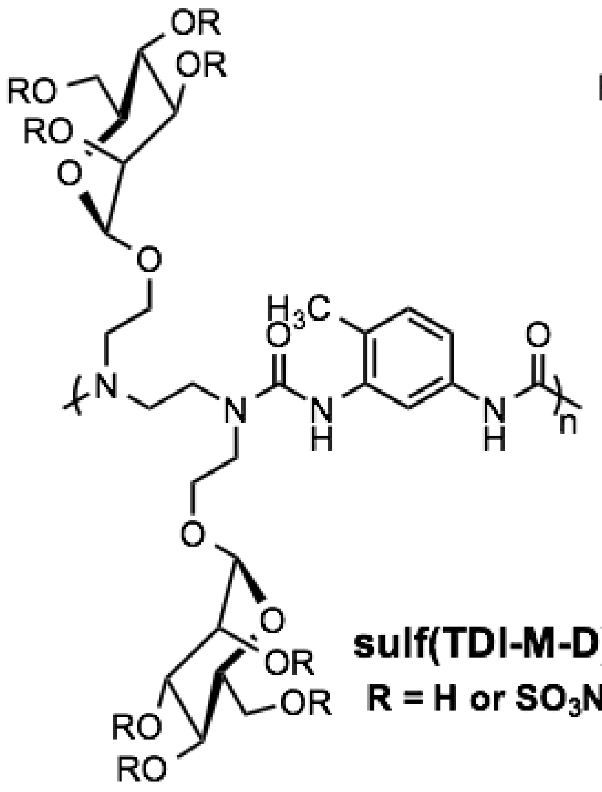
PT times (s)

polymer	polymer concentration (μg/mL)			
	0.5	5.0	50	500
G-T-09	15.0	14.0	14.0	13.5
M-T-09	14.0	13.5	13.5	15.5
G-D-11	14.0	14.0	>60	>60
M-D-11	14.0	14.0	>60	>60
L-D-11	14.5	13.0	>60	>60
GA-D-12	14.0	14.0	14.0	>60

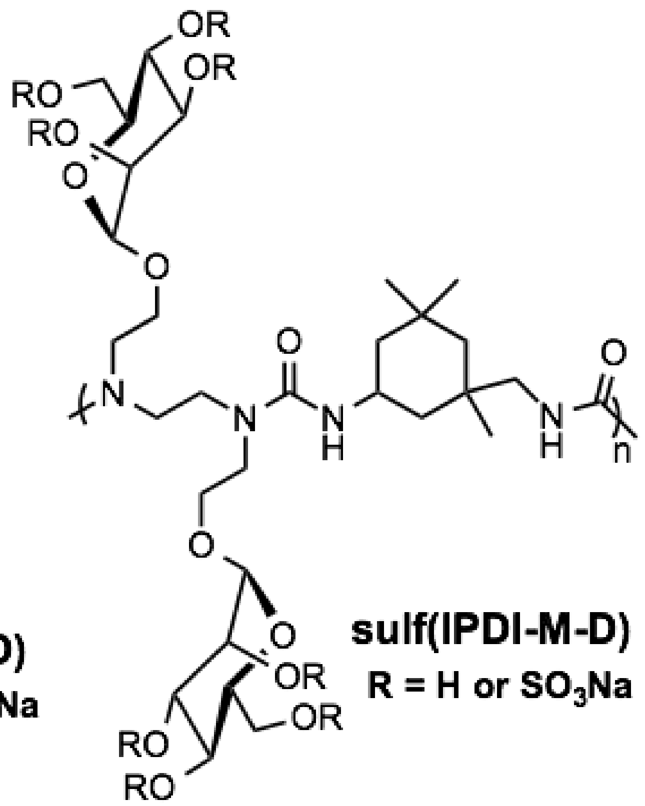
TT times (s)

polymer	polymer concentration (μg/mL)			
	0.5	5.0	50	500
G-T-09	23.0	23.5	>75	>75
M-T-09	23.0	20.5	>75	>75
G-D-11	24.0	27.5	>75	>75
M-D-11	22.0	>75	>75	>75
L-D-11	25.0	>75	>75	>75
GA-D-12	23.0	24.5	>75	>75

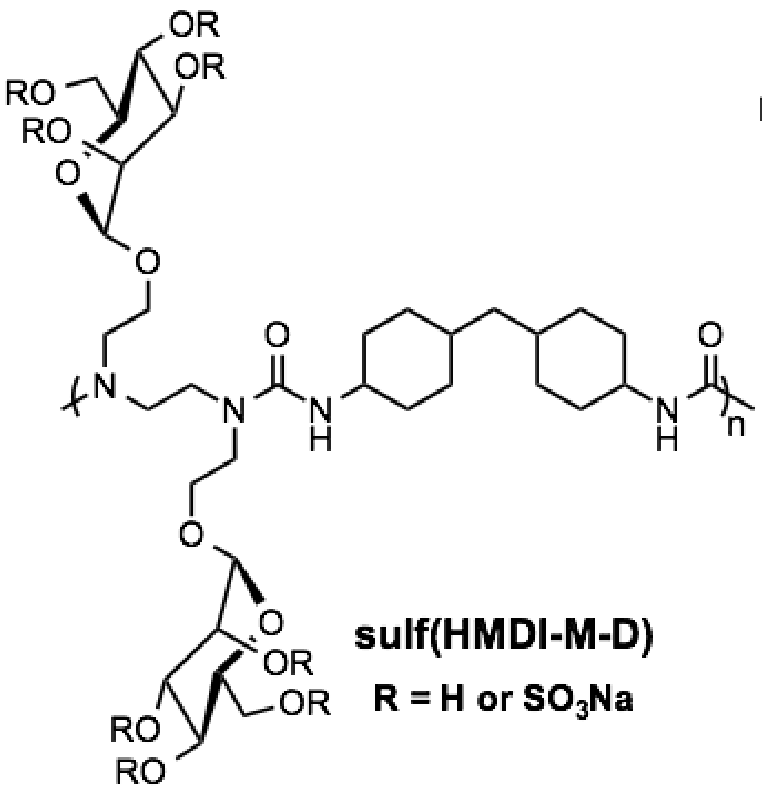
Varying the isocyanate comonomer



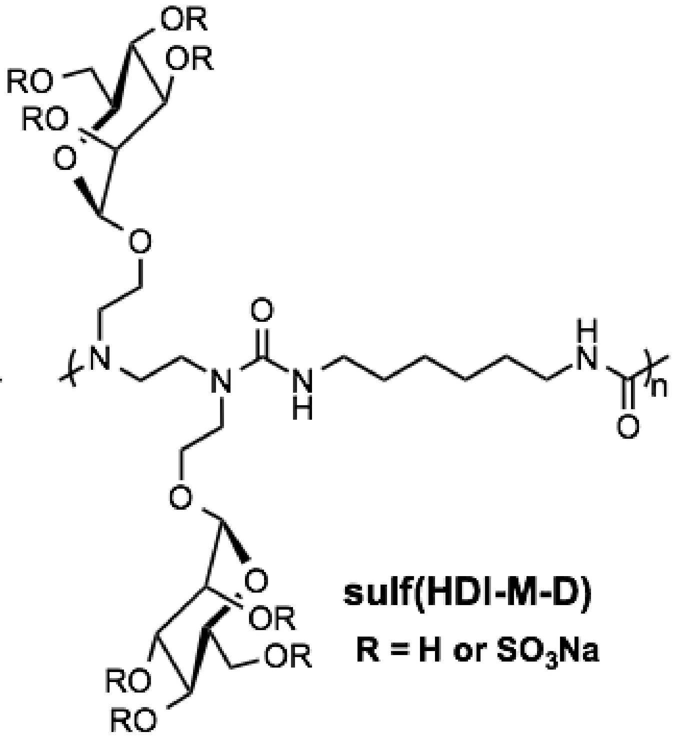
sulf(TDI-M-D)
R = H or SO_3Na



sulf(IPDI-M-D)
R = H or SO_3Na

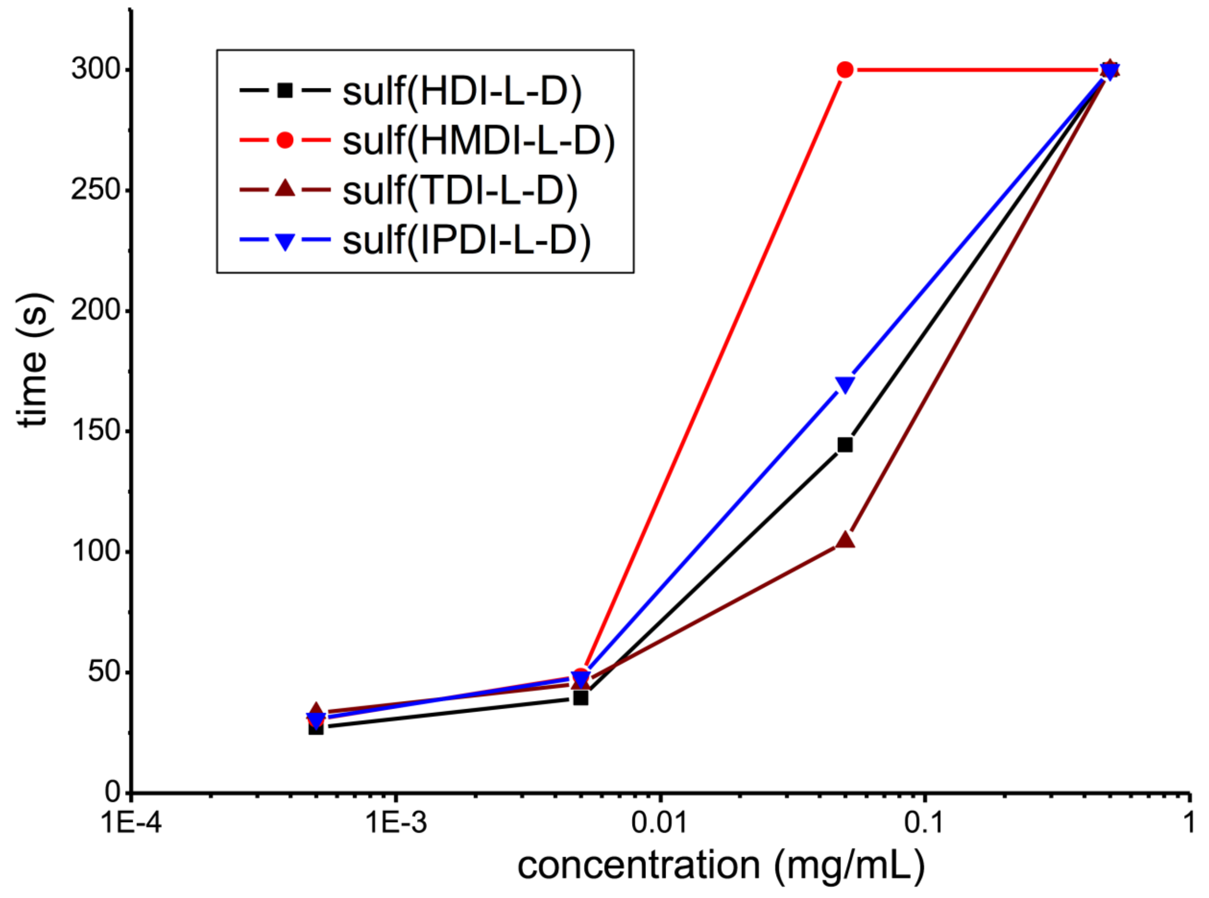


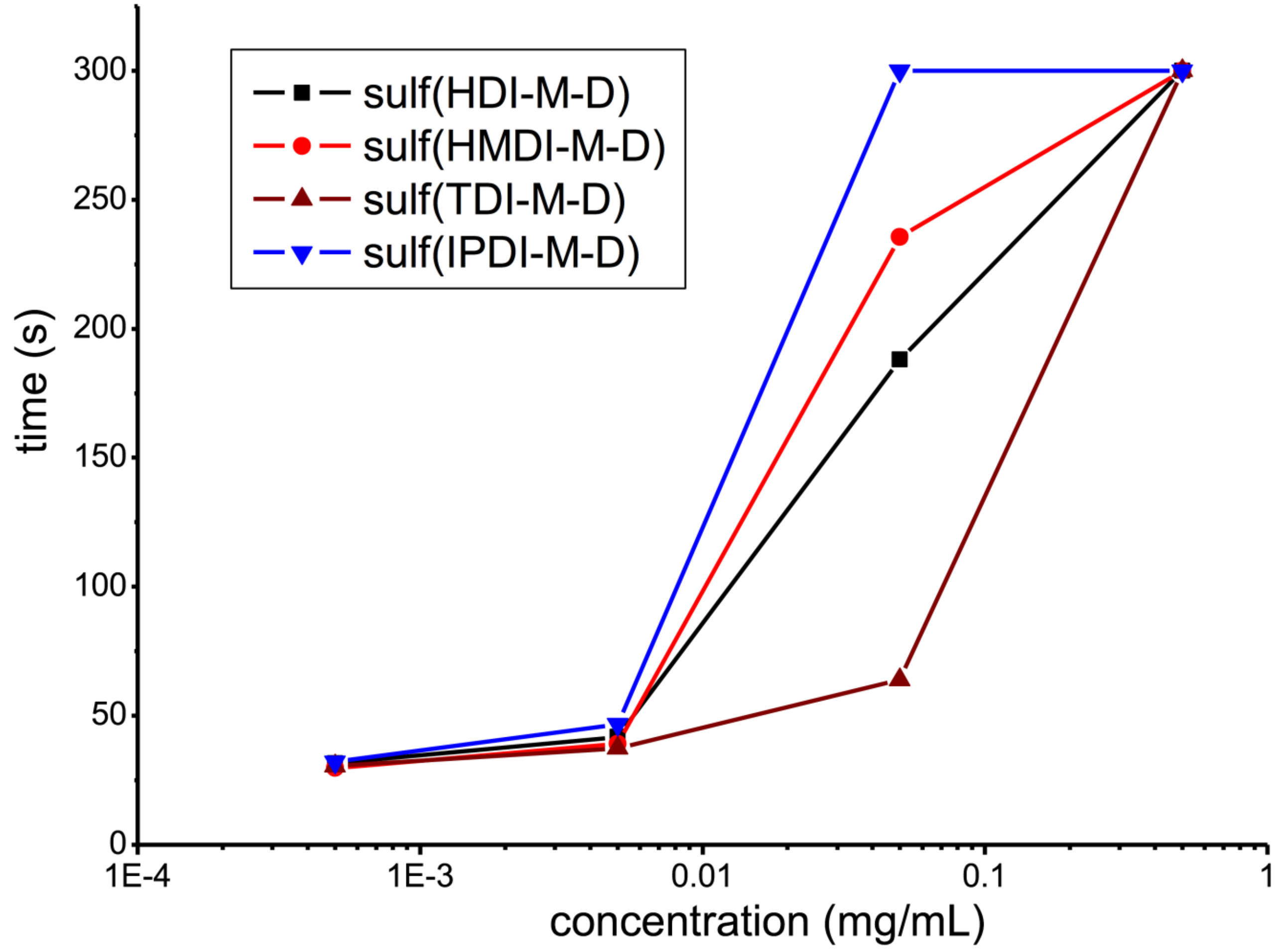
sulf(HMDI-M-D)
R = H or SO_3Na



sulf(HDI-M-D)
R = H or SO_3Na

Blood Compatibility



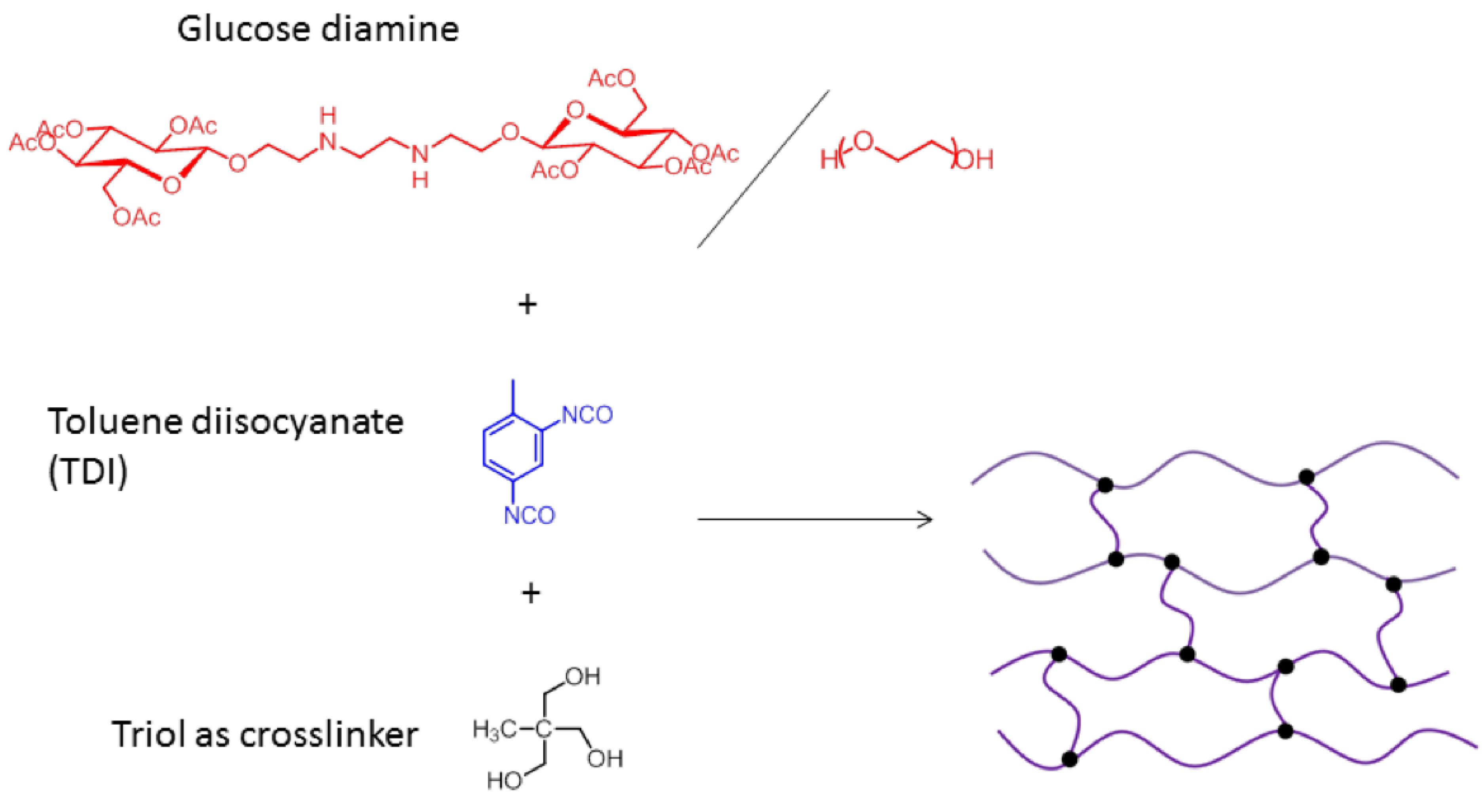


- Take-away: The isocyanate comonomer is important too!

