Electronic Supplementary Material (ESI) for Biomaterials Science.

Effect of carbon spacer length on antibacterial property of zwitterionic poly(sulfobetaine) type copolymeric brushes and its application in wound healing

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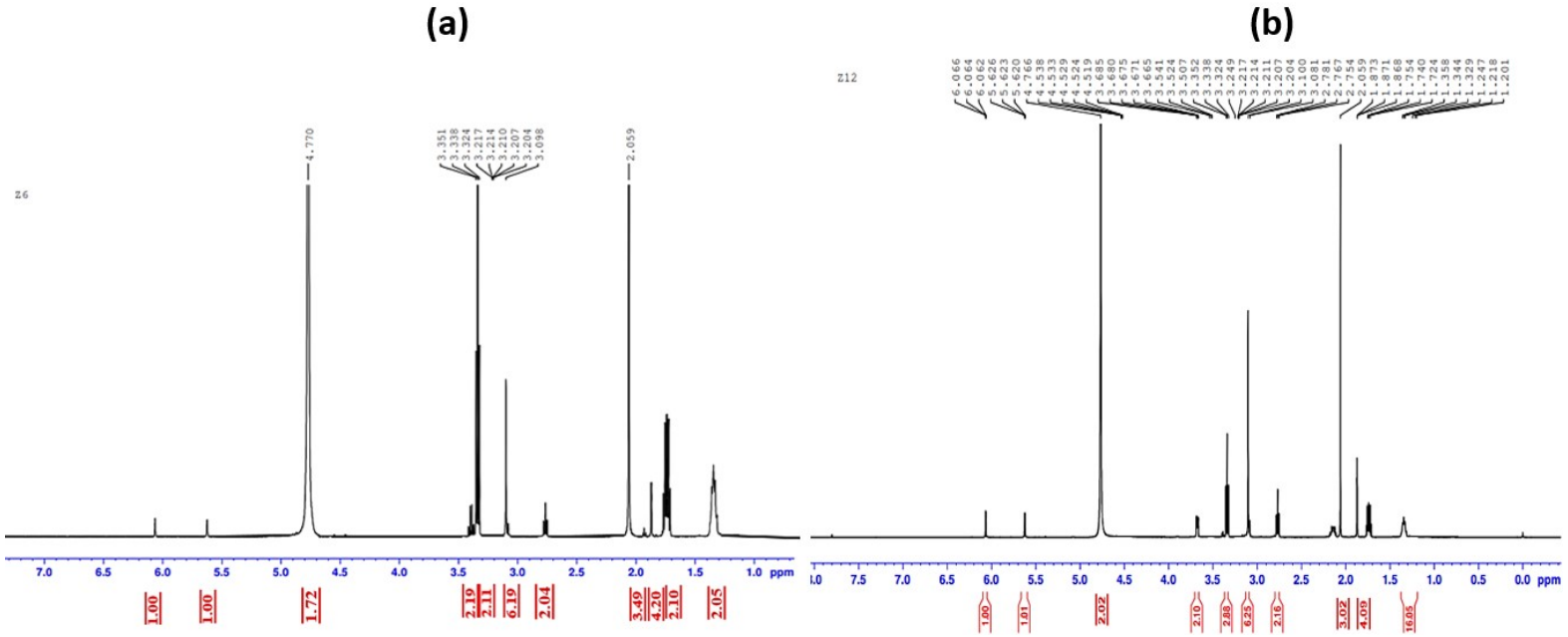


Figure S1. Proton NMR spectra for zwitterionic monomers (a) 6- {[2 (Methacryloyloxy)ethyl] dimethylammonio} hexane-1-sulfonate (Z6) (b) 12-{[2-(Methacryloyloxy) ethyl] dimethylammonio} dodecane-1-sulfonate (Z12)

