Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

for

Design of functional cationic microgels as conjugation scaffolds

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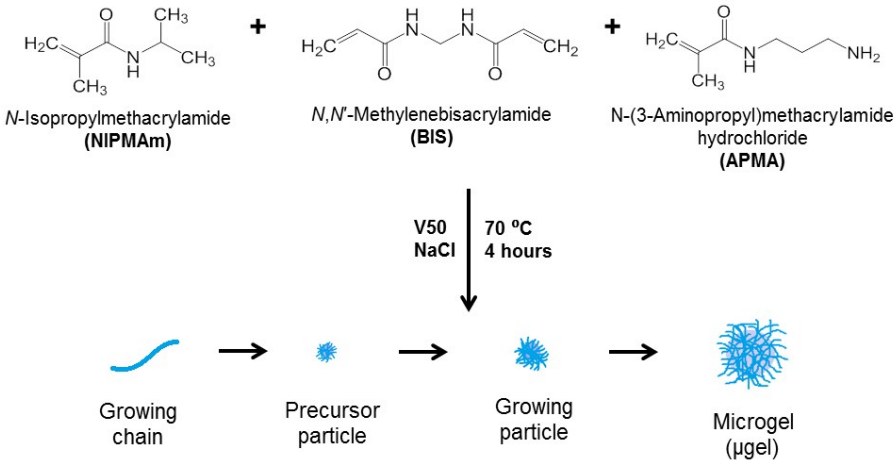


Scheme S1. General representation of a one step synthesis of p(NIPMAm-co-APMA) microgels.

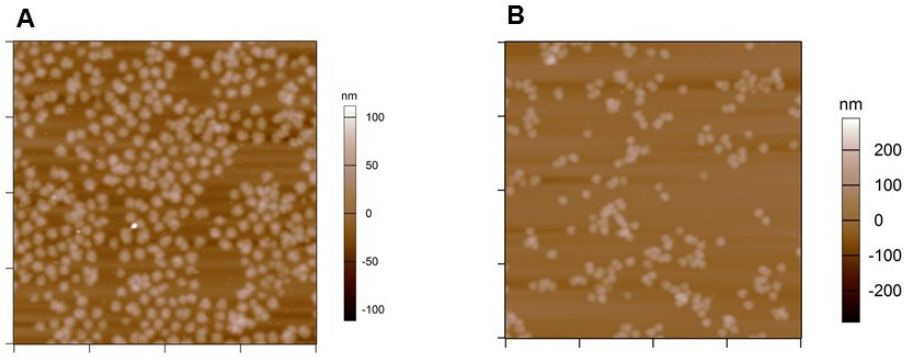


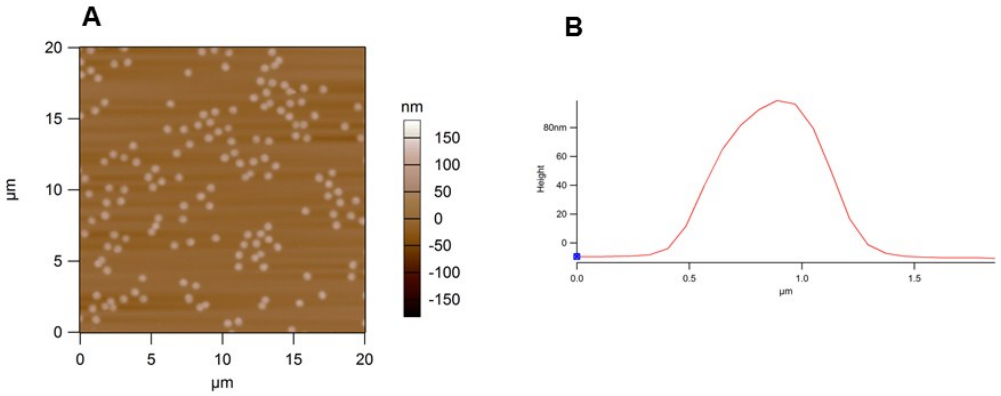
Figure S1. AFM height traces of microgels synthesized with [NaCl] (A) and [APS] (B) above a threshold (specific to the synthesis conditions) leads to the formation of misshapen particles (all images are 20μm × 20μm). Microgels were deposited on pre-functionalized glass by centrifugal deposition at 2250 × *g* for 15 min at 25 °C.