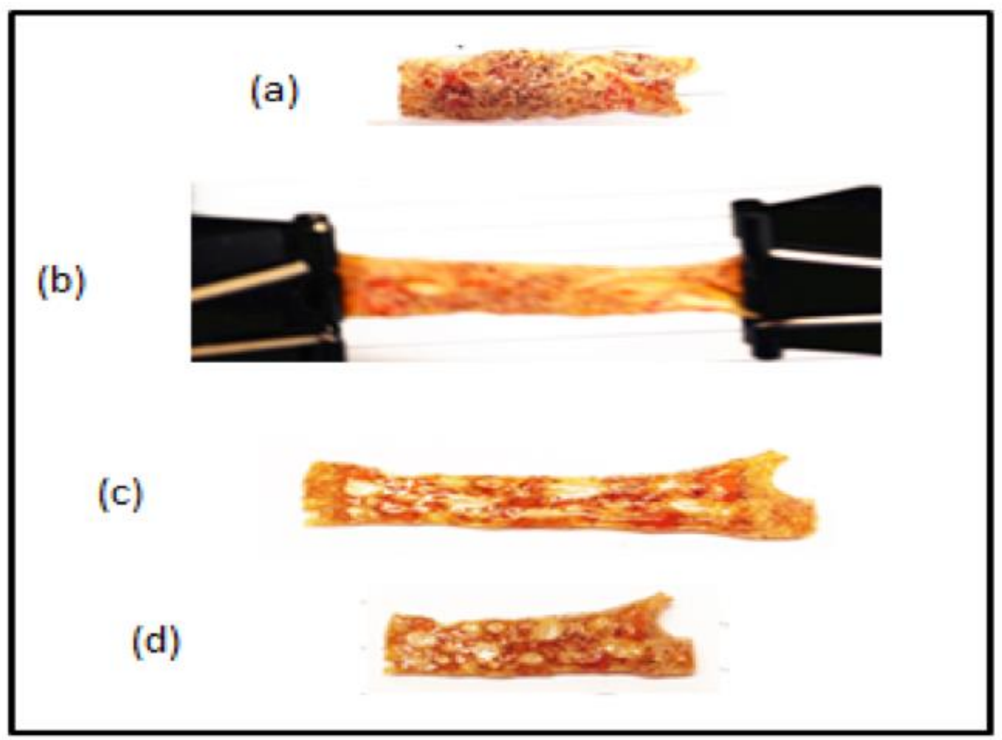
Huang Y., Shaw M.A., Warmin, M.R., Mullins E.S., Ayres N. *Polymer Chemistry,* 2016, *7*, 3897-3905

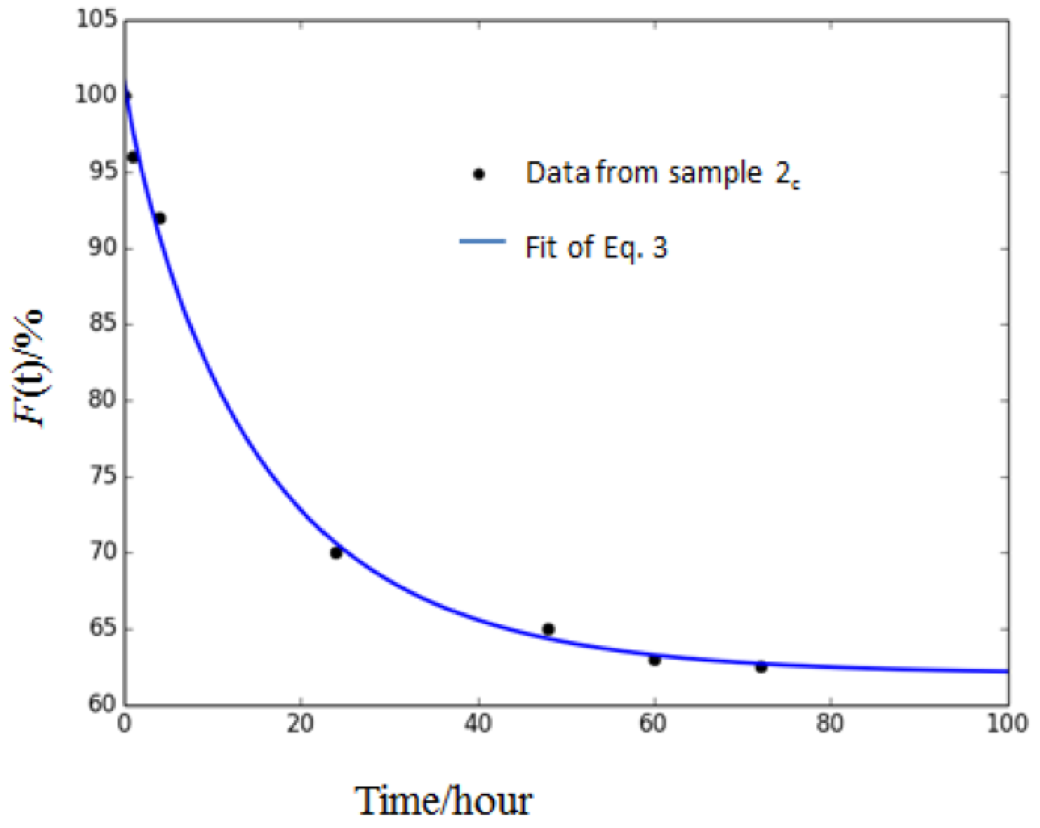
Cross-linking the polymers to make materials

- So far we have focused on the polymer synthesis and characterization.
- We are also a materials group, so we prepared films of one of the polymers.
- We used various ratios of PEG:Diamine to tune the T_g of the films.



Shape Memory behavior



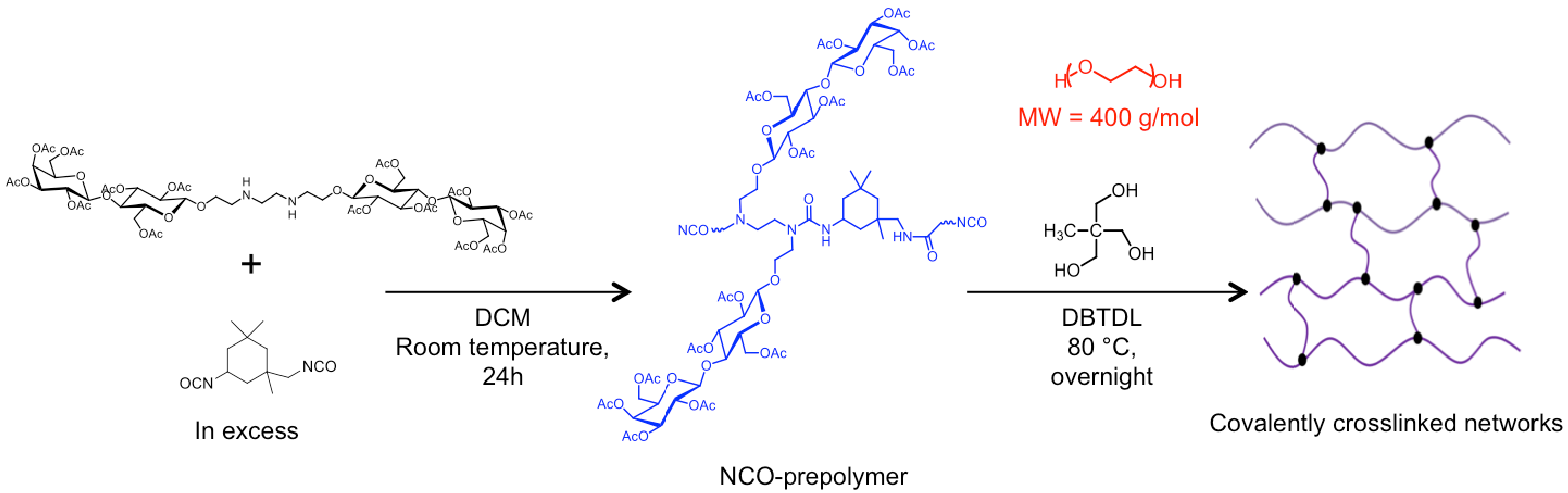


Sample	***F*(t)/%**		***R*/%**		
	1h	4h	1st cycle	2nd cycle	3rd cycle
1c	80	74	91	94	100
2c	96	92	88	90	96

Q. Chai, Y. Huang, and N. Ayres *Journal of Polymer Science, Part A: Polymer Chemistry* 2015 53(19) 2252-2257

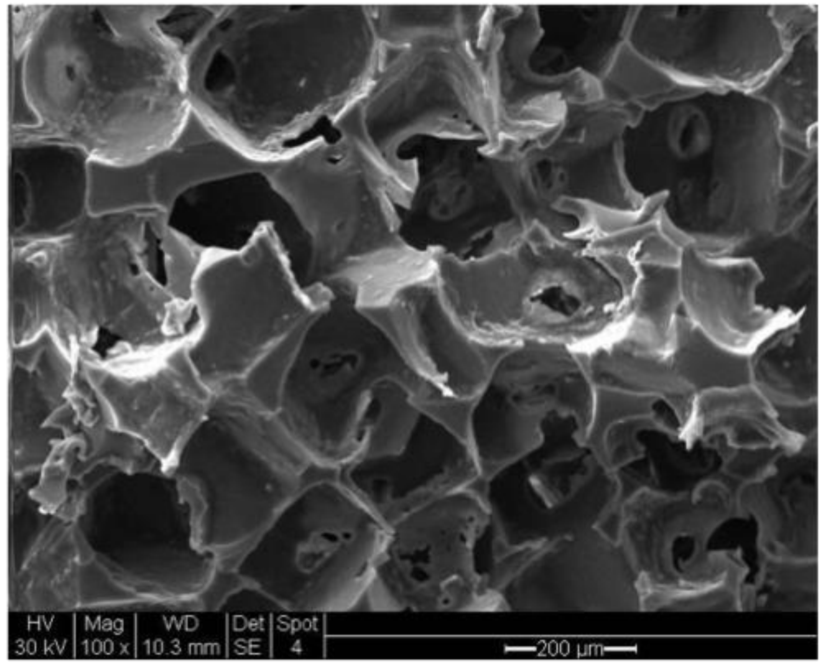
Moving from foams to films

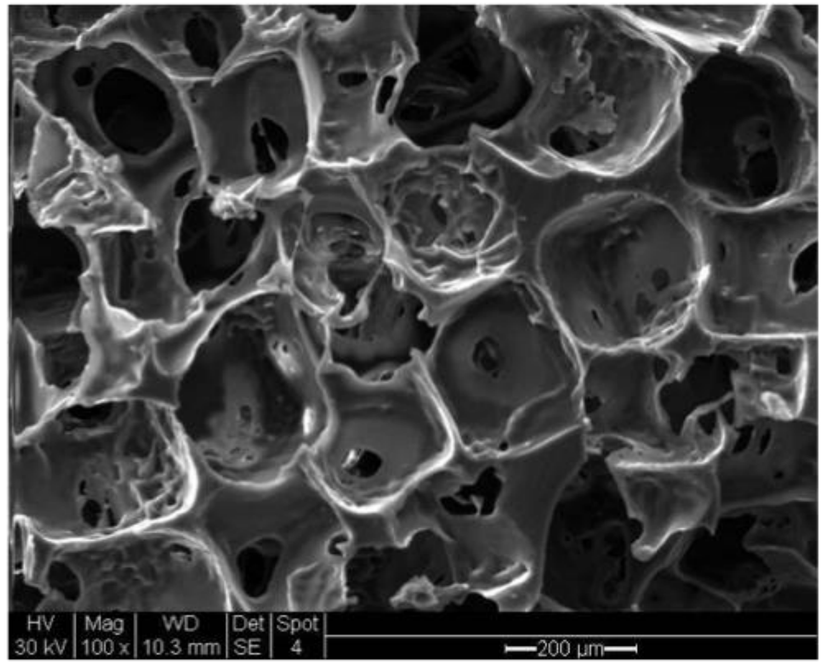
- Having prepared films of our material we moved into porous foams.
- Foams are used in several biomaterials applications, including embolizations.
- We used the best performing sugar/isocyanate combination in our synthesis.

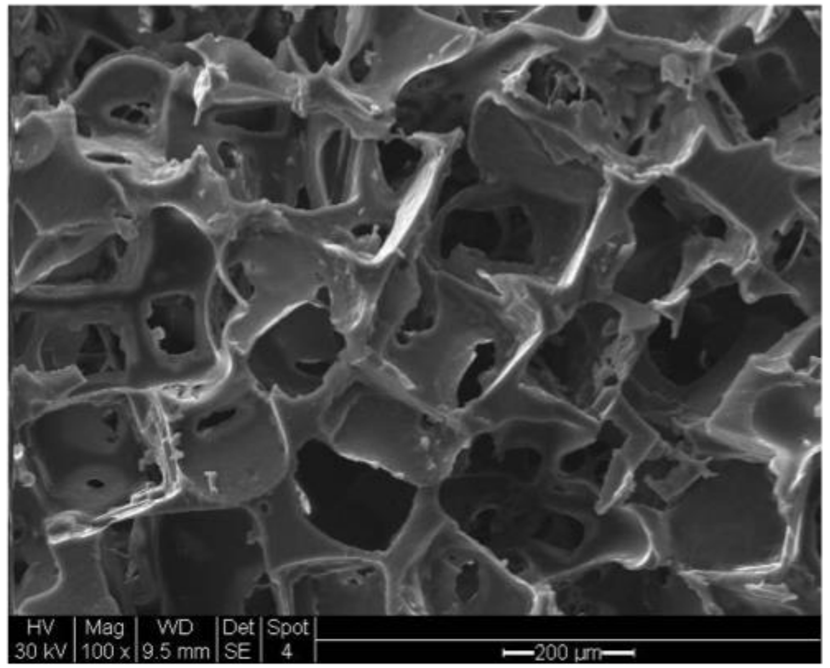


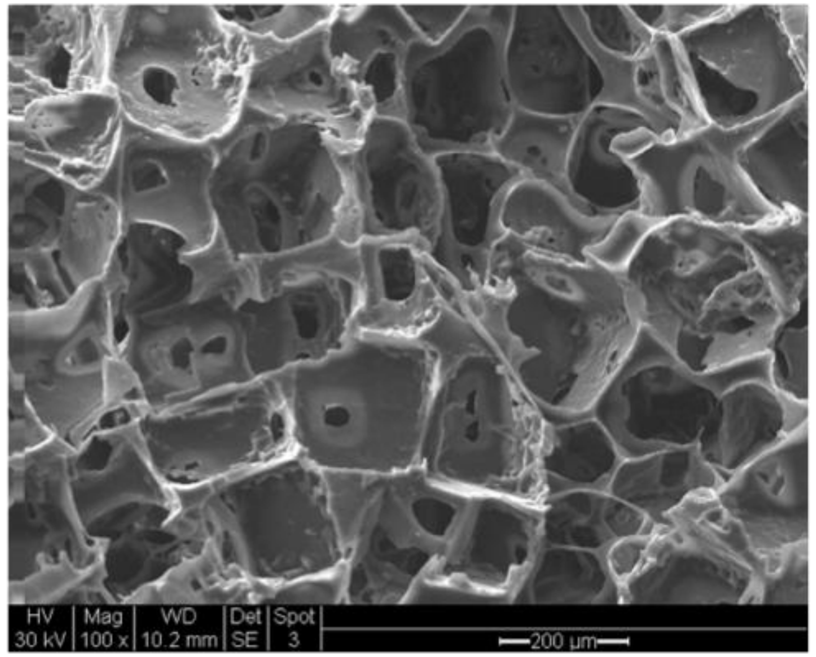
Q. Chai, Y. Huang, T. Kirley and N. Ayres *Polymer Chemistry* 2017 8 5039 - 5048