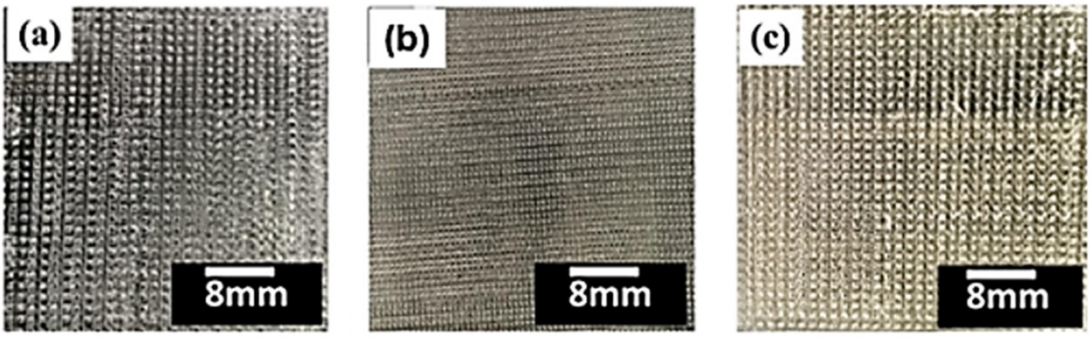


Figure S2. Pictures of Scaffold fabricated by 3D printer (a) PLA (b) Scaffold P2 (P1/PLA=5/95) [3,4], Pore size: 0.5 mm (c) Scaffold P2-g-polyZ6-co-polyZ12 (after 48 h of brush growth) Pore size: 0.456 mm (porosity ~85%).

Reference:

3. Dhingra, S., et al., *Infection resistant polymer brush coating on the surface of biodegradable polyester.* Materials Science and Engineering: C, 2021. **118**: p. 111465.

4. Dhingra, S., et al., *Cytocompatible, soft and thick brush-modified scaffolds with prolonged antibacterial effect to mitigate wound infections.* Biomaterials Science, 2022. **10**(14): p. 3856-3877.

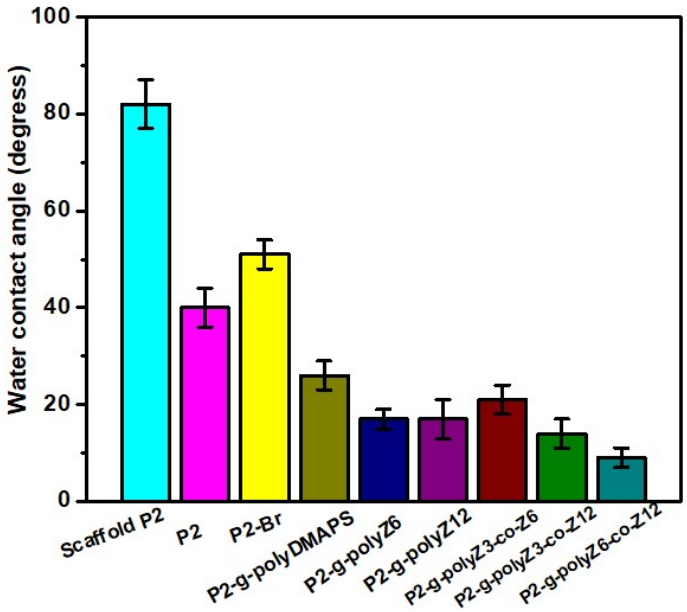


Figure S3. Variation of water contact angle after each step of surface modifications of Scaffold P2 substrate with various zwitterionic brushes. All data are shown as average + standard deviation (error bar). For all the samples, polymerization time was kept at 48 h.