Table S1. Effect of increasing V50 concentration on microgel size

Microgel identity	[NIPMAm] (mol%)	[BIS] (mol%)	[APMA] (mol%)	[APS] (mM)	[V50] (mM)	[NaCl] (mM)	R_H (nm) at pH 7
µgel13	89	2	9	-	1.0	50	195±6
µgel14	89	2	9	-	0.5	50	249±14

[Total monomer] = 140 mM, Temperature = 70 °C

DLS measurements performed in phosphate buffer (pH 7, 100 mM ionic strength)



C

R_H (nm) at pH 5	412 ± 27
R_H (nm) at pH 7	404 ± 21
R_H (nm) at pH 9.8	412 ± 17

Figure S2. AFM height trace of µgelPEG (A), representative height profile (B) and R_H measured in buffers of different pH values (C). Microgels were deposited on pre-functionalized glass by centrifugal deposition at 2250 × *g* for 15 min at 25 °C.

Analysis of µgelPEG using the CBQCA assay revealed the number of moles of APMA in the analyzed solution to be $(1.97\pm0.04)\times10^{-8}$, with the efficiency of primary amine incorporation being 39±1%.

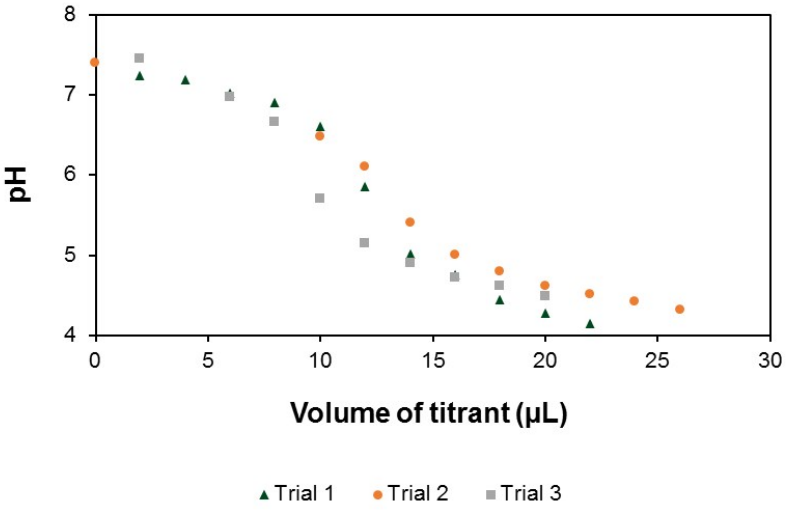
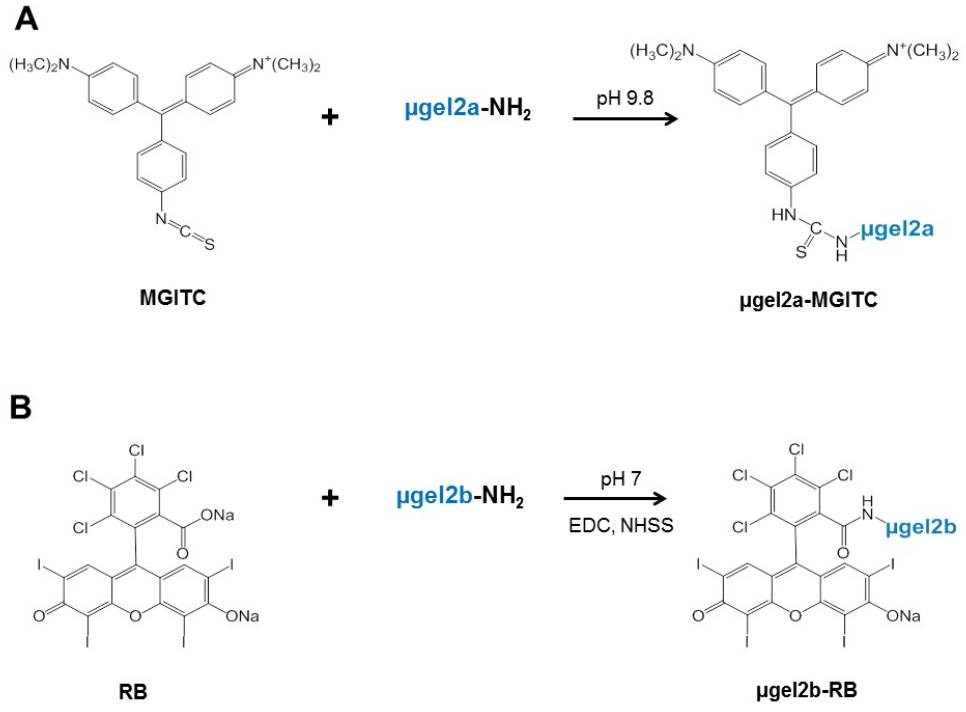