Control over the pore size using the template approach



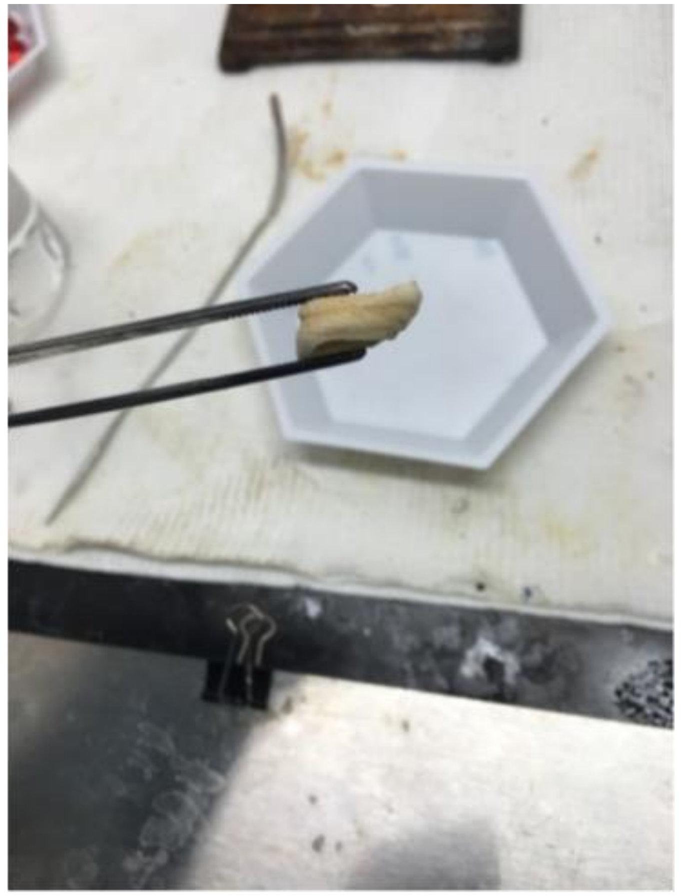




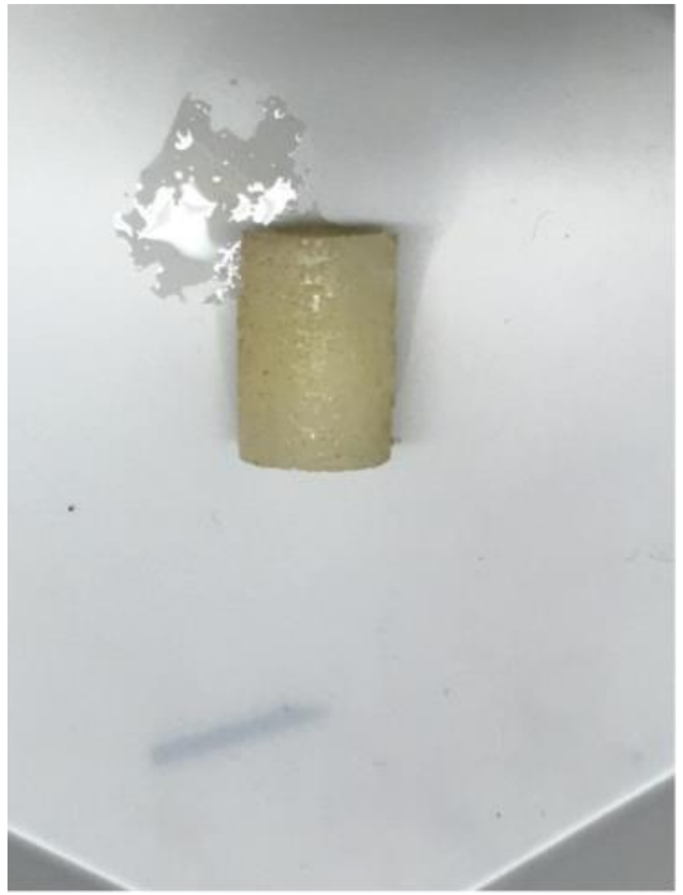


Shape memory properties of the foams

Permanent Shape



Fixed Shape



Recovered Shape

Hydrogel coated foams

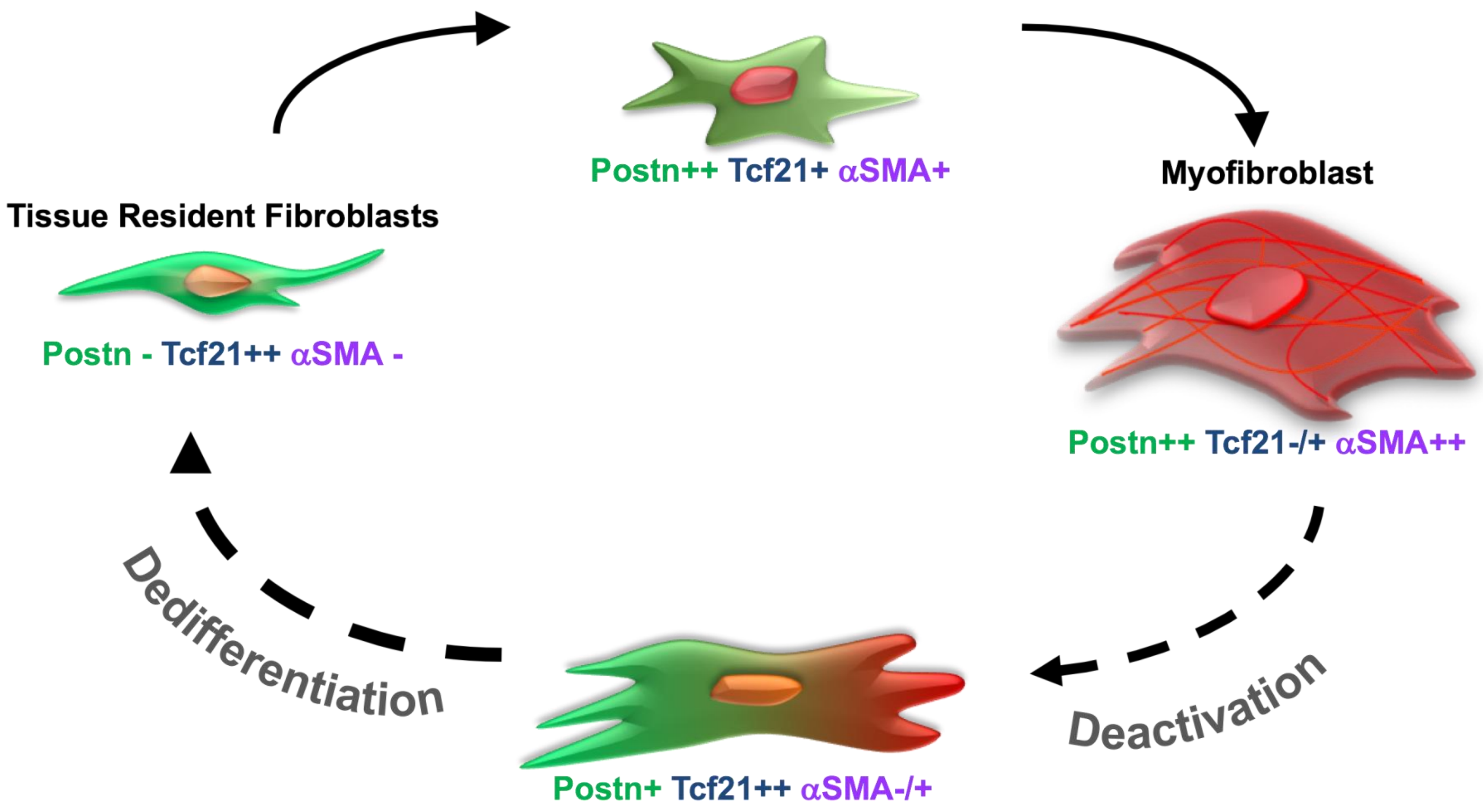
- We are becoming interested in coating the surface of the materials with hydrogels.
- This can either be to present a better surface for cell attachment and proliferation, or “pre-clotting” of small diameter vascular grafts.

E. Dalton, Q. Chai, M. Shaw, T. McKenzie, E. Mullins, and N. Ayres *Journal of Polymer Science, Part A: Polymer Chemistry* (2019) 57 1389-1395

Hydrogels with Dynamic Changes in Moduli

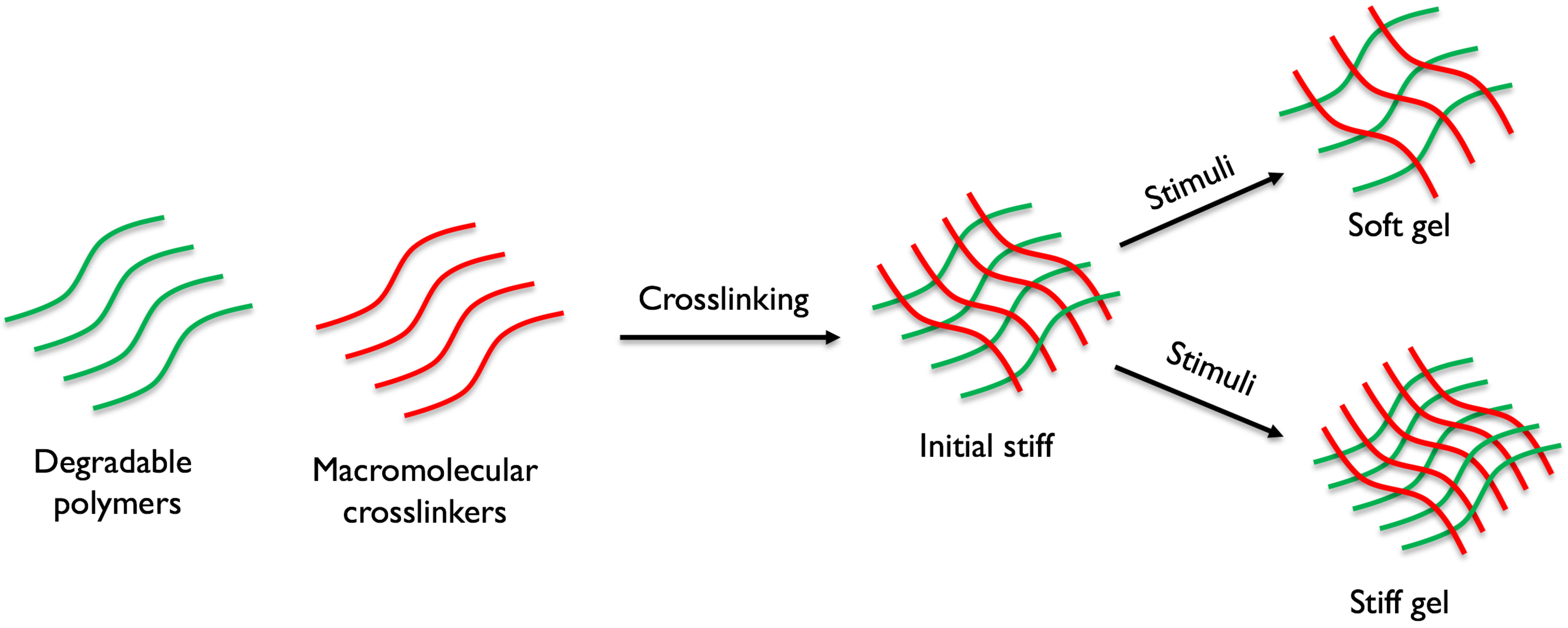
Fibroblast activation post-myocardial infarction

- Around 6 million Americans suffer from heart failure, resulting in a 50% 5-year mortality rate and health care cost of >$34 billion.
- Myocardial Infarction is the underlying cause in 70% of heart failure cases.
- Fibrosis is required Post-MI in the infarct zone to replace dead cardiomyocytes, however, excessive fibrosis leads to stiffening of the heart wall and impairing cardiac physiology.

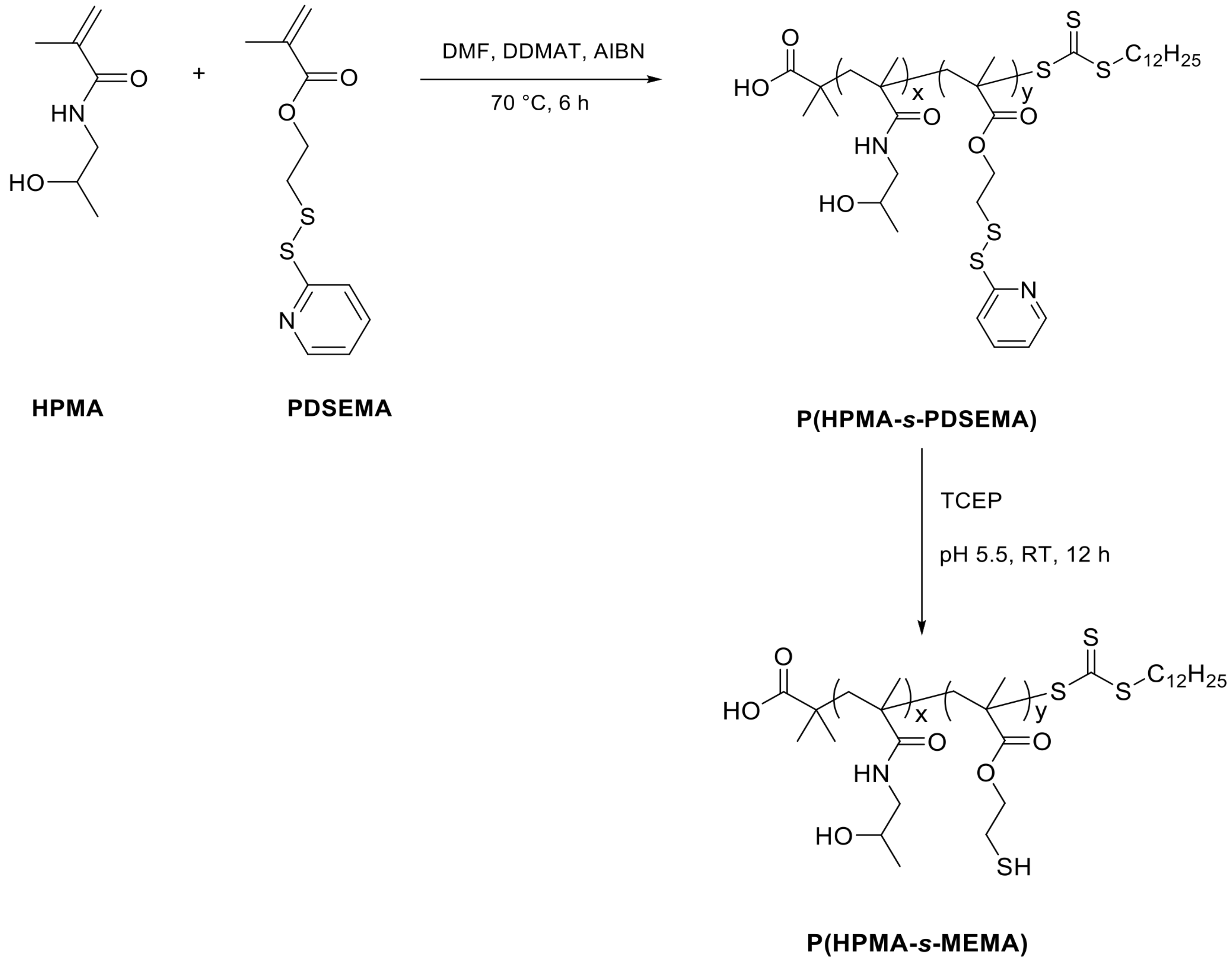


Ma, Y.; Lindsey, M.L.+ *Trends in Pharmacological Sciences* 2017 38 448-458

Our approach – combine natural and synthetic polymers



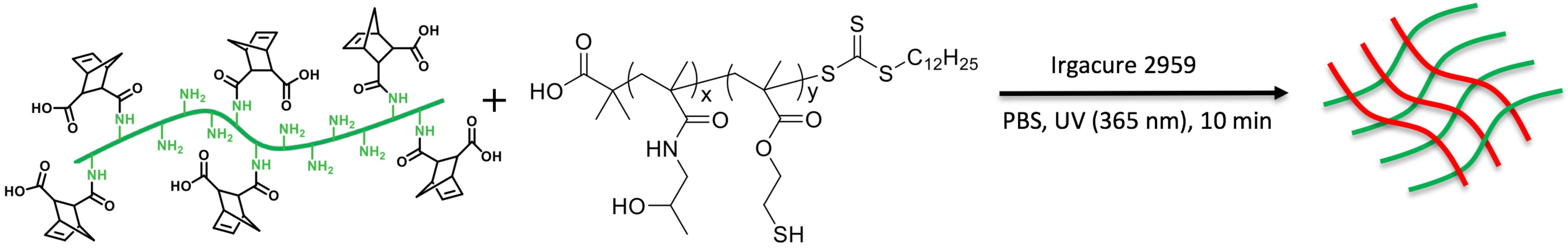
The cross linker is a ‘controlled’ polythiol from RAFT polymerization



Polymer	M_n (g/mol)	Đ
Poly(HPMA$_{77}$-*s*-PDSEMA$_5$)	12,500	1.25
Poly(HPMA$_{57}$-*s*-PDSEMA$_{15}$)	11,900	1.12

Polymer	[Thiol] mM	[Thiol] mmol/g of polymer
Poly(HPMA$_{77}$-*s*-MEMA$_5$)	0.43	0.37
Poly(HPMA$_{57}$-*s*-MEMA$_{15}$)	1.31	1.23

Hydrogel synthesis



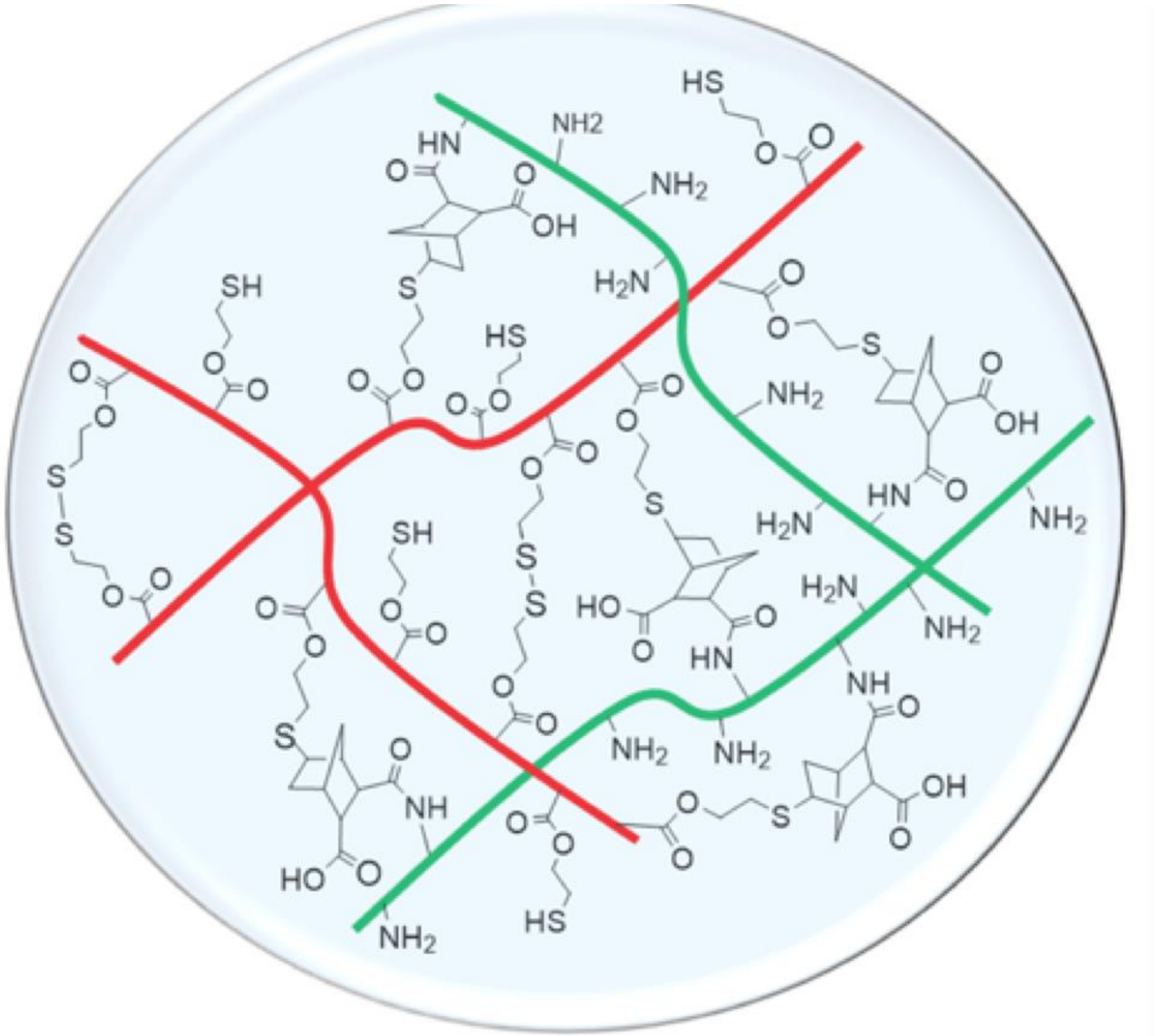
Hydrogel

Poly(HPMA$_{77}$-*s*-MEMA$_5$)

Thiol : Ene	Swelling ratio	Storage modulus (G′)
1:1	1200%	9.8 kPa
2:1	900%	12.0 kPa
3:1	880%	12.8 kPa

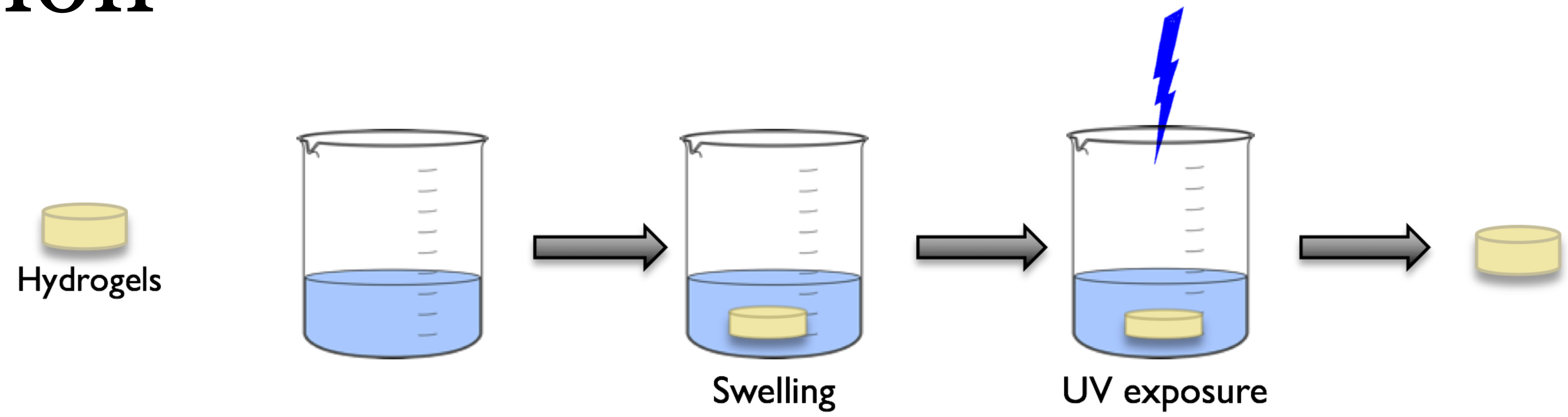
Poly(HPMA$_{57}$-*s*-MEMA$_{15}$)

