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Antibacterial, electrospun nanofibers of novel poly(sulfobetaine) and poly(sulfabetaine)s

Vivek Arjunan Vasantha,*[a] Siti Zarina Zainul Rahim,[b,c] Sundaramurthy Jayaraman,[d,e] Gabriel Han Junyuan,[d] Sreenivasa Reddy Punireddy,[f] Seeram Ramakrishna,[e] Serena Lay-Ming Teo[c] and Anbanandam Parthiban*[a]

[a]*Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (A*STAR), 1 Pesek Road, Jurong Island, Singapore 627833.*

[b]*Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, Singapore 119227*

[c]*Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543*

[d]*Environmental & Water Technology Centre of Innovation, Ngee Ann Polytechnic, 535 Clementi Road, Singapore 599489*

[e]Department of Mechanical Engineering, *National University of Singapore, 2 Engineering Drive 3, Singapore 117576*

[f]Institute of Materials Research and Engineering (IMRE)*, Agency for Science, Technology and Research (A*STAR), 2 Fusionopolis Way, Innovis, Singapore 138634.*

**E-mail: vivek_vasantha@ices.a-star.edu.sg; aparthiban@ices.a-star.edu.sg*

Supporting Information

1. Biological Evaluation – Static bacterial adhesion assay

1.1 Growth of biofilm

Coupons were pre-wet in sterile, 0.22μM filtered, 27ppt seawater (FSW). A vacuum was applied in order to remove bubbles from hydrophobic surfaces and then coupons allowed to soak overnight. Six coupons from each treatment were cleaned by dipping into sterile FSW three times using tweezers. Coupons were then randomized and placed into separate containers (6 well plates) filled with 5ml of sand-filtered natural seawater at the Tropical Marine Science Institute marine station, St John's Island.

1.2 Extraction of DNA

After 48 hours of incubation at 27 °C, coupons were rinsed in an excess of sterile FSW twice and all water removed using a micropipette. Four coupons from each treatment were selected for DNA extraction whilst the remainder were selected for microscopy. Biofilms were removed from coupon surfaces via 2 hour digestion at RT with 1% enzymatic cleaner (3M) diluted in calcium and magnesium free artificial seawater (CMFASW). [1] DNA was extracted from the enzyme solution using a modified phenol:chloroform:isoamyl alcohol (Sigma) protocol followed by DNeasy blood and tissue kit (Qiagen) spin column purification as previously described.[2]

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1.3 Biofilm fingerprinting

Fingerprinting was conducted via ribosomal intergenic spacer analysis (RISA) and terminal restriction fragment length polymorphism (TRFLP). For RISA, universal primers S-D-Bact-1522-b-S-20 and L-D-Bact-132-a-A-18 [3] were used to amplify the intergenic regions. Amplicons were run on 2% agarose gel to qualitatively compare the bacterial community. For TRFLP, the 16s rDNA was amplified using universal bacterial primers 926F [4] and 1392R [5] modified with 6-FAM and ATTO565 fluorescent dyes respectively. PCR amplicons were then purified using Qiaquick PCR purification kit (Qiagen) and digested with AluI and Bsu361 (New England Biolabs). Capillary electrophoresis and fragment analysis were then conducted using the ABI 3730 sequencer (Applied Biosystems).

1.4 Fluorescence microscopy

Bacterial biofilms were preserved by fixing in 2.5% glutaraldehyde for 30 minutes before staining with Baclight Live/Dead bacterial viability kit (Molecular probes) as previously desribed.[6, 7] Each coupon was washed by dipping twice into sterile FSW before being visualized under the microscope under fluorescence. Twenty micrographs were taken at randomly selected locations on each coupon at 400X air objective using an Olympus BX51 microscope. Each micrograph covered approximately 0.04 mm^2 area. Percentage and total number of viable cells (fluorescing green) were calculated using data obtained via a modified cell counter macro for analysis of micrographs using ImageJ software (NIH). As an overwhelming majority of bacteria stained live (less than 0.01% dead, or red, bacteria in total), only total number of viable cells were compared.

1.5 Statistical analysis

TRFLP data was transformed into an absence/presence matrix and exported using Genemapper (Applied Biosystems), then analysed via non-metric multidimensional scaling (NMDS) using Bray-Curtis distance measures and k=2 in conjunction with the function “ordiellipse” in package “vegan” to delimit 95 % confidence intervals around the clusters of treatments. Bacterial density data were organized in Microsoft Excel 2007 and normalized to cells per mm^2. Then, data were analyzed for normality and homoscedasticity using the Wilkes-Shapiro and Bartlett tests followed by a Kruskal-Wallis test in package “agricolae” in order to determine significant differences between each treatment. In both cases, significance was

defined as $p < 0.05$. Data were analysed using the R software package (Development Core Team 2010).