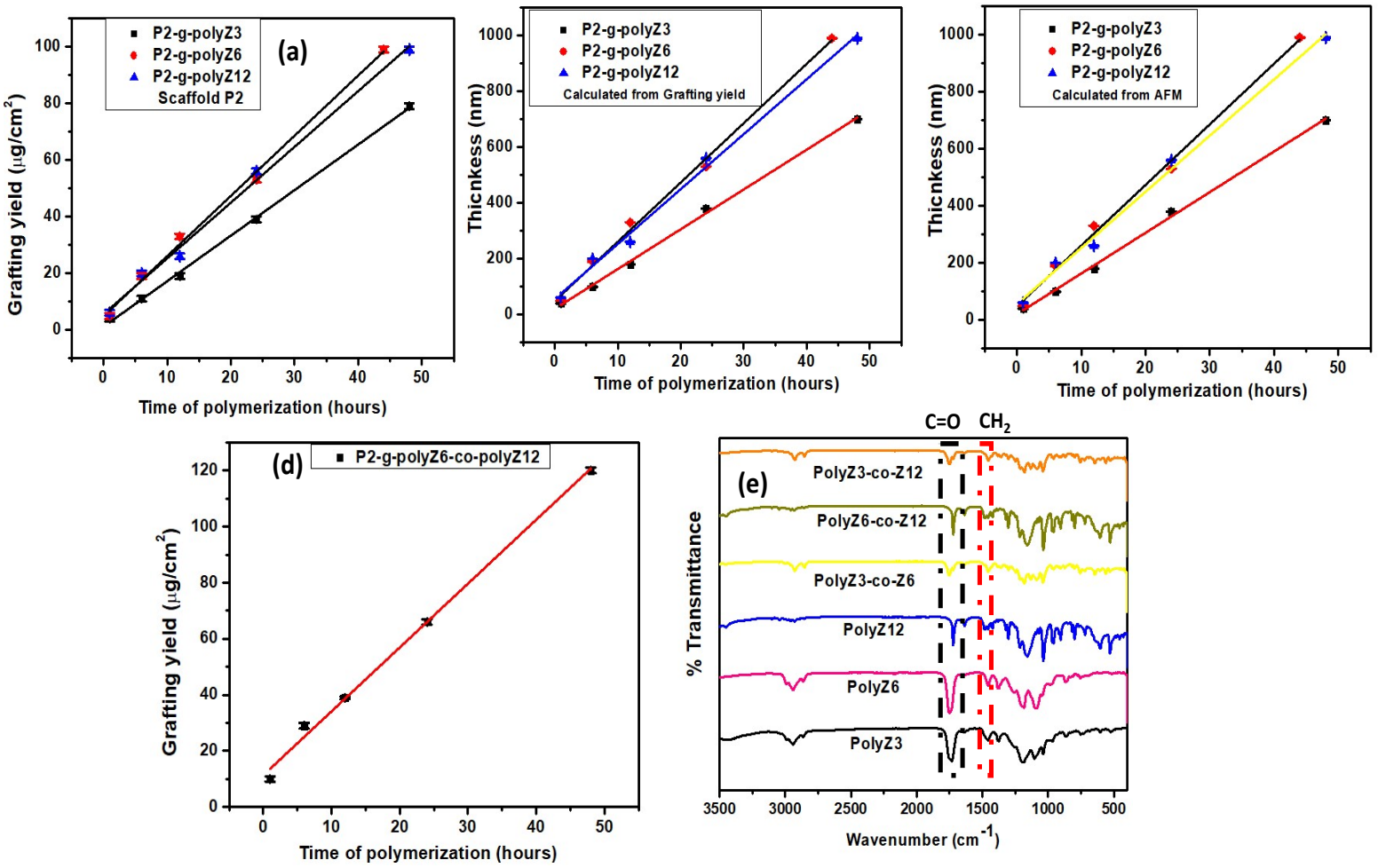


Figure S4. Surface initiated polymerization of various zwitterionic brushes on Scaffold P2 surface represented by measuring (a) 'Grafting yield' (b) Thickness calculated from grafting yield values (c) Thickness determined from AFM images; at every time interval. (d) Grafting yield' with respect to polymerization time for copolymer brush grafted on P2 surface (e) ATR-IR spectra for various copolymer brushes grafted on Scaffold P2, where polymerization time was kept at 48 h. All data points are shown as average + standard deviation (error bar).