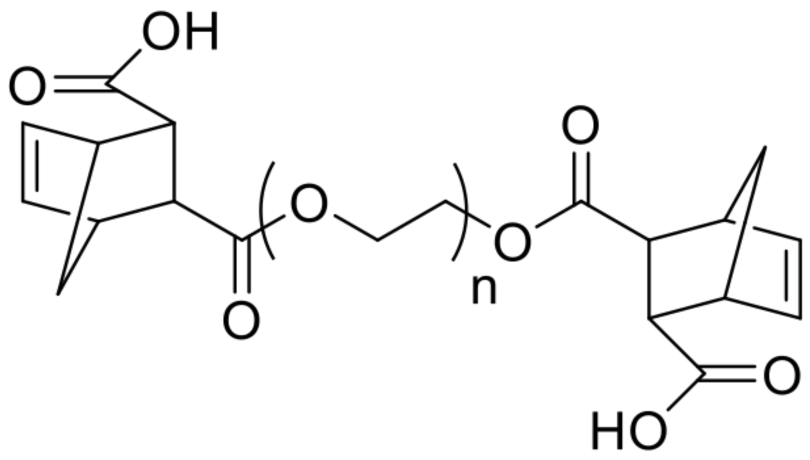
Thiol : Ene	Swelling ratio	Storage modulus (G′)
3:1	840%	13.2 kPa
6:1	650%	15.3 kPa
9:1	590%	17.8 kPa



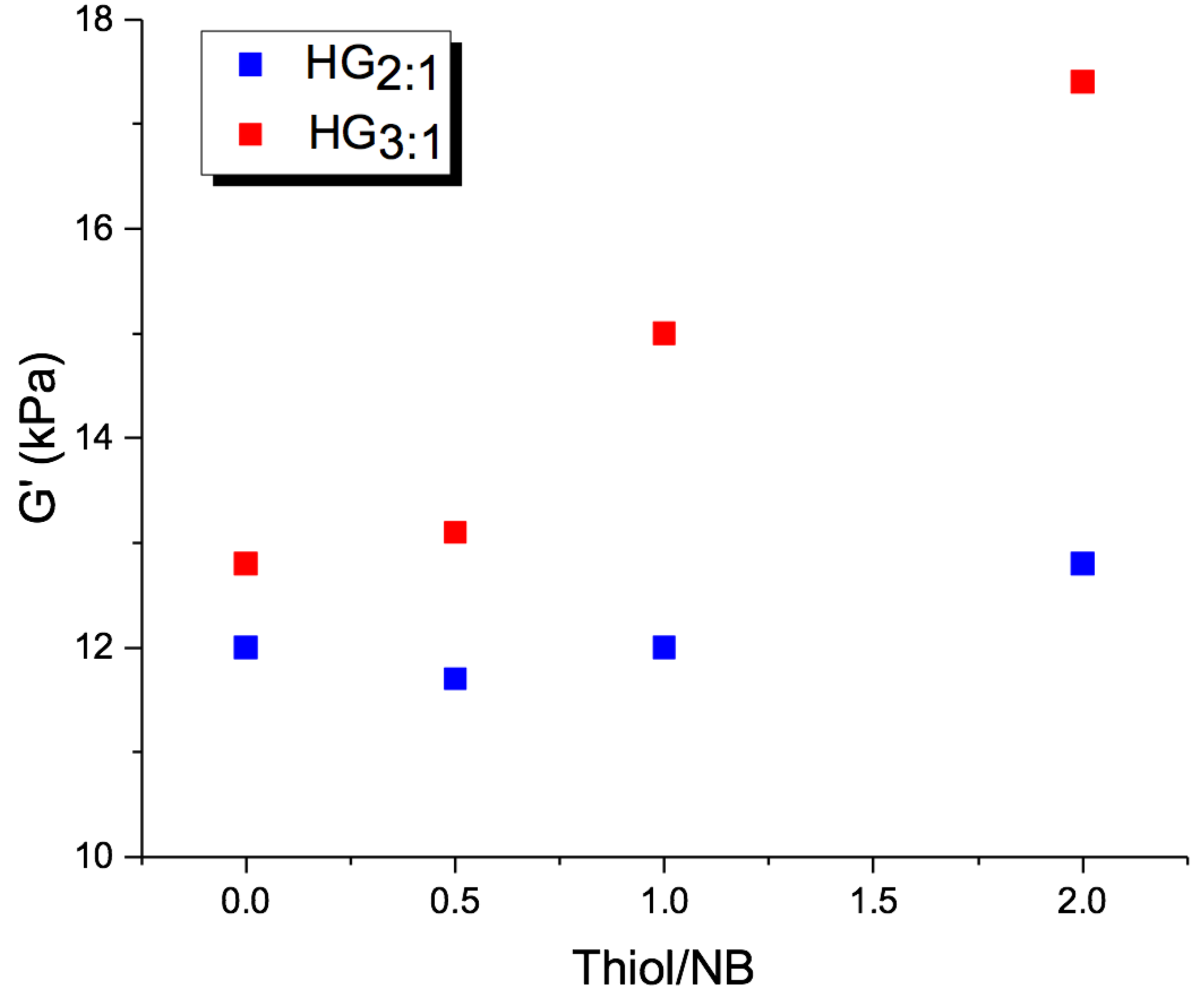
M. Perera and N. Ayres *Polym Chem* **2017** 8 6741-6749

The gels can be stiffened with a secondary cross-linking reaction

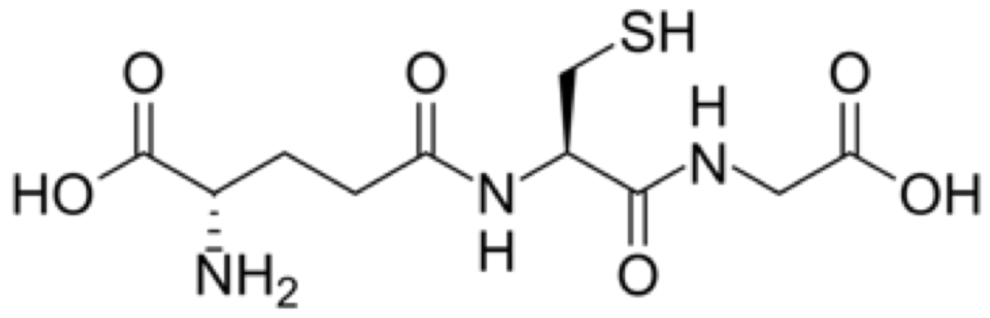


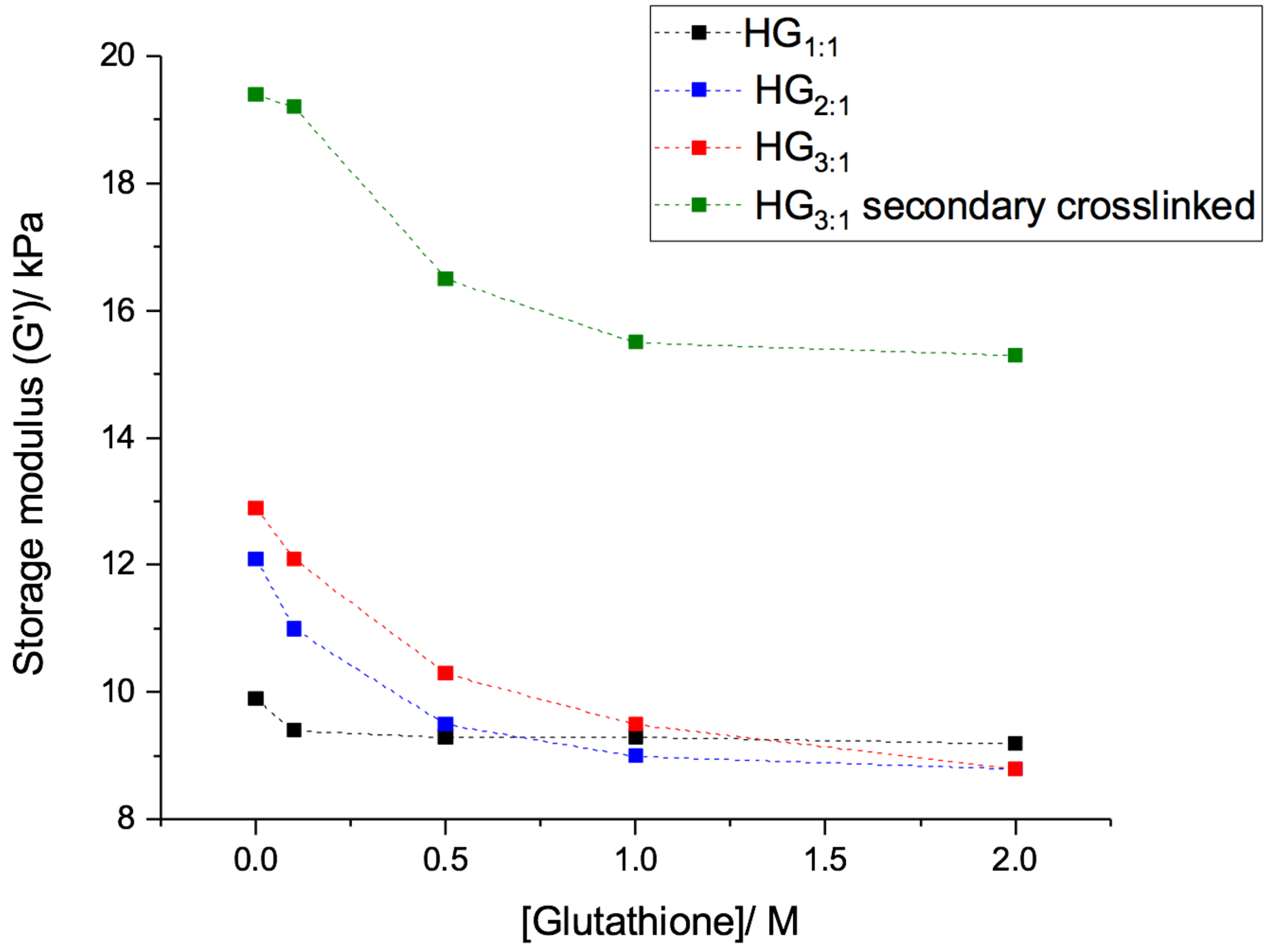


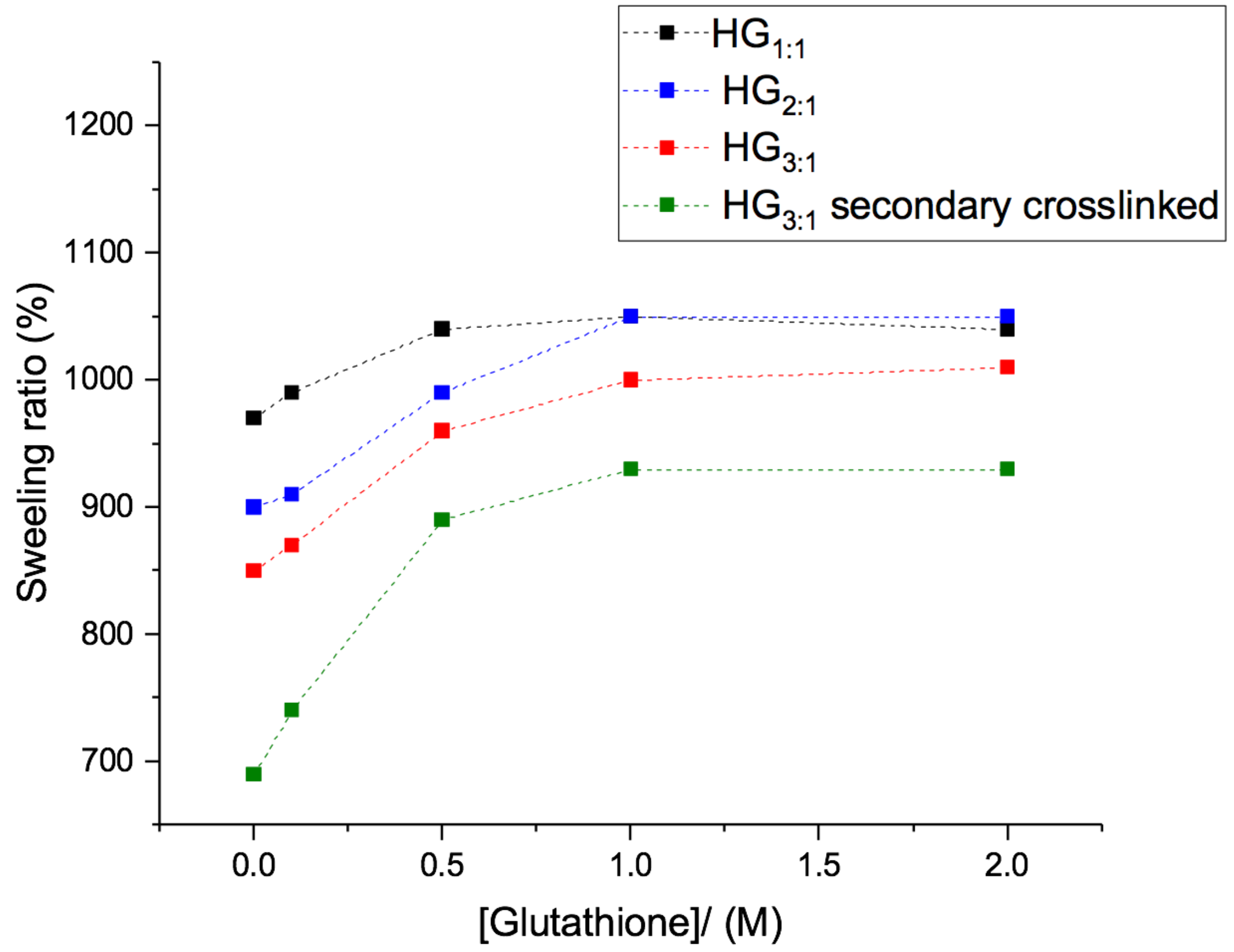
PEG-diNB- 8000 g/mol



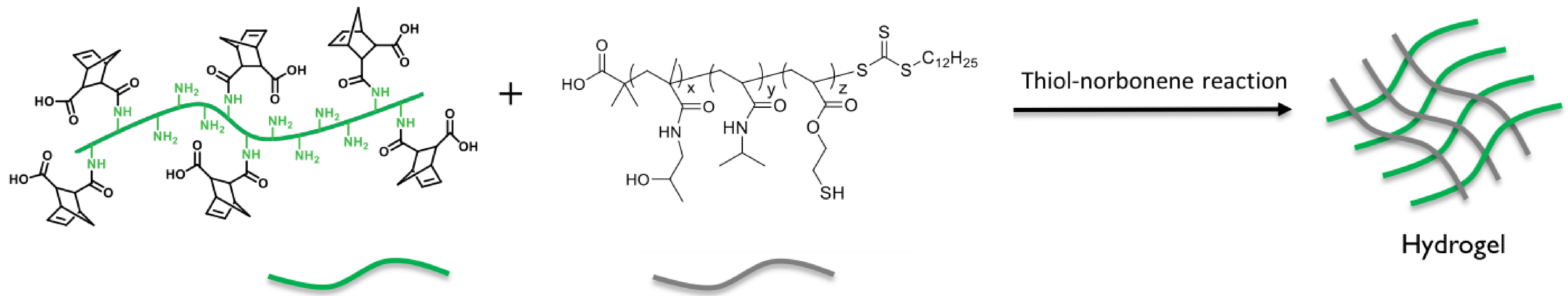
The gels can be softened by thiol exchange reactions with a small molecule

