Nanoscale Growth Factor Patterns by Immobilization on a Heparin-Mimicking Polymer

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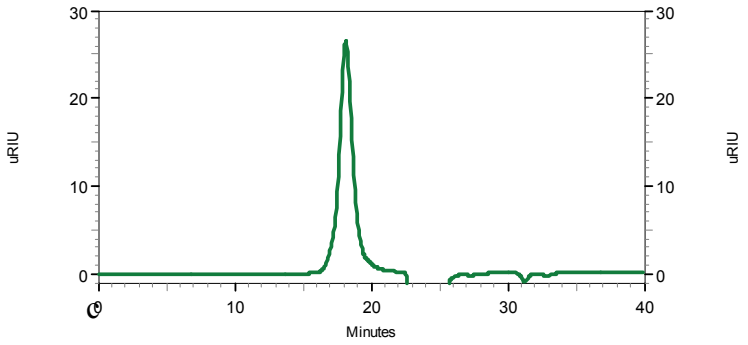


Figure S1. GPC chromatogram of PSS-co-PEGMA **1**. Solvent: DMF with 0.1 M LiBr, 40 °C, flow rate: 0.8 mL/min.

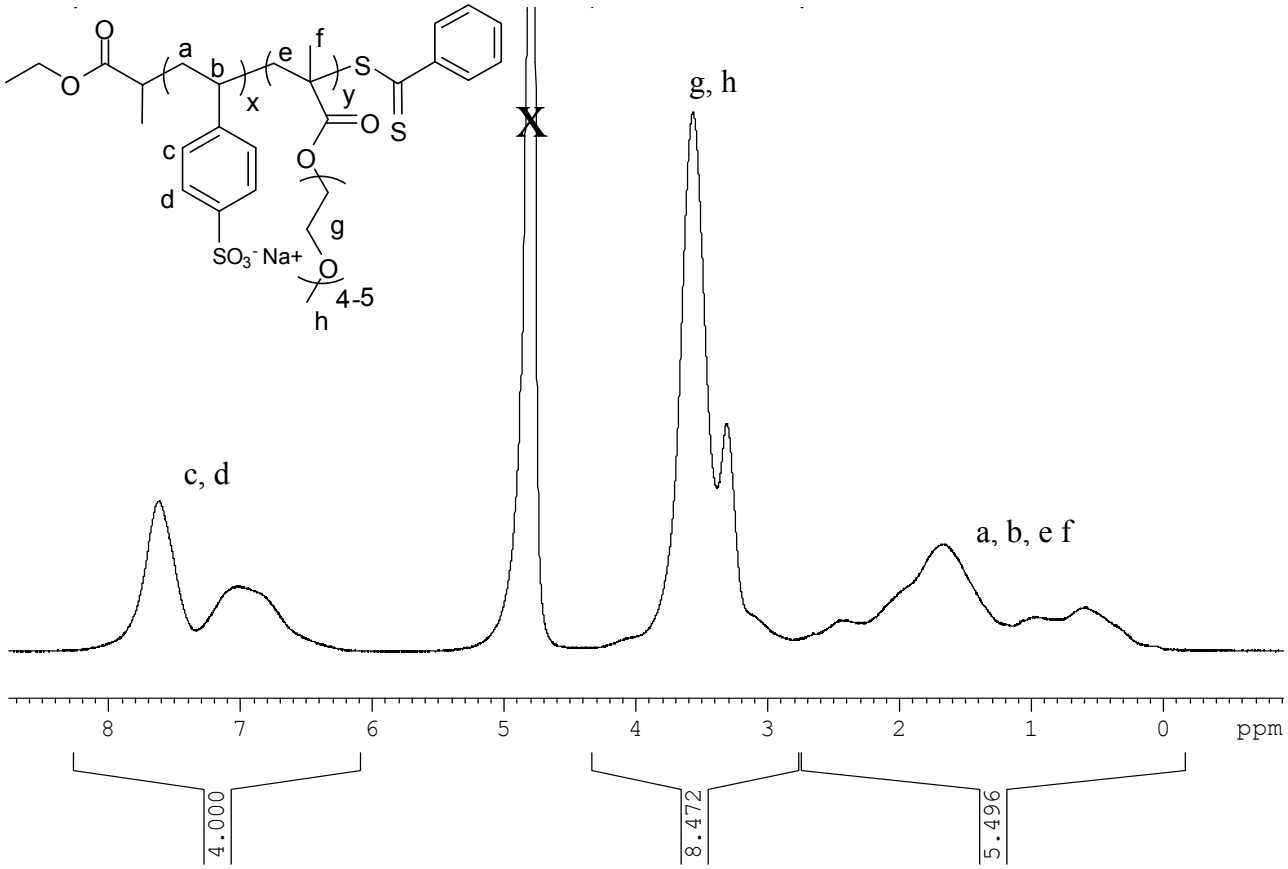
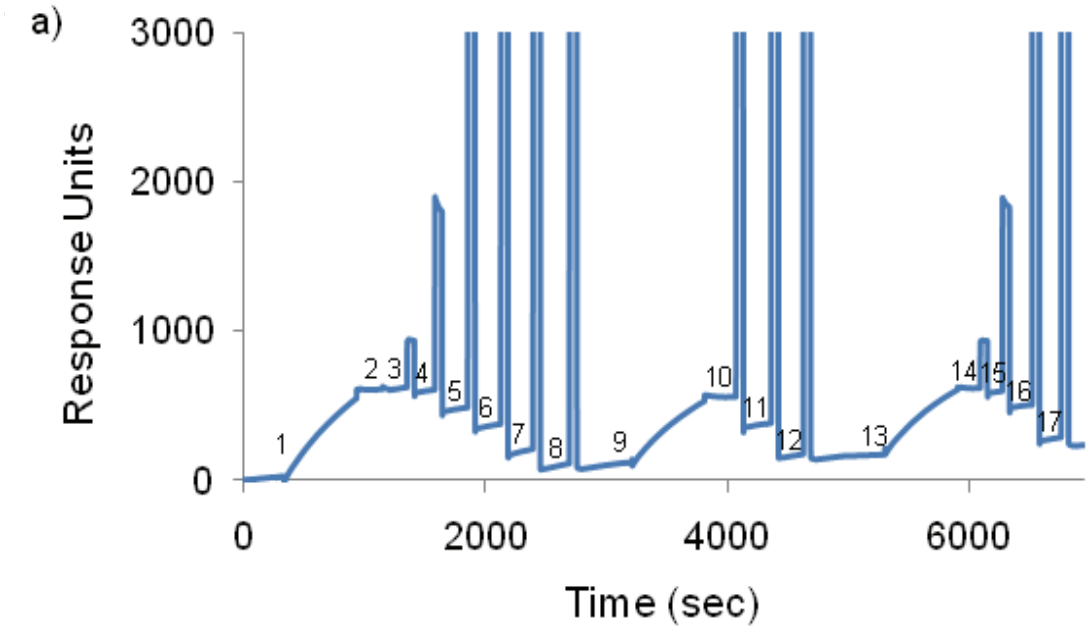