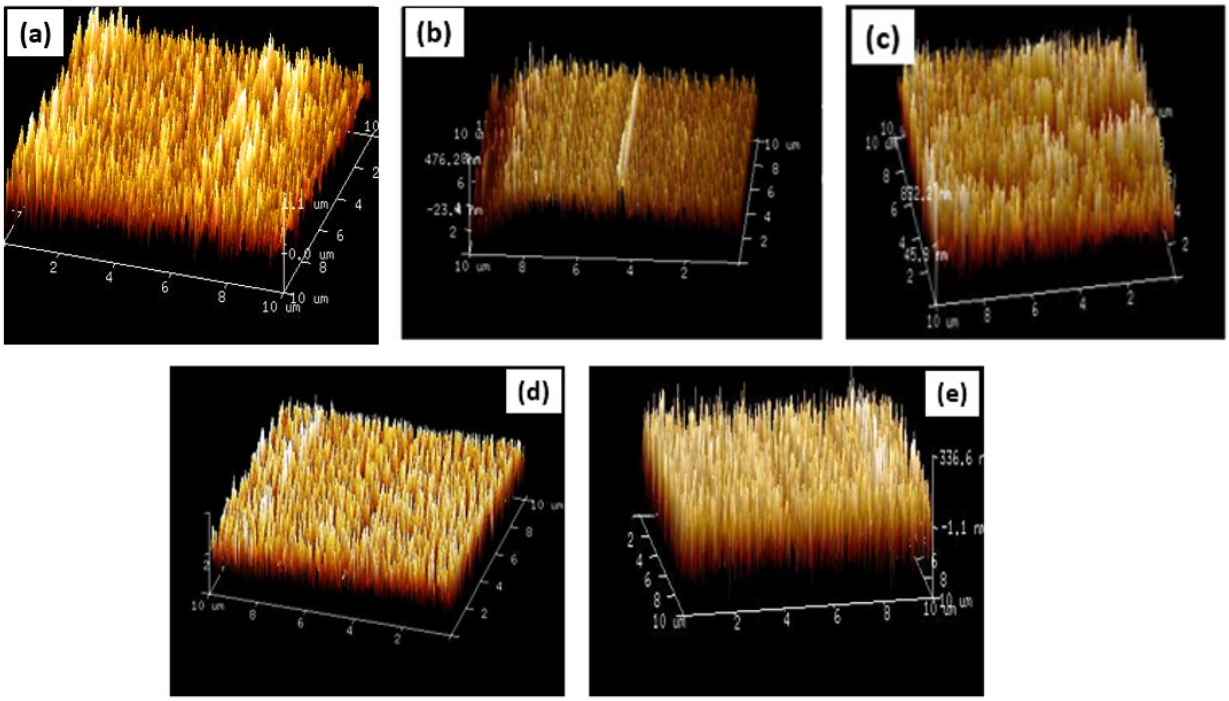


Figure S5. AFM topography images obtained for (a) P2-g-polyZ3 (b) P2-g-polyZ6 (c) P2-g-polyZ12(d) P2-g-polyZ3-*co*-polyZ6 (e) P2-g-polyZ3-*co*-polyZ12 Scale bar: 10 microns. For all the samples, polymerization time was kept at 48 h.