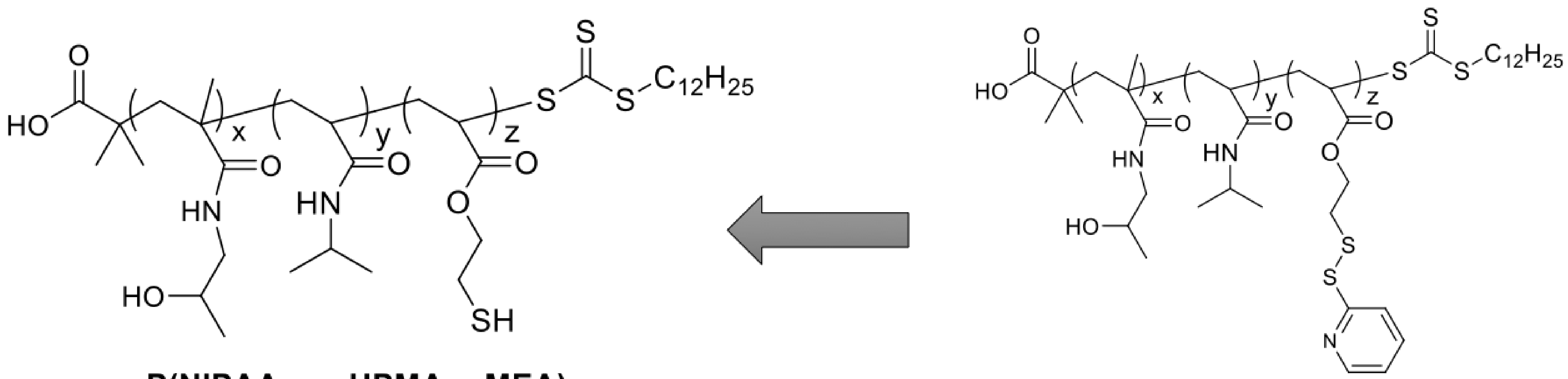






Adding the thermoresponsive NIPAAm to the crosslinker





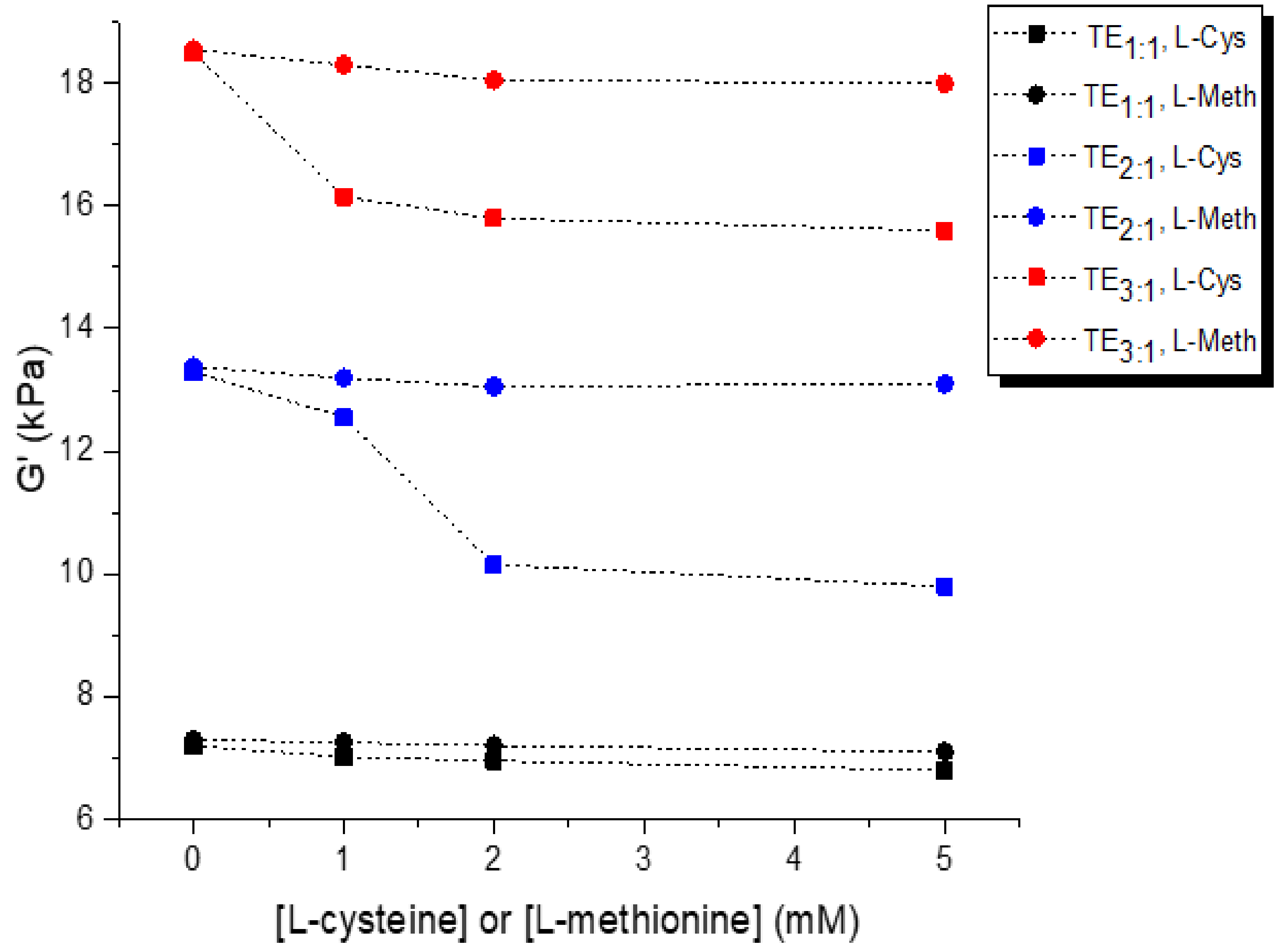
P(NIPAAm-*s*-HPMA-*s*-MEA)

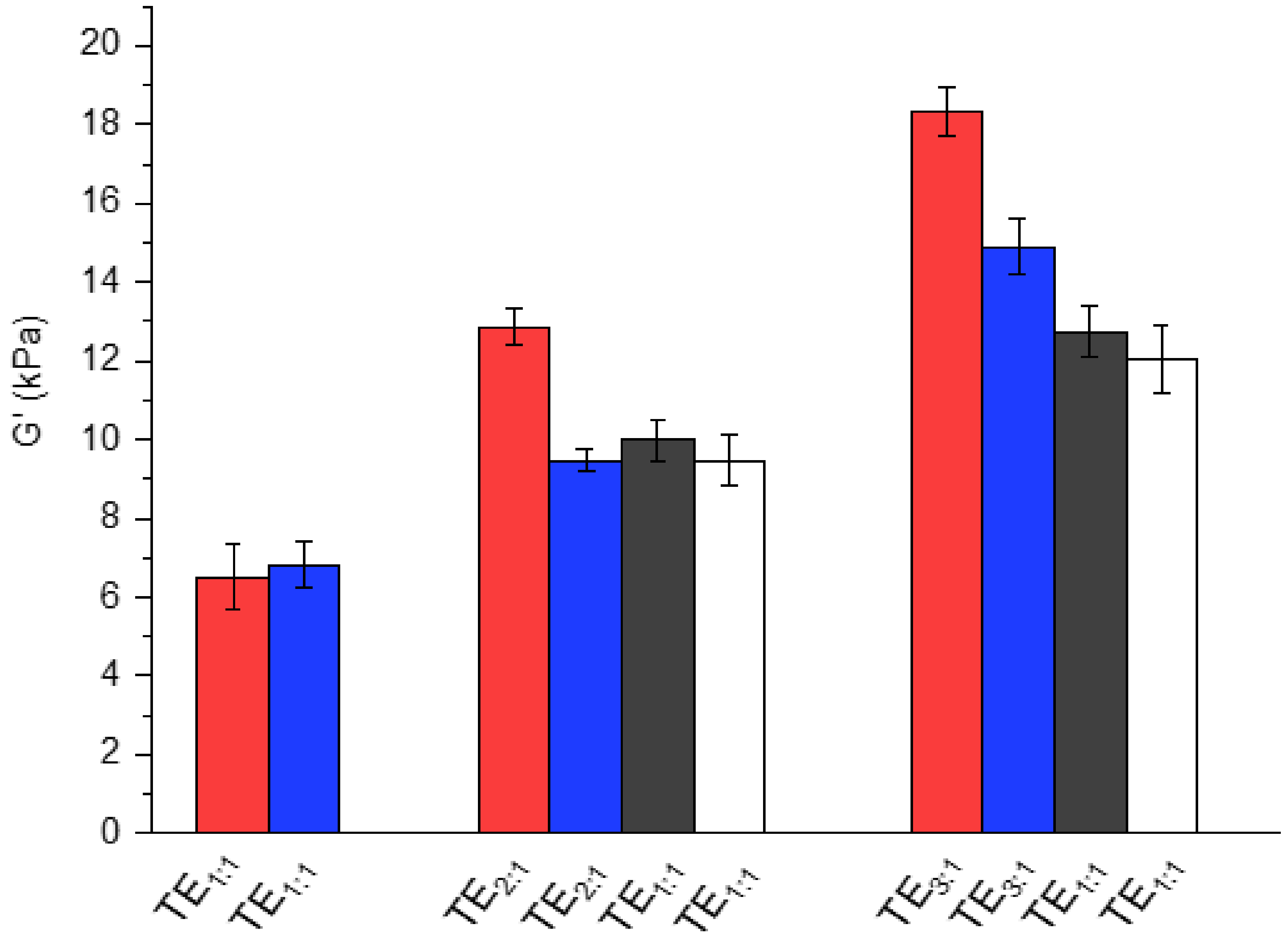
P(NIPAAm-*s*-HPMA-*s*-PDSA)

M_n=15,200 g/mol
Đ = 1.35

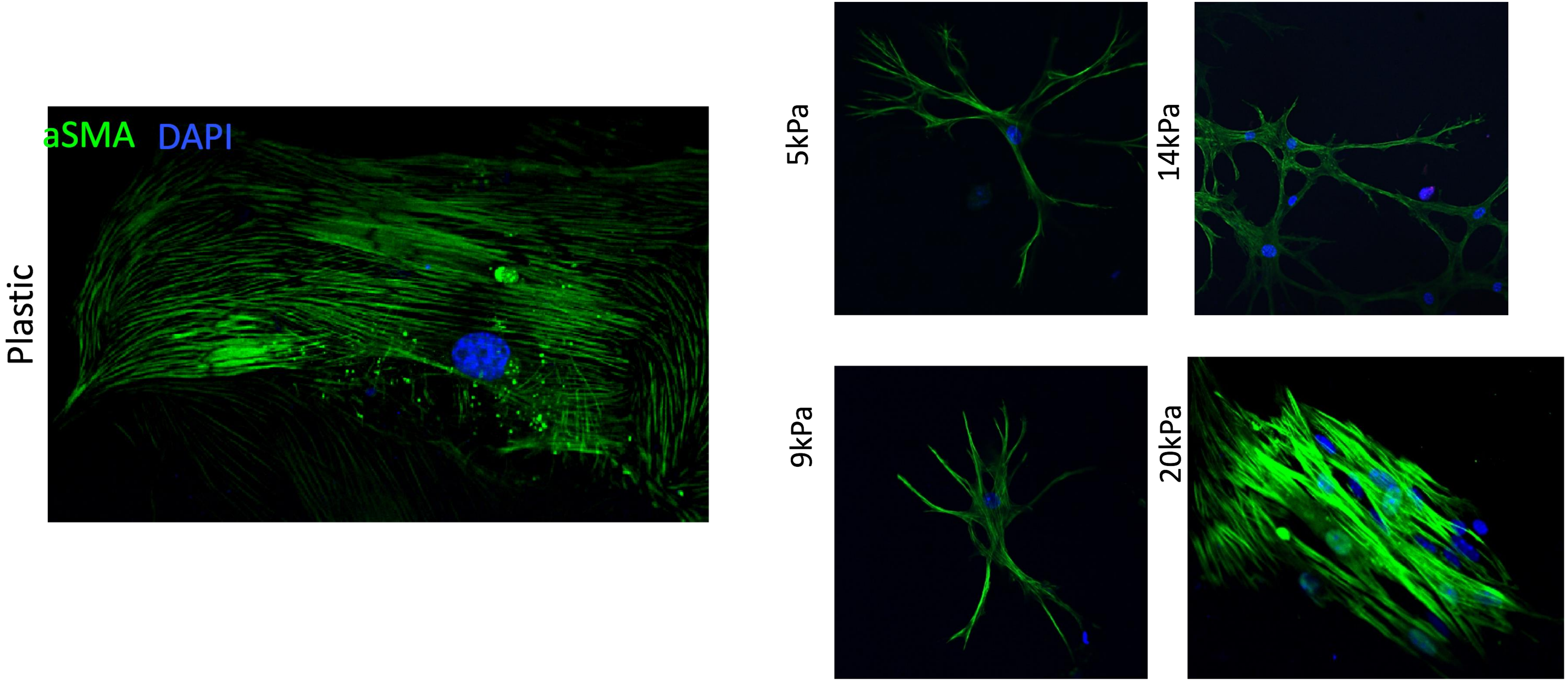
M. Perera, D. M. Fischesser, N. Ayres+ *Polym Chem* **2019** 10 6360-63679

Disulfide exchange using cysteine spiked media changes

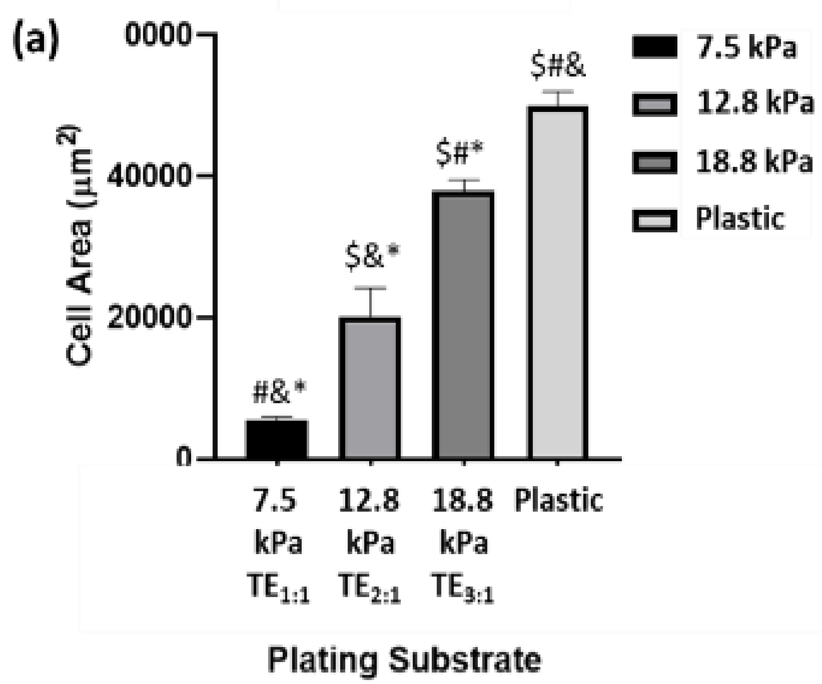


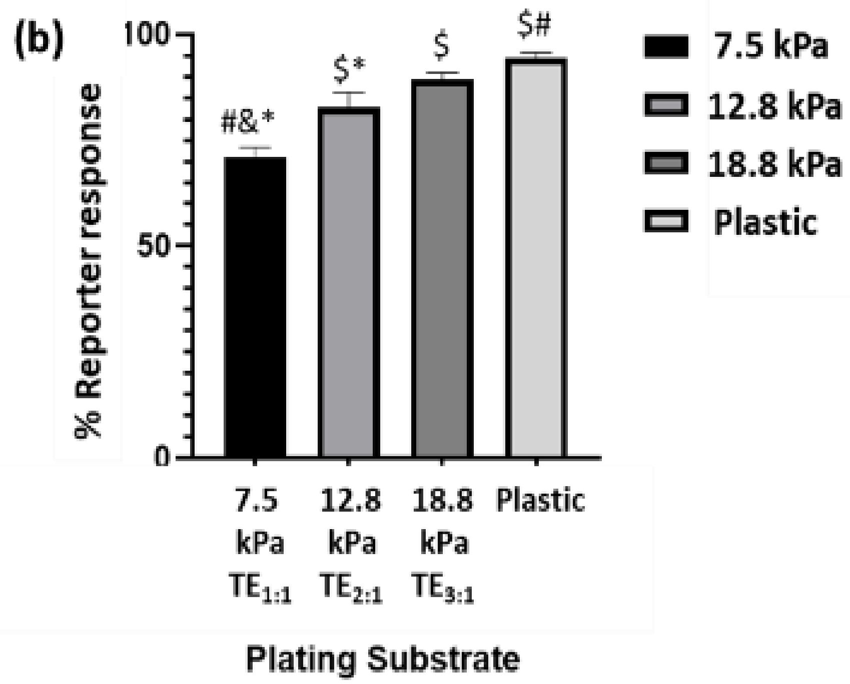


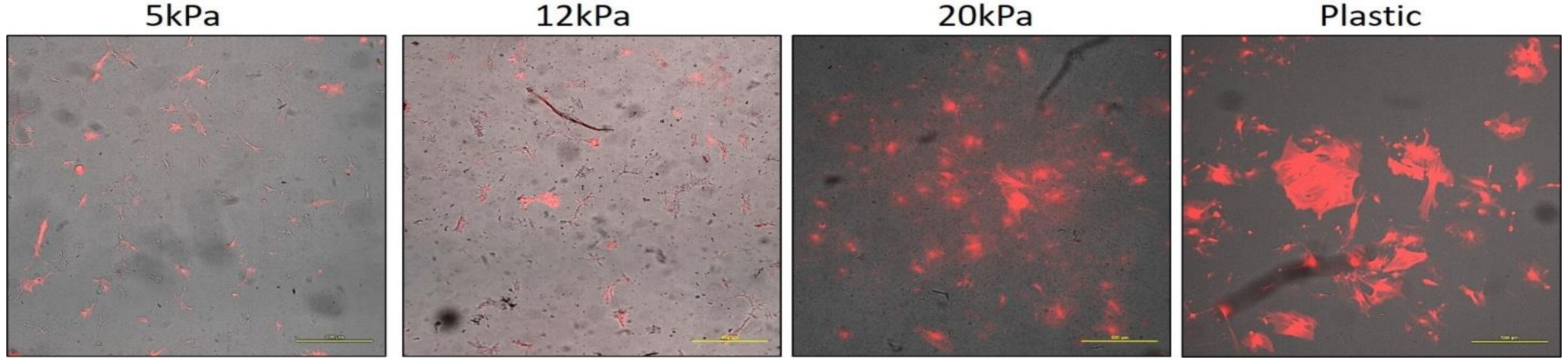
Fibroblasts show similar morphology on soft gelatin-based hydrogels to *in vivo*

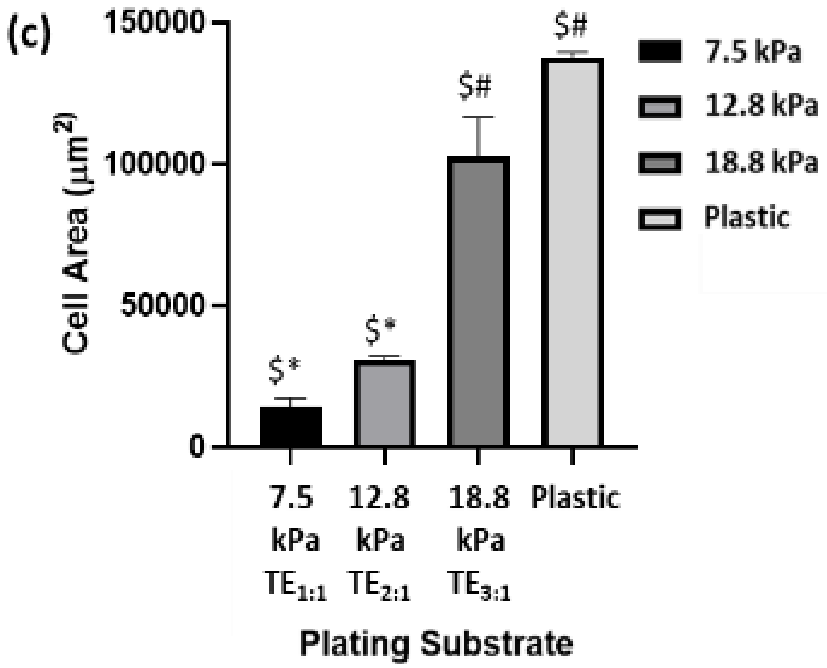


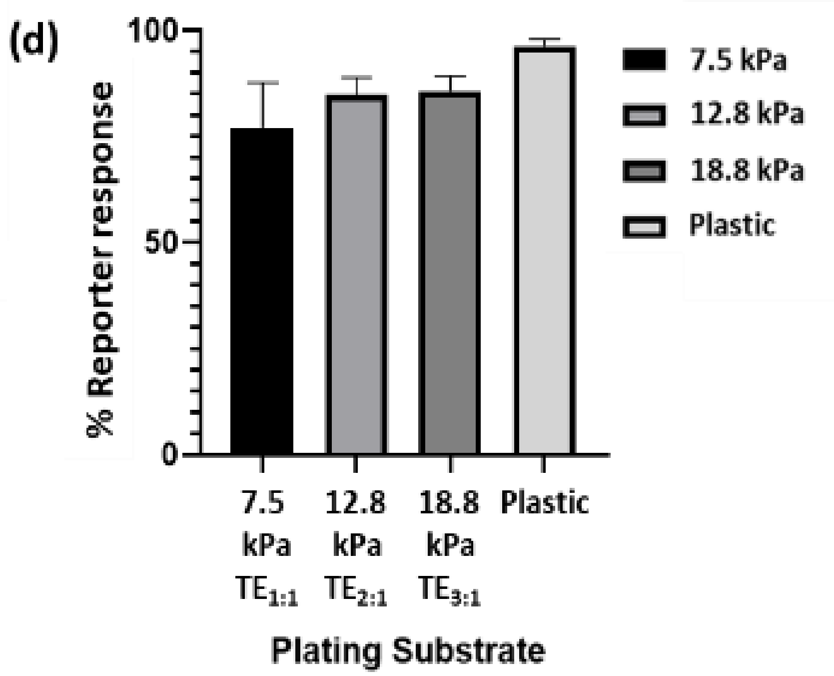
Cell area and αSMA activation in culture for 7 and14 days