Figure S2. ^{1}H NMR spectrum (D_2O) of PSS-co-PEGMA **1**.

Determination of copolymer ratio: We determined the SS:PEGMA ratio by two different methods: First, we compared the integration of the four protons of the aromatic ring (6.2 to 8 ppm) of pSS to the backbone protons of the copolymer (2.9 to 0 ppm). This provided a SS:PEGMA ratio of 2:1. Second, we compared the integration of the four protons of the aromatic ring (6 to 8 ppm) of pSS to the PEGMA side chain protons (4.3 to 2.9 ppm). Commercial PEGMA has a mixture of PEG side chain lengths. Thus, we first determined from the spectrum of the PEGMA monomer that there were 20.5 protons in the region of 4.3 to 2.9 ppm. Using this value, the calculated SS:PEGMA ratio was 2.4:1. We report the average of these two ratios, 2.2:1 to be the composition of SS:PEGMA in the copolymer.



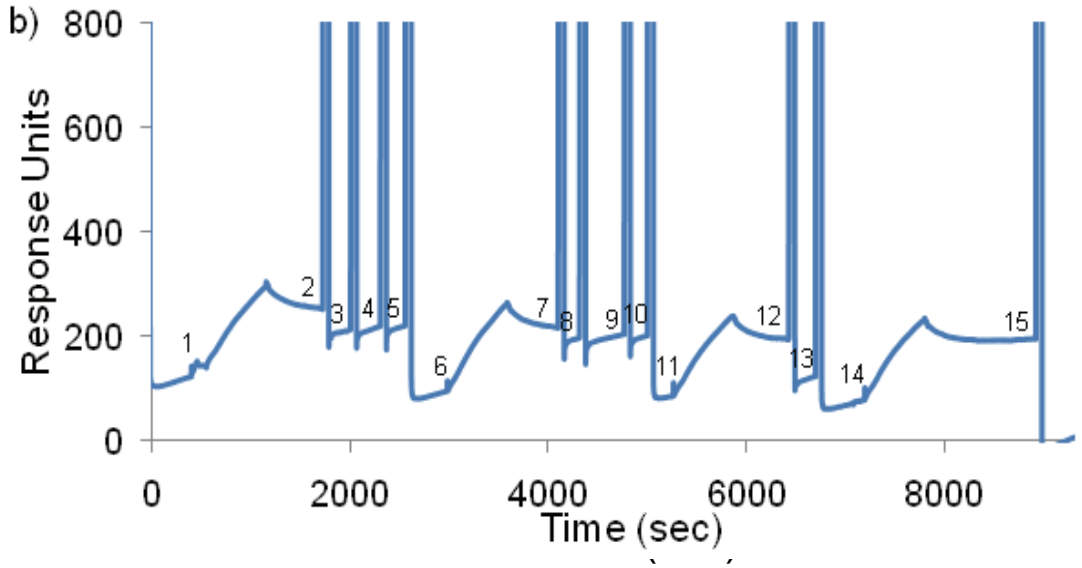


Figure S3. Salt concentration studies for a) bFGF and b) VEGF. Time course: 50 µL of 0.2 ug/mL solution of protein, 600 sec running buffer, 40 uL/ min of salt buffer for 60 sec; flow rate of 5 µL/min.

Table S1. Change in RU units upon injection of salt.

bFGF		
Injection #	Solution	Δ RU
1	0.2 µg/mLbFGF	+607
2	0.15 M NaCl	0
3	0.25 M NaCl	-18
4	0.35 M NaCl	-144
5	0.50 M NaCl	-264
6	1.0 M NaCl	-422
7	4.0 M NaCl	-532
8	Sat. NaCl	-532
9	0.2 µg/mLbFGF	+448
10	0.50 M NaCl	-214
11	4.0 M NaCl	-413
12	Sat. NaCl	-423
13	0.2 µg/mLbFGF	+453
14	0.25 M NaCl	-43
15	0.35 M NaCl	-132
16	1.0 M NaCl	-356
17	Sat. NaCl	-393

VEGF		
Injection #	Solution	Δ RU
1	0.2 µg/mL VEGF	+137
2	0.50 M NaCl	-49
3	0.50 M NaCl	0
4	0.50 M NaCl	0
5	4.0 M NaCl	-125
6	0.2 µg/mL VEGF	+126
7	0.35 M NaCl	-29
8	0.35 M NaCl	0
9	0.35 M NaCl	0
10	4.0 M NaCl	-105
11	0.2 µg/mL VEGF	+111
12	1.0 M NaCl	-82
13	4.0 M NaCl	-44
14	0.2 µg/mL VEGF	+116
15	4.0 M NaCl	-210

*Determined from the data shown in Figure S3.

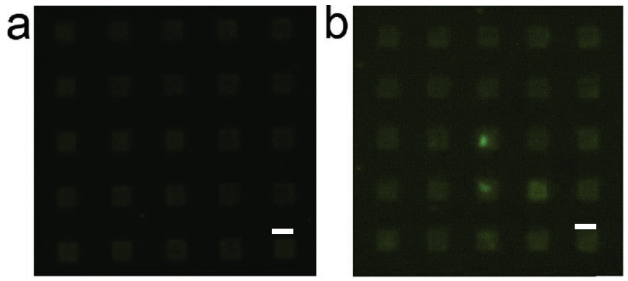


Figure S4. Control studies: Fluorescent images of a) bFGF and b) VEGF immobilized onto pSS-co-PEGMA micropatterns in the presence of heparin with antibody staining. Scale bar = 5 microns.

Determination of signal-to-noise ratio of fluorescent images. Signal-to-noise ratios (S/N) were calculated using Carl ZeissAxioVision LE v. 4.6. The S/N measured for the bFGF and VEGF micropatterns (Figure 3) were 6.7 and 2.7, respectively. The use of 10% SuperBlock solution (ThermoScientific) during protein and antibody staining steps significantly increased the measured S/N for the bFGF and VEGF micropatterns.