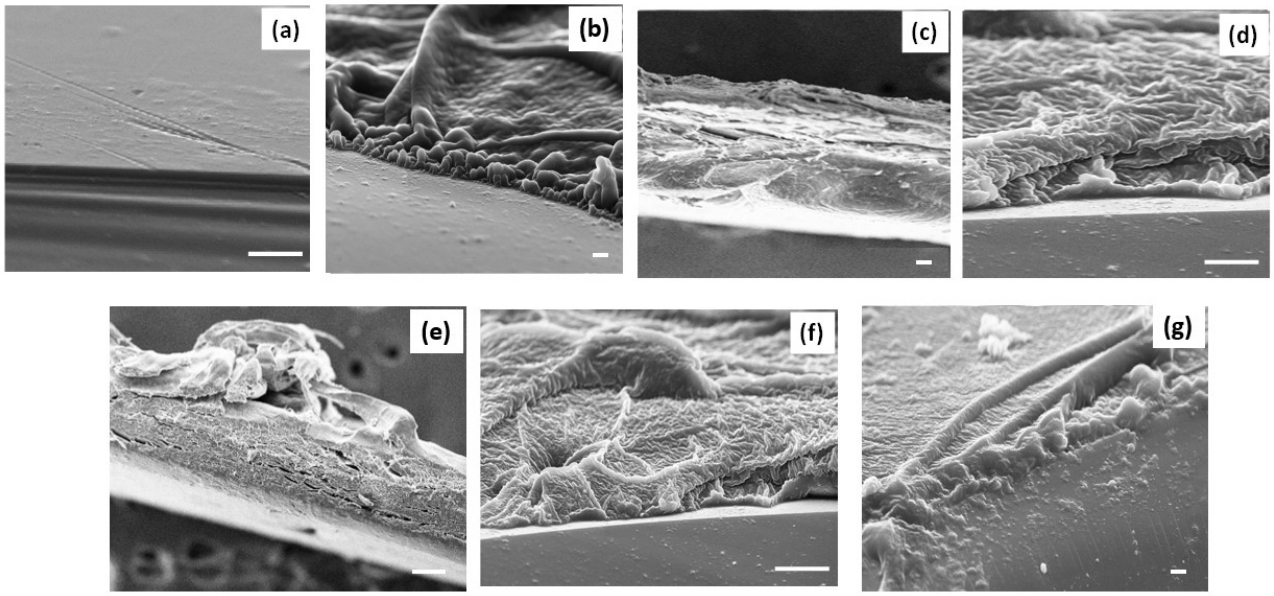


Figure S6. SEM images obtained for (a) Scaffold P2 (0.4 micron) (b) P2-g-polyZ3 (0.2 micron) (c) P2-g-polyZ6 (0.1 micron) (d) P2-g-polyZ12 (0.2 micron) (e) P2-g-polyZ3-*co*-

polyZ6 (0.3 micron) (f) P2-g-polyZ3-*co*-polyZ12 (0.2 micron) (g) P2-g-polyZ6-*co*-polyZ12 (0.4 micron). For all the samples, polymerization time was kept at 48 h. Scale bars are mentioned in the brackets.

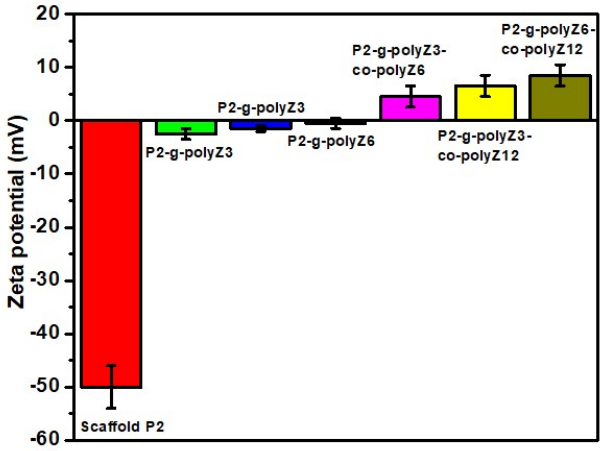


Figure S7. Evaluation of surface charge (zeta potential) for Scaffold P2 and zwitterionic brush modified Scaffold P2 surfaces at pH 7.

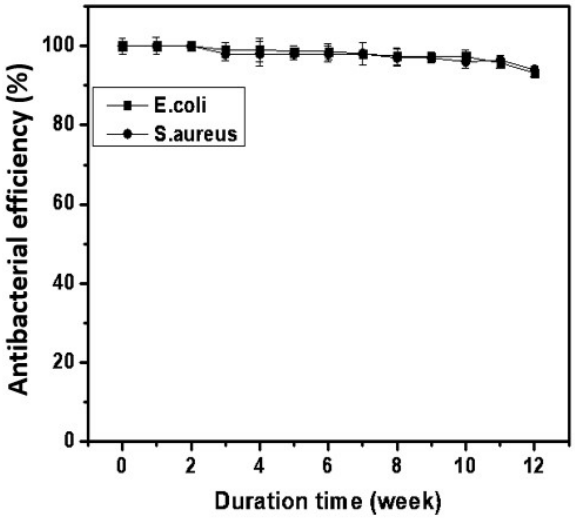
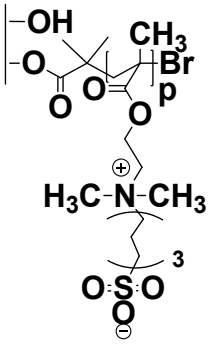
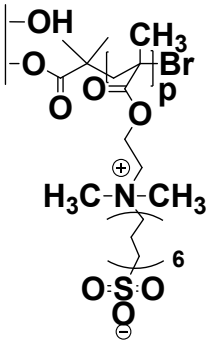
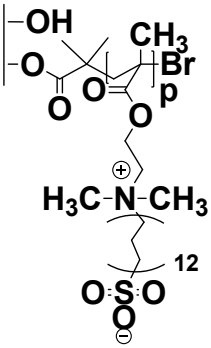


Figure S8. Statistical data for antibacterial activity determined from P2-g-polyZ6-*co*-Z12 brush modified scaffold against *E. coli* and *S. aureus* bacteria for 3 months (12 weeks). All data points are shown as average + standard deviation represented by error bar ($p \leq 0.05$).