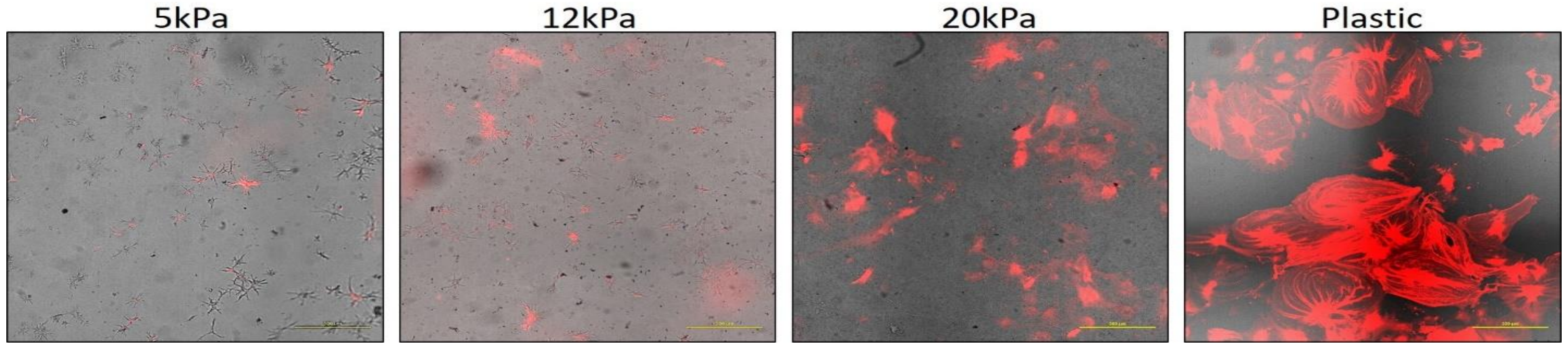




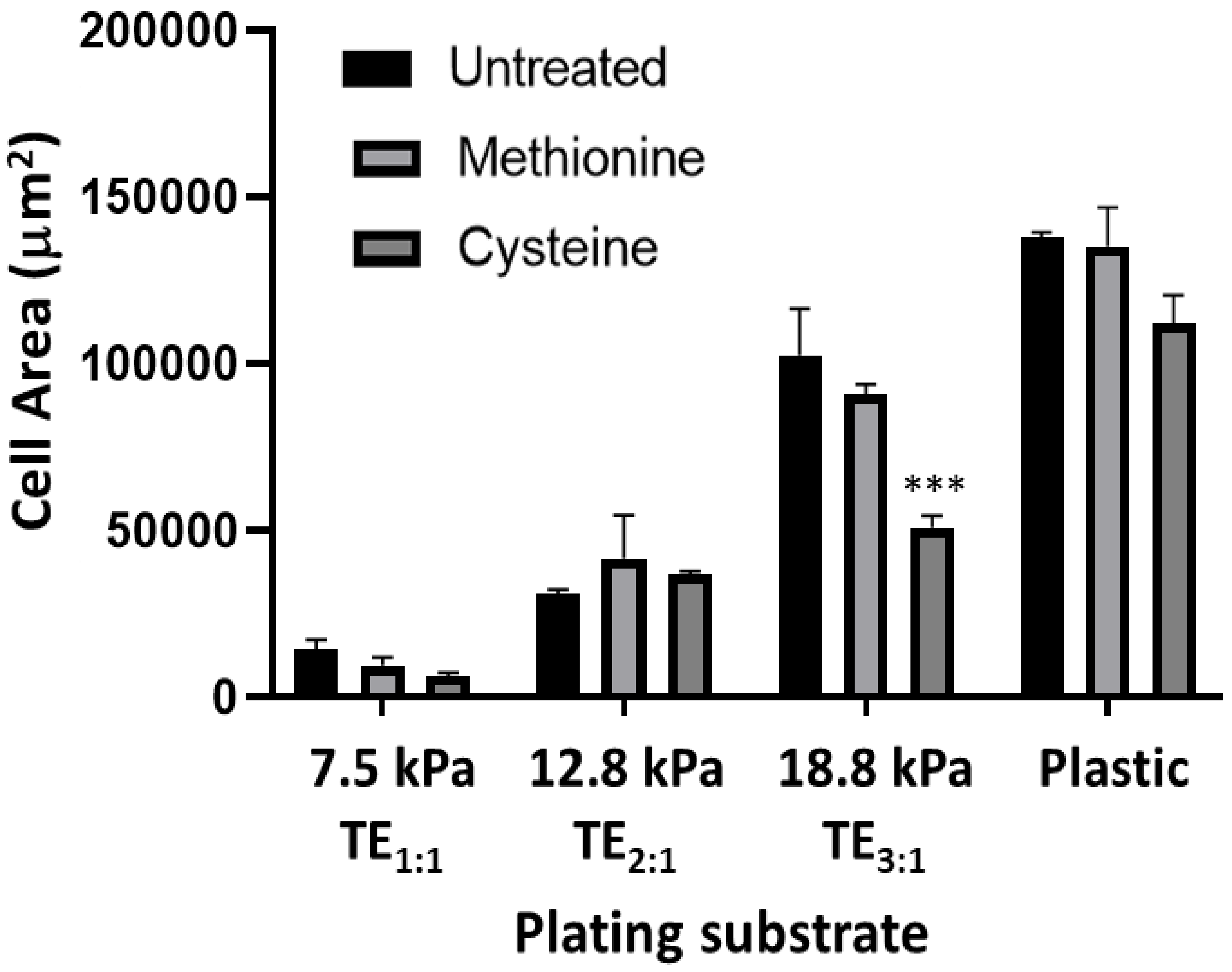






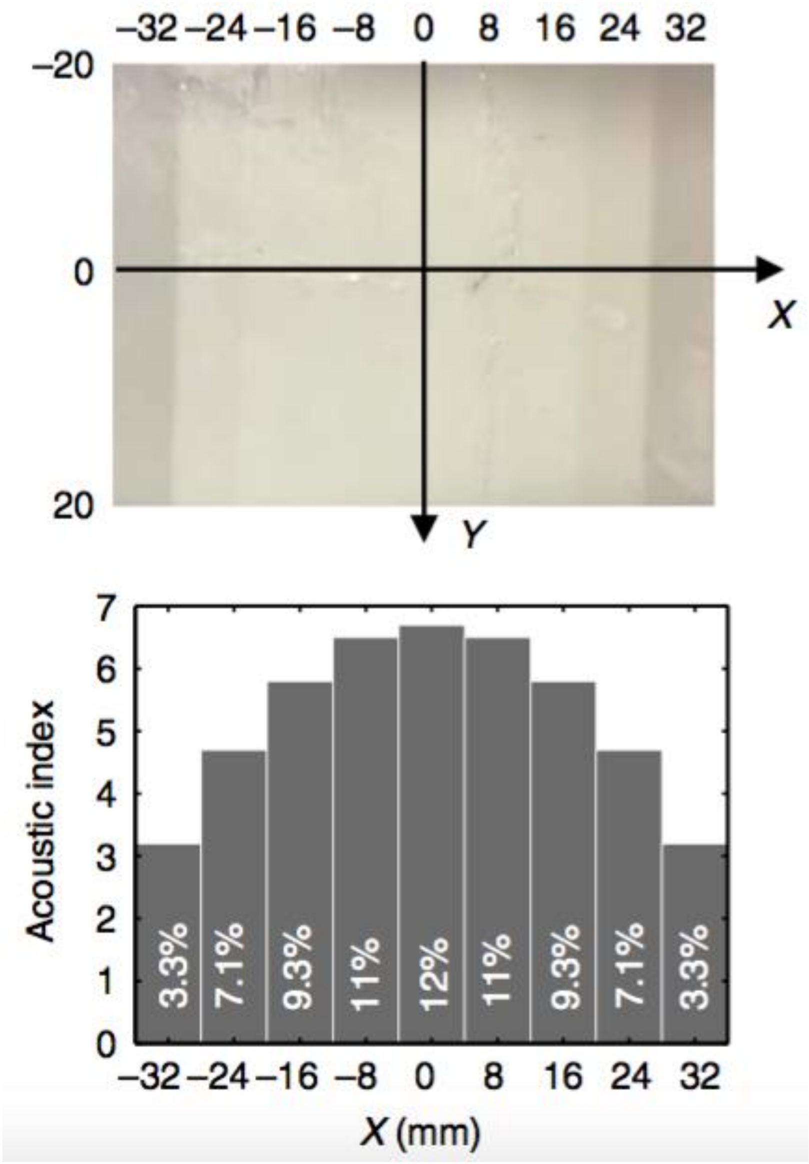


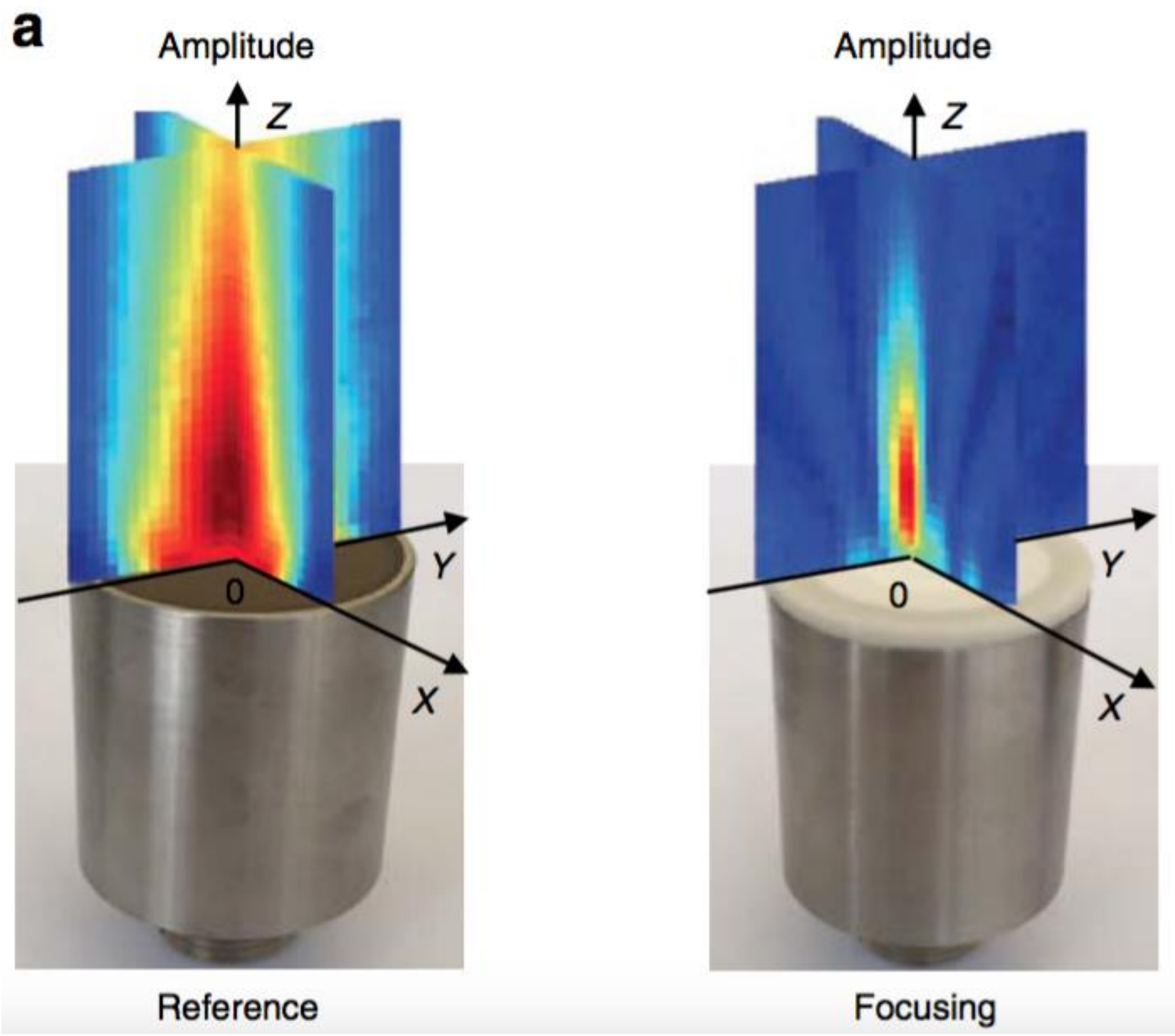
Cell areas after culture for 14 days and treated with cysteine



Porous polymers as acoustic metamaterials

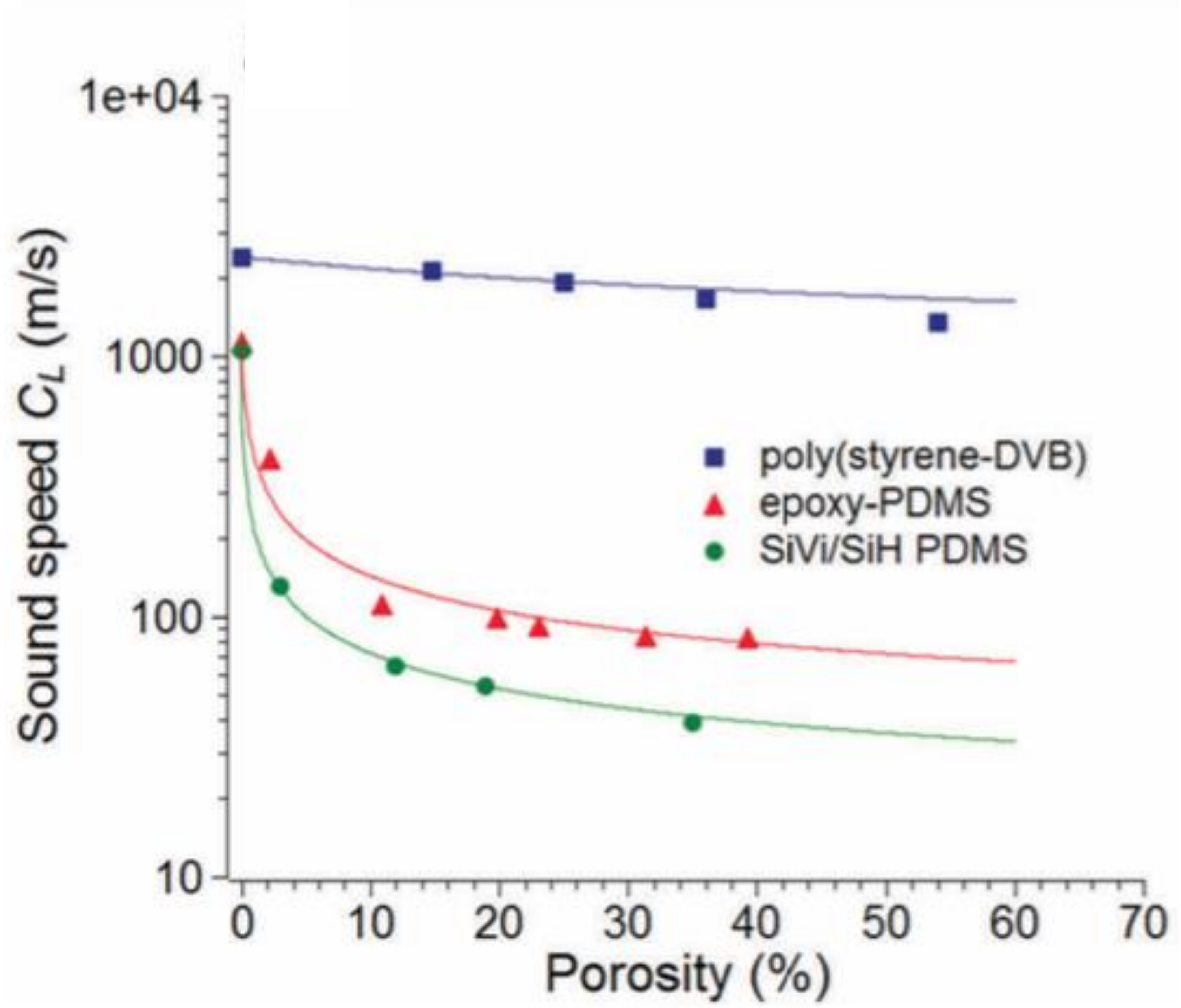
‘Soft’ Metamaterials for acoustics





Jin, Y.; Kumar, R.; Poncelet, O.; Mondain-Monval, O.; Brunet, T. *Nature Communications* 2019, *10* (1).

Stiffness and porosity of the matrix are crucial

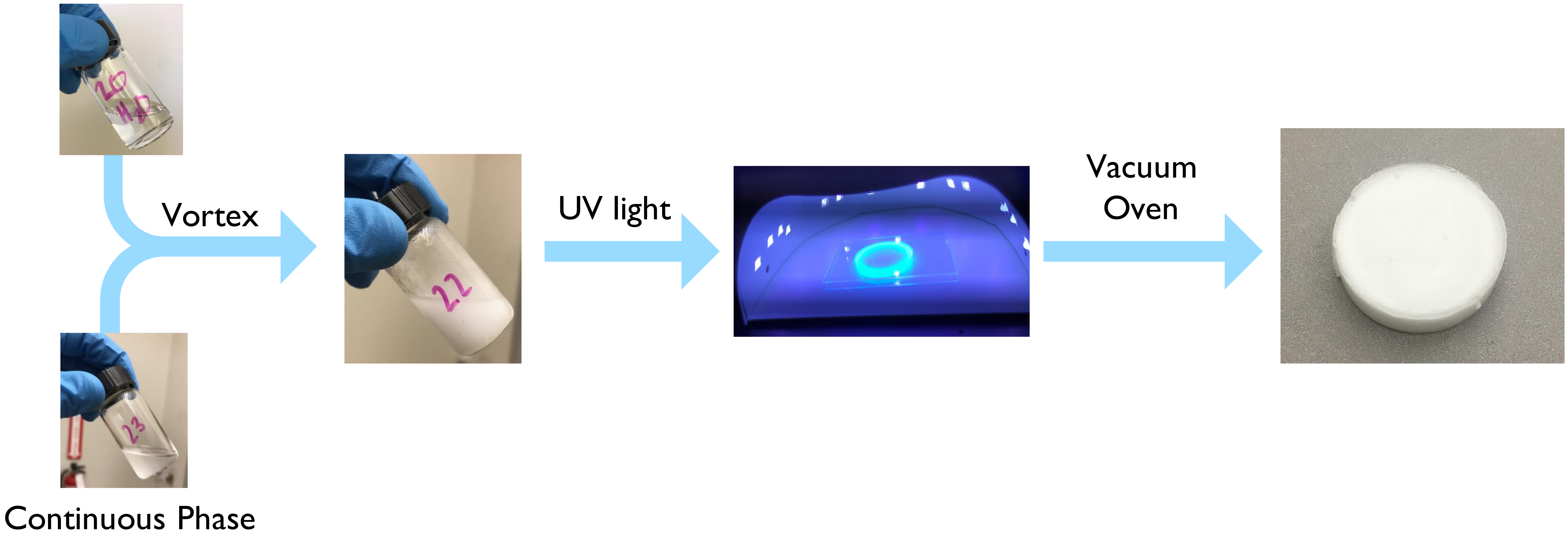


- 'Soft' materials prepared using PDMS performed better than polystyrene materials.