Figure S3. pH titration curves of µgel2 solutions using 0.101 M HCl as a titrant for quantitation of primary amine groups.

pH titrations were performed using a Vernier pH Electrode and LabQuest Software (Vernier Software and Technology, LLC., Beaverton, OR). Microgels were diluted to a total concentration of 1 mg/mL in 20 mL distilled, deionized water. Titration was performed against 0.101 M HCl (standardized against 0.099 M Na_2CO_3) under a N_2 purge with stirring, at ambient temperature. Measurements of solution pH were made after 300 s equilibration following each addition of titrant.



Scheme S2. Conjugation reactions utilized for synthesis of µgel2a-MGITC (A) and µgel1-RB, µgel2-RB, µgel2b-RB and µgel6-RB (B) conjugates.

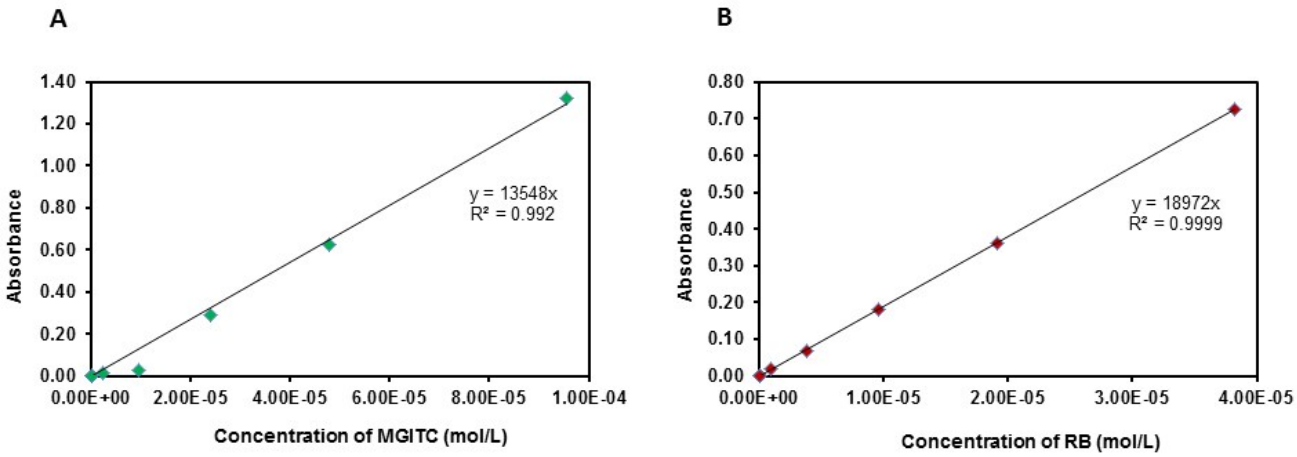


Figure S4. Standard curves for absorbance measurements of malachite green isothiocyanate (A) and rose bengal (B) solutions at λ_{max} values of 625 nm and 540 nm respectively.