Table S1 Chemical structure of various zwitterionic homo and copolymer brushes and their corresponding surface roughness obtained from their AFM images.

S. No	Polymer combinations	Structure of polymer/copolymer	Surface roughness	Ratio of C=O/C-H (as estimated from Figure S4e)
1	**polyZ3** poly(3-dimethyl-(methacryloyloxyethyl) ammonium propane sulfonate) and polysulfobetaine methacrylate		179 nm	2.0
2	**polyZ6** poly6-Bromo-N-(2-hydroxyethyl)-N,N dimethyl hexane-1-ammonium bromide (b)		188 nm	1.33
3	**polyZ12** poly12-Bromo-N-(2-hydroxyethyl)-N,N dimethyldodecane-1-ammonium bromide		209 nm	1.05

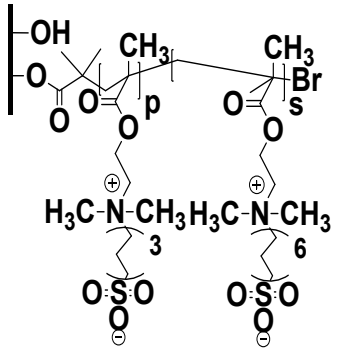
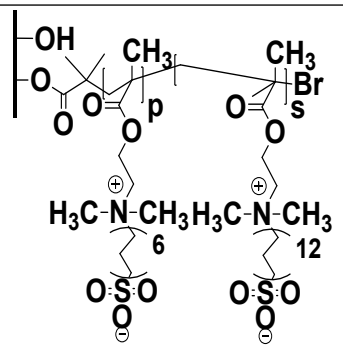
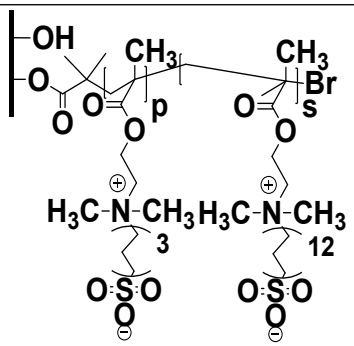
4	**polyZ3-co-polyZ6**		210 nm	1.59
5	**polyZ6-polyZ12**		389 nm	1.15
6	**polyZ3-co-polyZ12**		259 nm	1.48

Table S2 Characteristic IR peaks along with their corresponding assignments for various zwitterionic polymer brushes.

S. No	Functional group	Characteristic peak
1.	O-H stretching	3456 cm -1
2.	$\nu_{(C=O)}$	1757 cm^{-1}
3.	C-N^+ bending vibrations	1458 cm^{-1}
4.	CH_2 bending vibrations	1400 cm^{-1} to 1460 cm^{-1}
5.	S--O stretch	1055 cm -1 to 1270 cm -1
6.	resonating motion of the three oxygen atoms in sulfonate	690 cm^{-1}
7.	C-Br stretching	624 cm^{-1}

Table S3. Measurements of surface charges for homo and copolymer brushes at pH 7

S. No	Surface charge at pH 7
1 polyZ3	-2.5±0.8
2 polyZ6	-1.5±0.9
3 polyZ12	-0.5±0.09
4 polyZ3-co-polyZ6	+4.5±0.9
5 polyZ3-co-polyZ12	+6.5±0.6
6. polyZ6-co-polyZ12	+8.5±0.9

All data points are shown as average + standard deviation.

Table S4. Water absorption data (in percentage) for various brush modified scaffold surfaces

S. No	Sample Name	Water hydration in 24 hours
1.	polyZ6-co-polyZ12	25.9 μg/cm^2,23%
2	polyZ3-co-polyZ6	23.4 μg/cm^2,23.1%
3	polyZ6-co-polyZ12	24.8 μg/cm^2,24.1%
4	polyZ6	9.38 μg/cm^2, 10.4%
5	polyZ12	10.38 μg/cm^2, 11.4%
6	polyZ3	9 μg/cm^2, 10.0%

Note: The water adsorption was done initially by weighing the samples and then keeping the samples dipped in PBS buffer for 24 hours, Next day they were dried and weighed again and the percentage of water adsorbed was calculated by taking the difference between after and before weighing them.