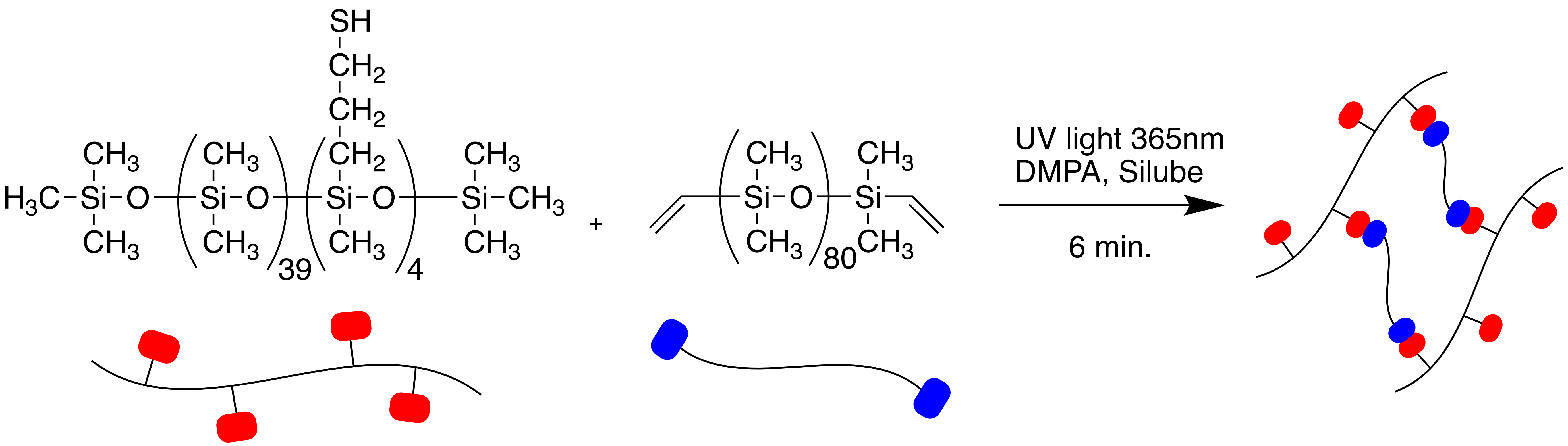
- The observed speed of sound through the materials were dependent on the materials properties of the polymer matrix, which in turn were dependent on the initial emulsion template.

Kovalenko, A.; Fauquignon, M.; Brunet, T.; Mondain-Monval, O. *Soft Matter* 2017, *13* (25), 4526–4532.

PolyMIPE synthesis strategy

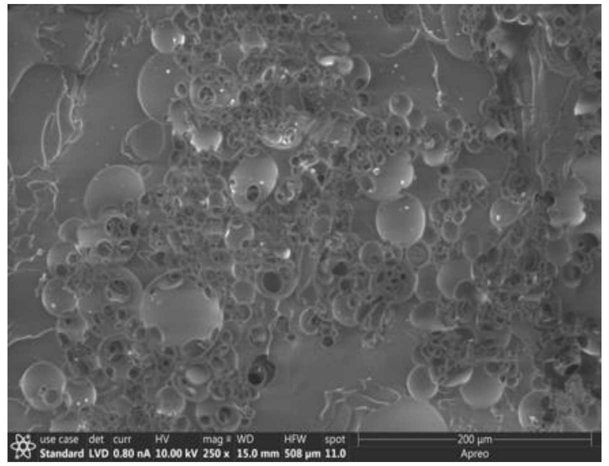


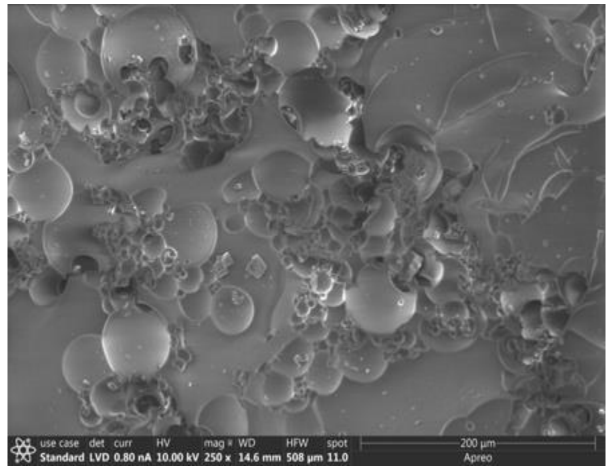
Synthesis of PDMS polyMIPEs

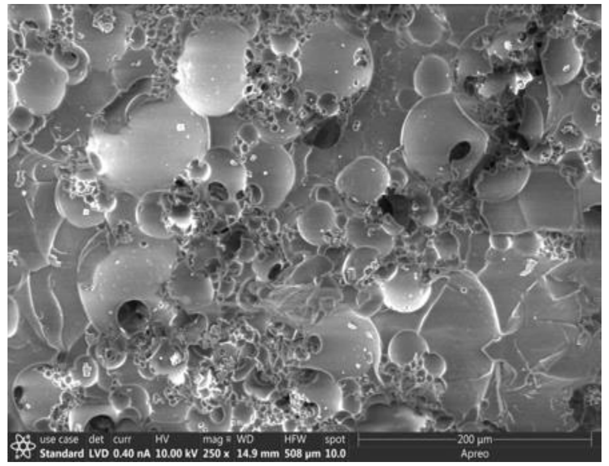


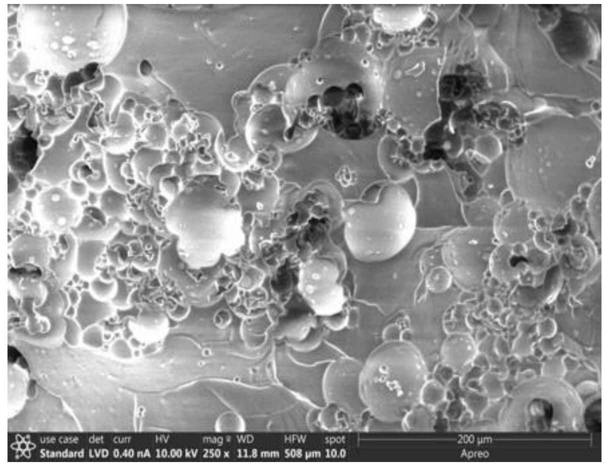
Characterization of the PolyMIPEs

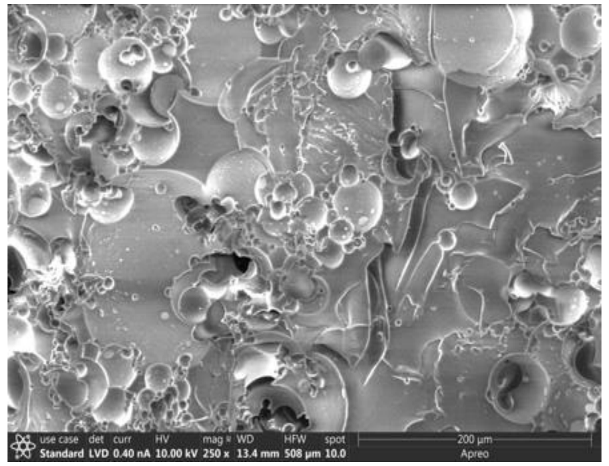
MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
1	1:2	40% (NaCl)	0.40%
2	1:1	40% (NaCl)	0.40%
3	2:1	40% (NaCl)	0.40%
4	1:2	40% ($CaCl_2$)	0.40%
5	1:1	40% ($CaCl_2$)	0.40%
6	2:1	40% ($CaCl_2$)	0.40%

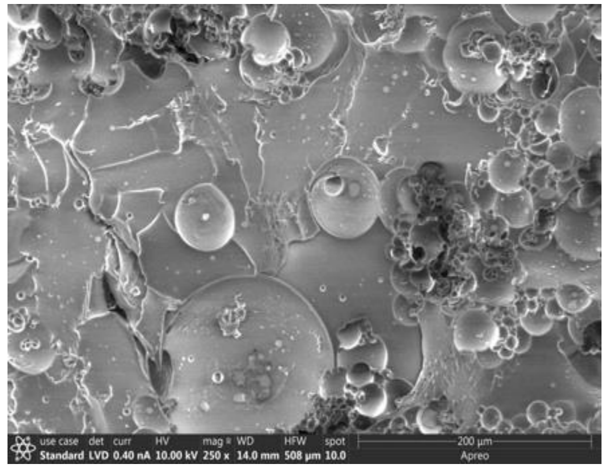

polyMIPE 1


polyMIPE 2


polyMIPE 3

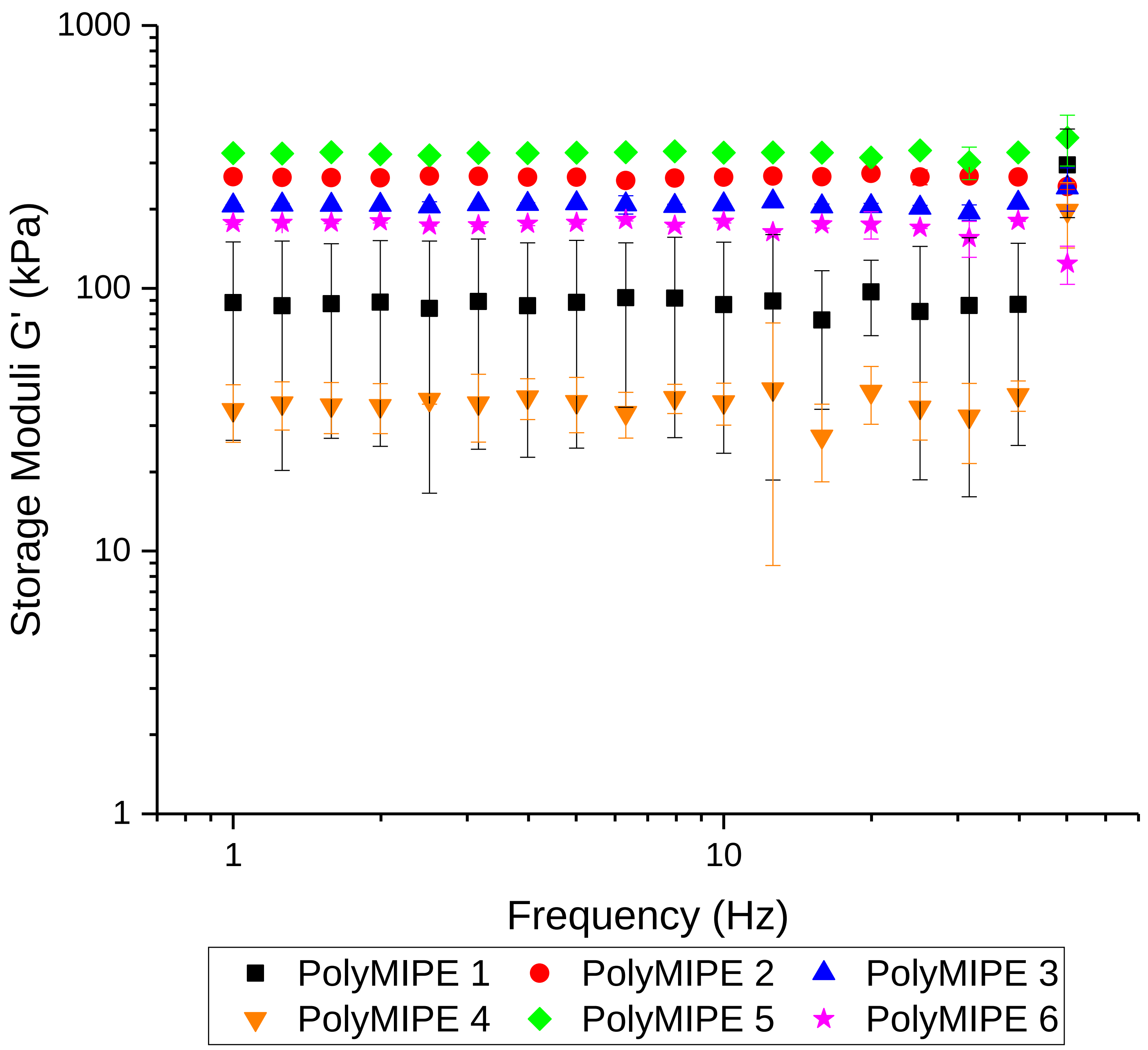

polyMIPE 4


polyMIPE 5


polyMIPE 6

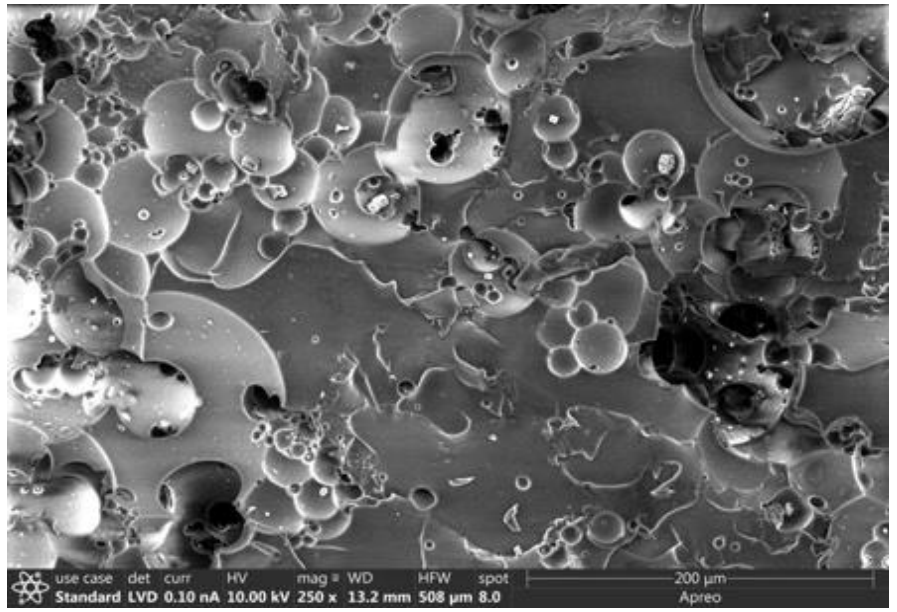
Characterization of the PolyMIPEs

MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
1	1:2	40% (NaCl)	0.40%
2	1:1	40% (NaCl)	0.40%
3	2:1	40% (NaCl)	0.40%
4	1:2	40% ($CaCl_2$)	0.40%
5	1:1	40% ($CaCl_2$)	0.40%
6	2:1	40% ($CaCl_2$)	0.40%

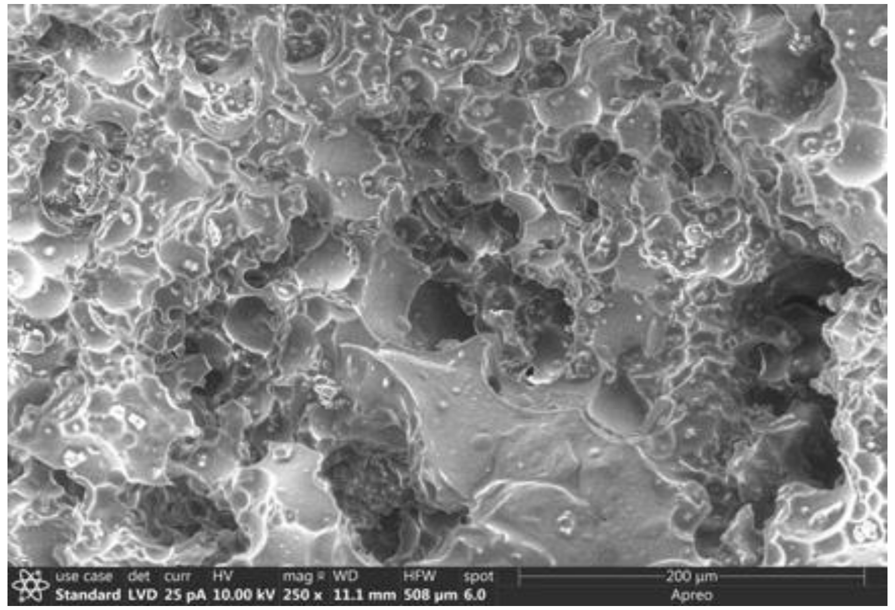


Characterization of the PolyMIPEs

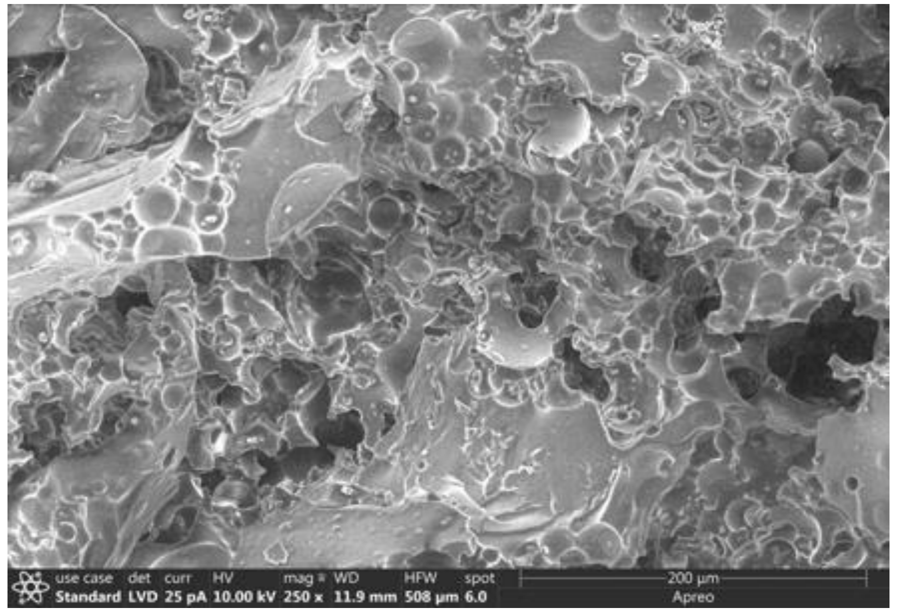
MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
7	1:1	40% (NaCl)	1.00%
8	1:1	40% (NaCl)	3.00%
9	1:1	40% (NaCl)	5.00%