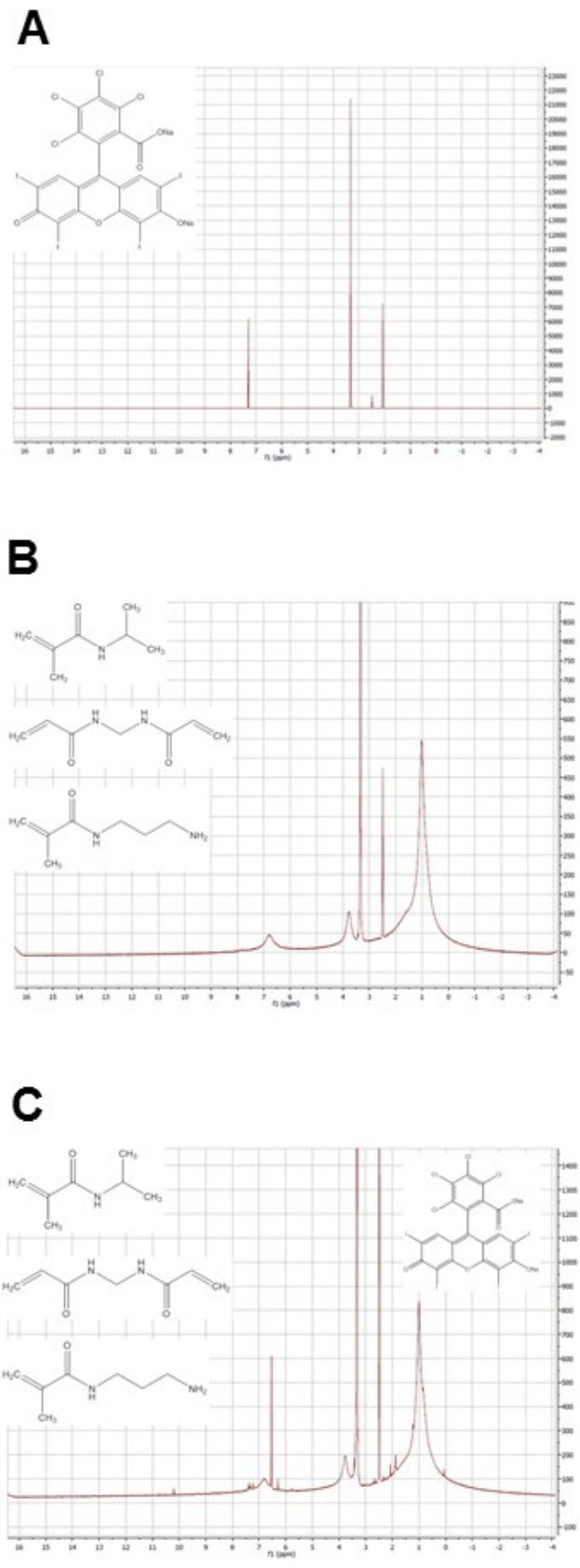


Figure S5. 1D ^{1}H NMR spectra of RB (A), μgel2b (B) and μgel2b-RB (C).