polyMIPE 7



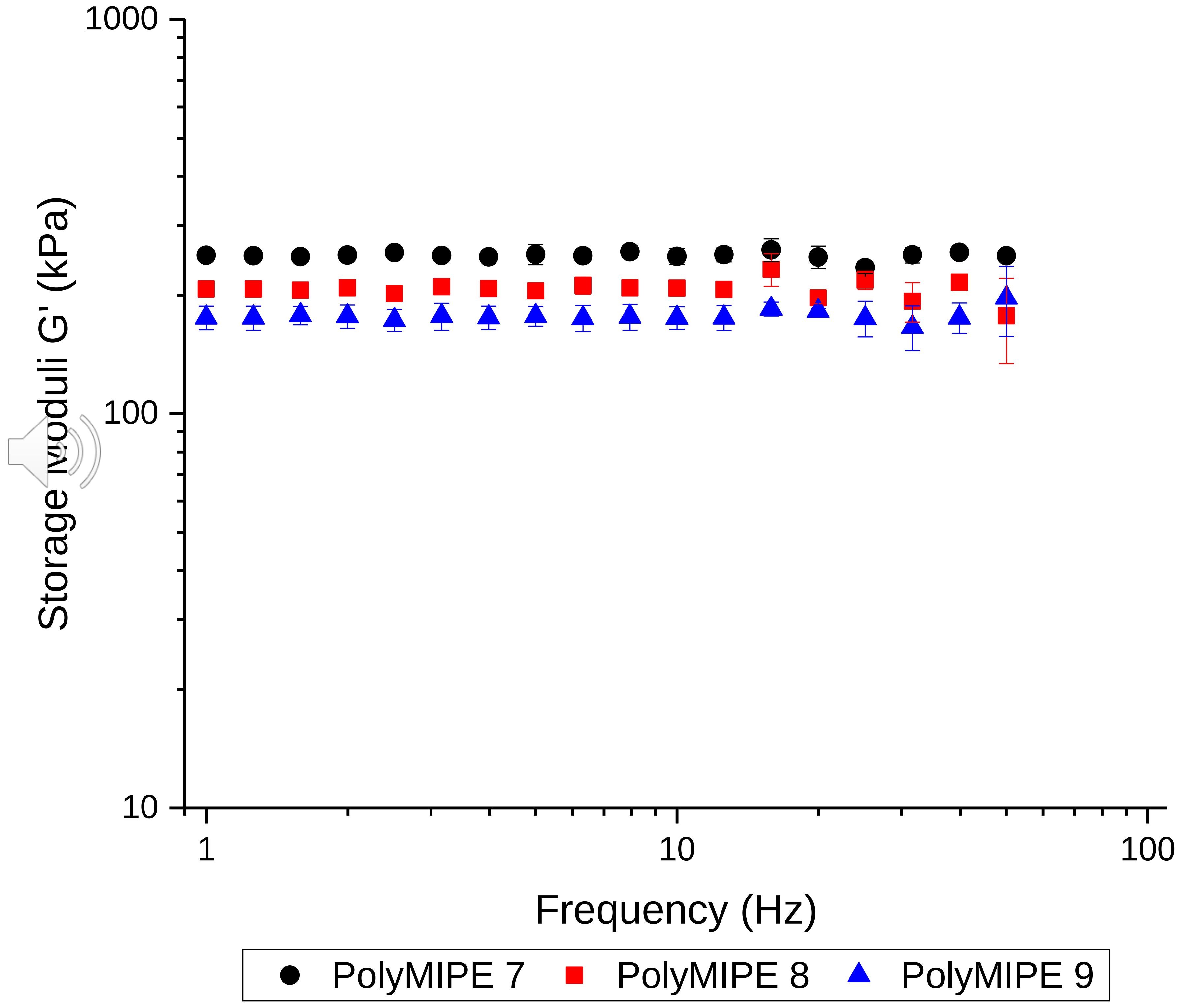
polyMIPE 8



polyMIPE 9

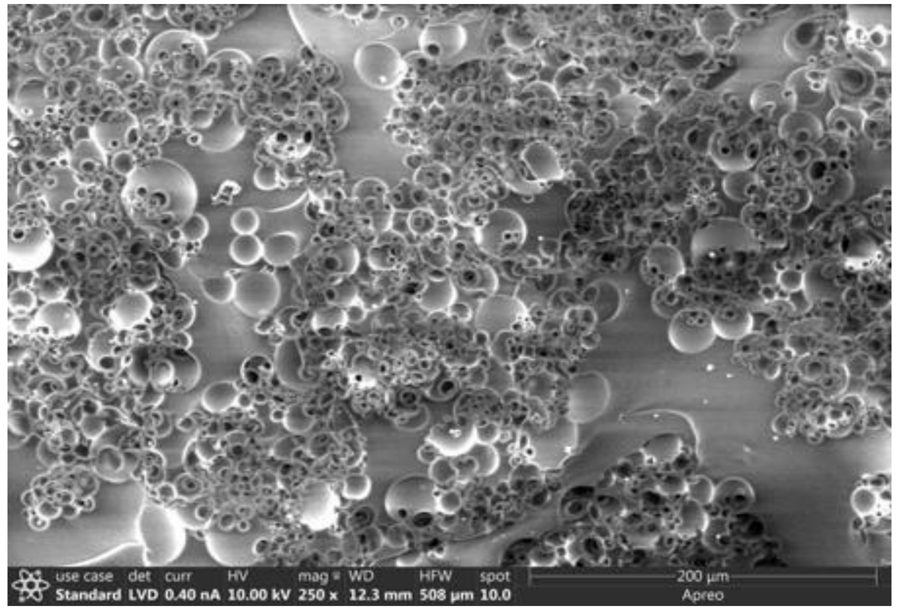
Characterization of the PolyMIPEs

MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
7	1:1	40% (NaCl)	1.00%
8	1:1	40% (NaCl)	3.00%
9	1:1	40% (NaCl)	5.00%

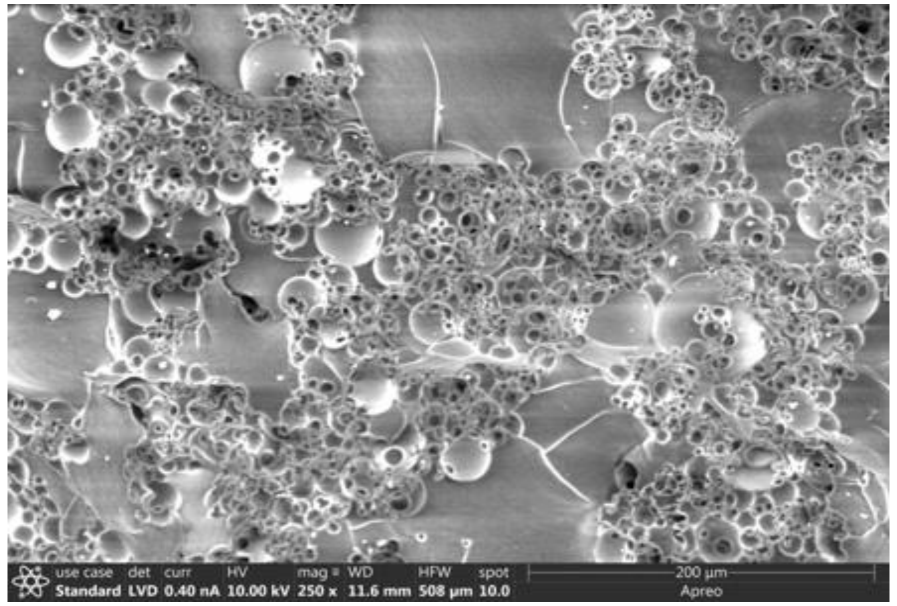


Characterization of the PolyMIPEs

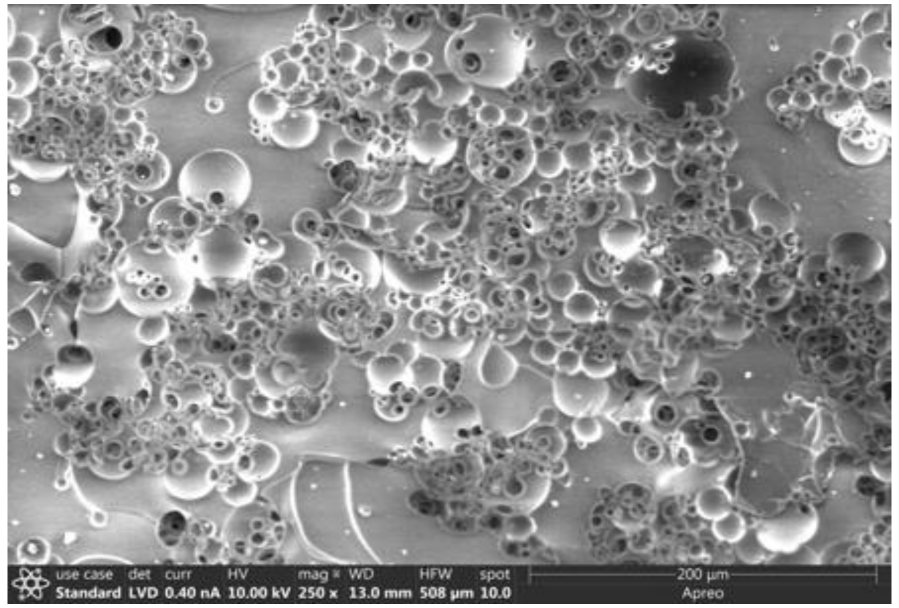
MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
10	1:1	50% (NaCl)	1.00%
11	1:1	60% (NaCl)	1.00%
12	1:1	70% (NaCl)	1.00%



polyMIPE 10



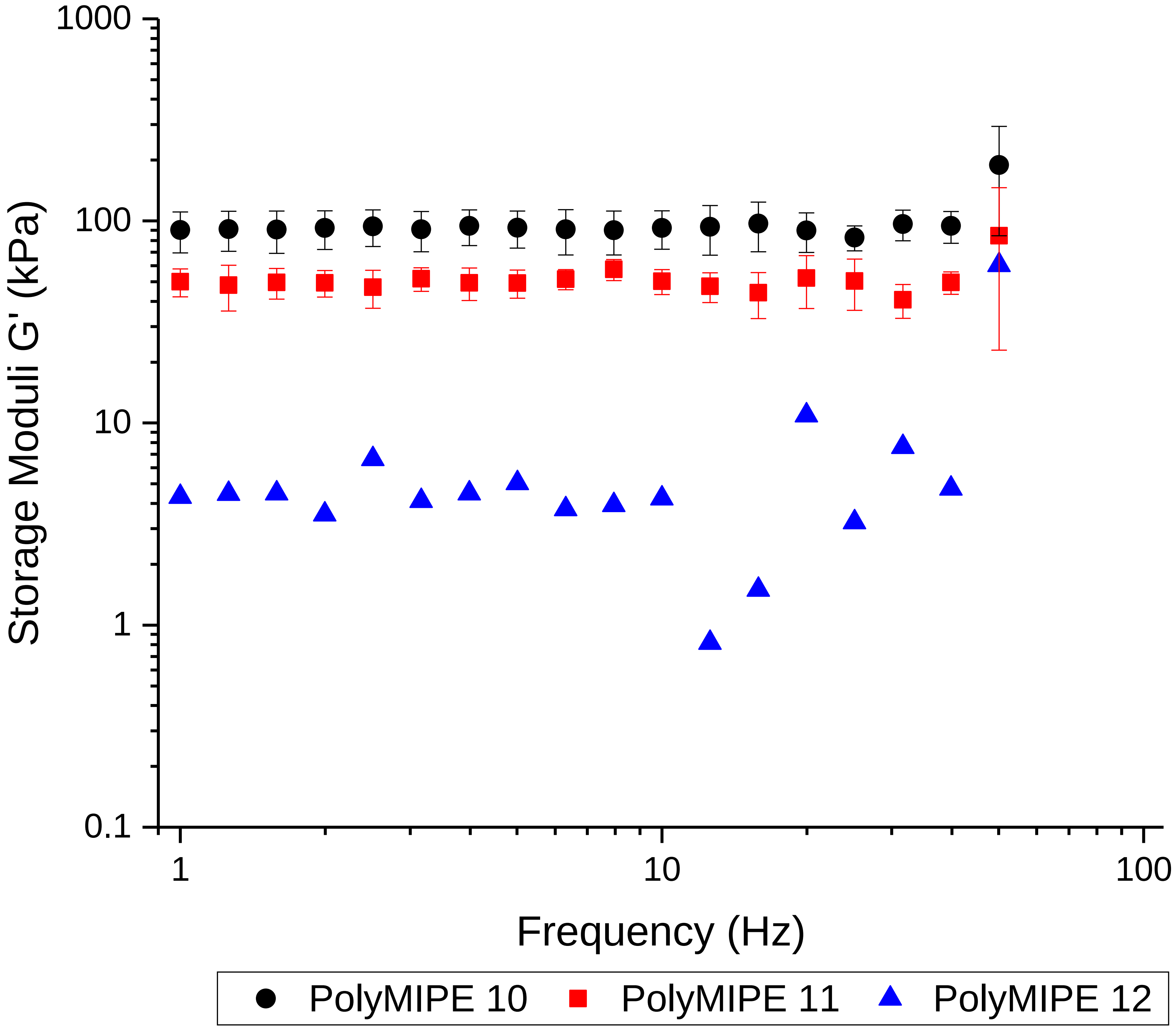
polyMIPE 11



polyMIPE 12

Characterization of the PolyMIPEs

MIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content
10	1:1	50% (NaCl)	1.00%
11	1:1	60% (NaCl)	1.00%
12	1:1	70% (NaCl)	1.00%



polyMIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content	Surface Area (cm^2/g)	Average Pore Size D (microns)	Total Porosity (+/- 2%)
1	1:2	40% (NaCl)	0.40%	586	164	38%
2	1:1	40% (NaCl)	0.40%	567	173	39%
3	2:1	40% (NaCl)	0.40%	727	136	38%
4	1:2	40% ($CaCl_2$)	0.40%	494	195	36%
5	1:1	40% ($CaCl_2$)	0.40%	635	153	38%
6	2:1	40% ($CaCl_2$)	0.40%	616	150	42%
7	1:1	40% (NaCl)	1.00%	810	123	40%
8	1:1	40% (NaCl)	3.00%	402	249	44%
9	1:1	40% (NaCl)	5.00%	352	272	42%
10	1:1	50% (NaCl)	1.00%	1151	104	49%
11	1:1	60% (NaCl)	1.00%	2557	56	60%
12	1:1	70% (NaCl)	1.00%	3743	48	66%

polyMIPE	Thiol:Ene Ratio	Volume of Dispersed Phase and Salt	Surfactant Content	Surface Area (cm^2/g)	Average Pore Size D (microns)	Total Porosity (+/- 2%)
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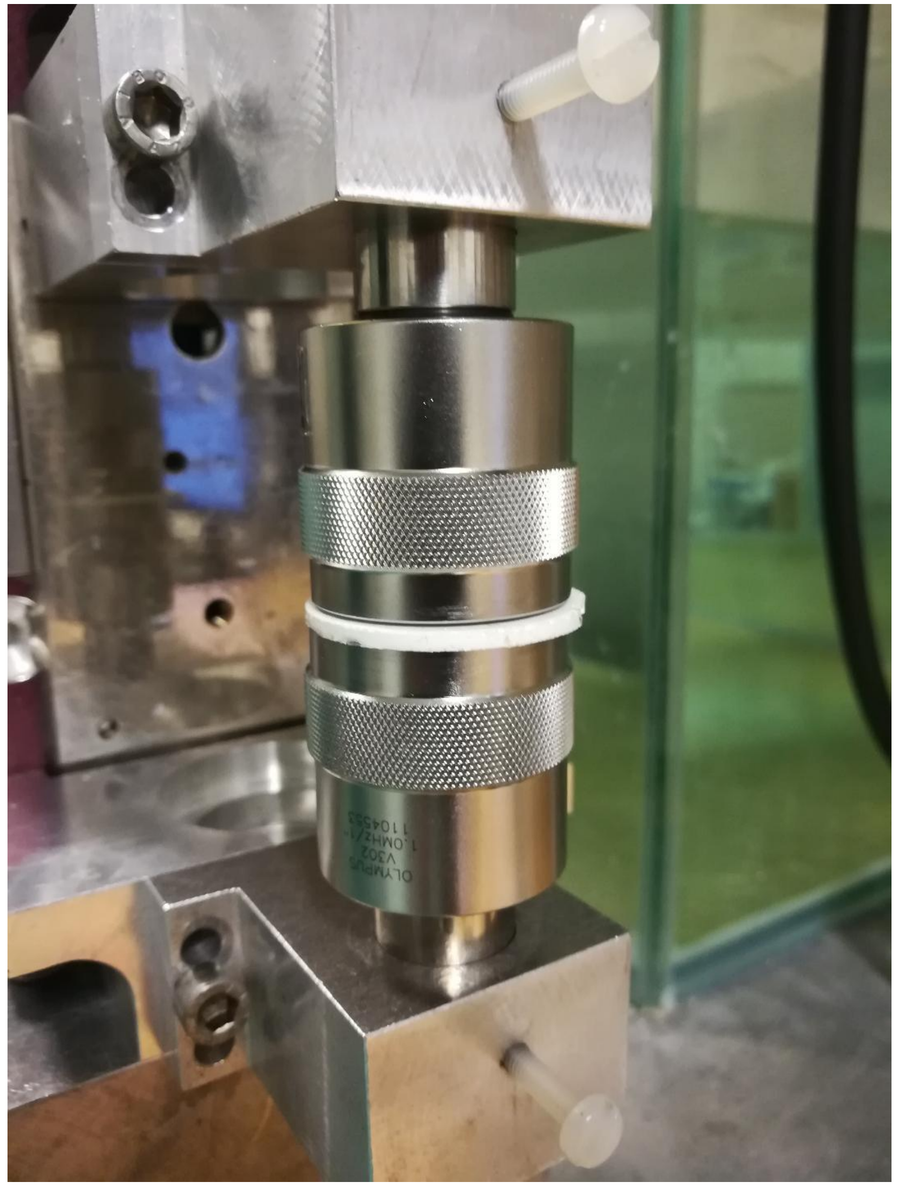
12	1:1	70% (NaCl)	1.00%	3743	48	66%

Acoustic Analysis

- Acoustic characterization of samples was performed on polyMIPEs at ultrasonic frequencies
  - Two different thicknesses were used to measure time of flight differences to confirm calculated speed of sound
- Longitudinal sound speed (c_L) is calculated
  - The distance traveled per unit time by a sound wave as it propagates through an elastic medium



Longitudinal sound speed was calculated to be ~40m/s

T. McKenzie, P. Heaton, K. Rishi, R. Kumar, T. Brunet, G. Beaucage, O. Mondain-Monval, and N. Ayres (2020) Macromolecules 53, 3719-3727

Conclusions

- We have several projects in various application areas
- All the projects share the same philosophy, where we take a hierarchical view.
- Specifically, how can we control polymer chemistry to dictate materials properties.

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- Molly Shaw (CCHMC)
- Molkentin Research Group (CCHMC)
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- Prof. Greg Beaucage (UC)
- Prof. Oliver Mondain-Monval (UB)

- Find us on the web at ayres.group

or on twitter @AyresLab

Thank you