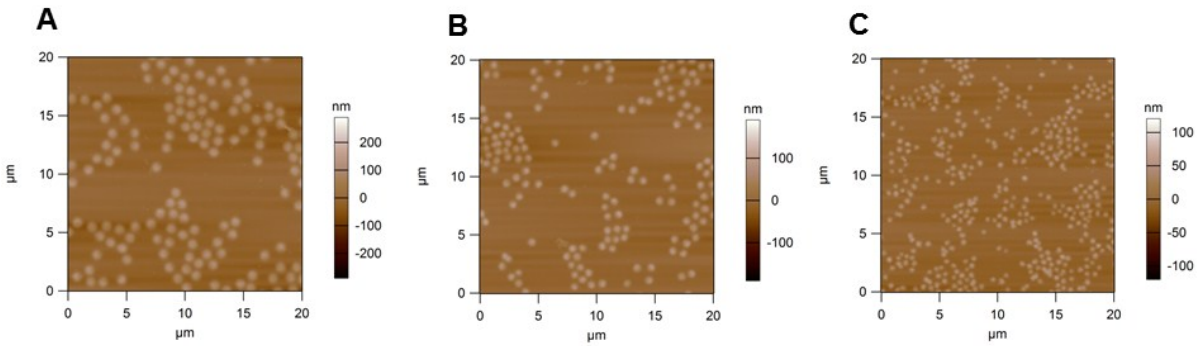


Figure S6. AFM height traces of µgel1-RB (A), µgel2-RB (B) and µgel6-RB (C).

Table S2. Deswelling data for µgel2b-RB at 37 °C vs. 20 °C

Temperature (^{0}C)	R_H (nm)
20	401±21
37	349±18

DLS performed in phosphate buffer (pH 7, 50 mM ionic strength)

The average percent deswelling of the microgels is 33.33% by volume, demonstrating that as a result of pNIPMAm having an LCST of ~44 °C, the microgels are not completely deswollen at physiological temperature.

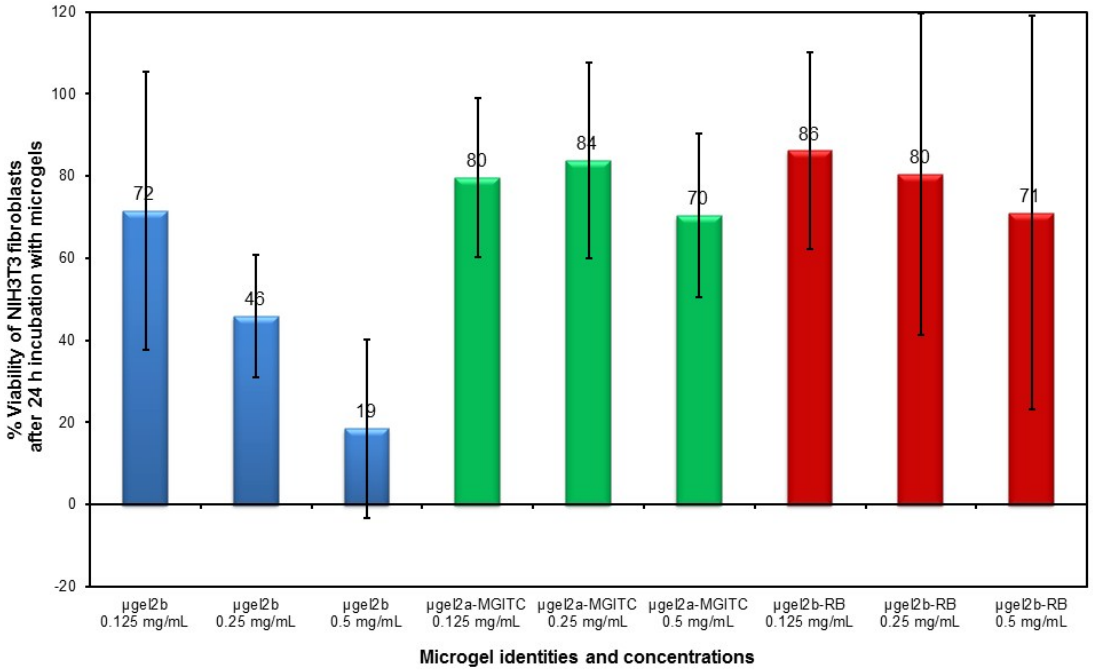


Figure S7. Viability data for NIH 3T3 fibroblasts incubated for 24 h with unconjugated microgels (blue bars), microgels conjugated to malachite green (green bars) and microgels conjugated to rose bengal (red bars) *(Values presented as averages of three trials, error bars represent standard deviations of these three values)*.