2. Thermal stability of polysulfo(a)betaines

Table 1S. Thermal stability of the polyzwitterionics

Polymer	Sample weight (mg)	5 % Weight loss Temperature (°C)	First Stage		Second Stage		Solid[b] (%)
			[a]Thermal Decomposition (Stage I)	Weight Loss	[a]Thermal Decomposition (Stage II)	Weight Loss	
PSB **1**	19.4	358	380	16	460	34	56
PSB **2**	18.7	320	406	33	514	39	58
PSB **3**	17.7	285	289	14	422	58	34
PSB 4	20.4	296	301	20	421	64	30

Experimental conditions: Rate 10 °C /min under nitrogen; [a]thermal decomposition temperature from DrTGA; [b]solids residues at 600 °C.

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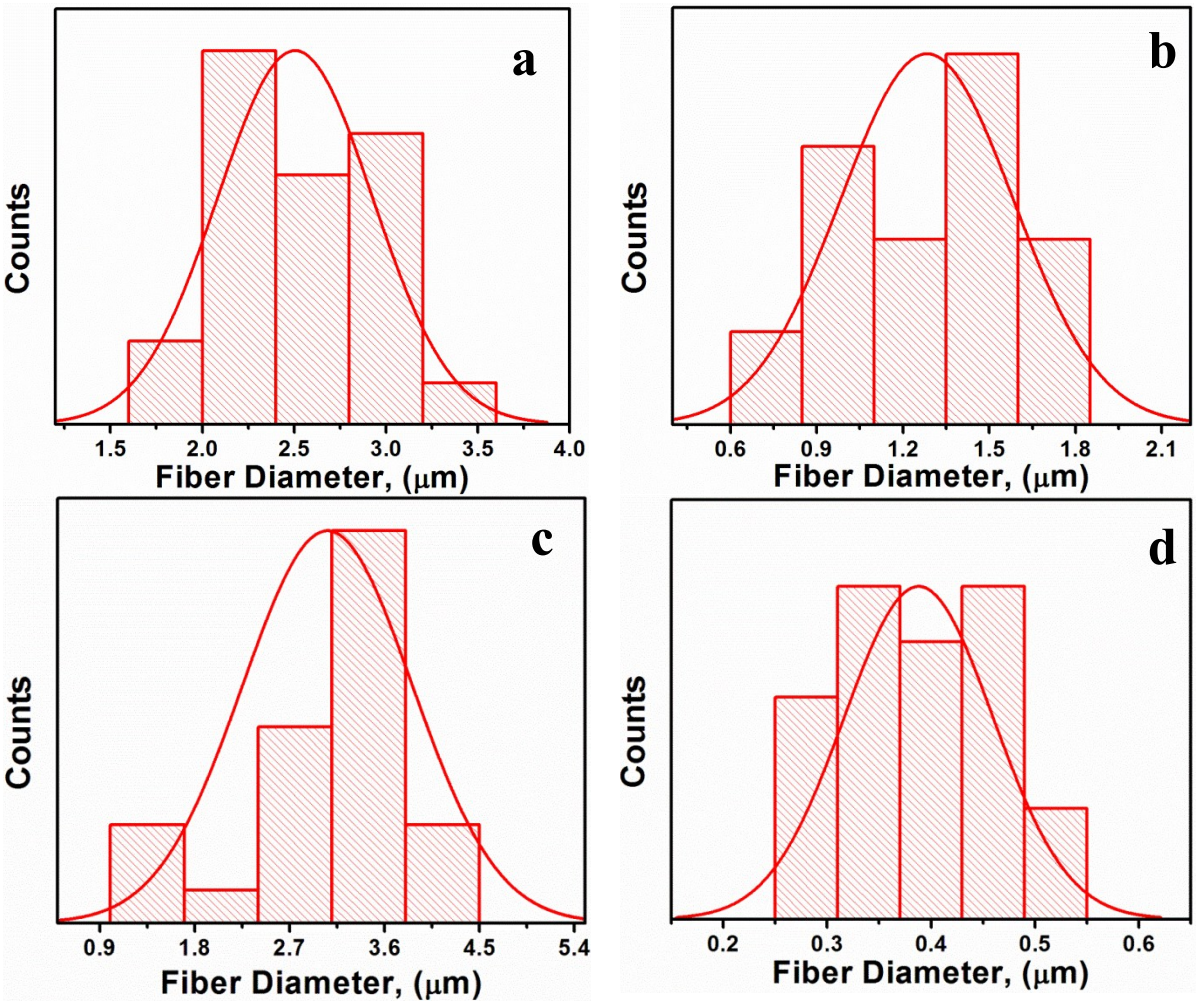

Fig S1 Fiber distribution of PSBs, PSB 1 (a); PSB 2 (b); PSB 3 (c); PSB 4 (d).

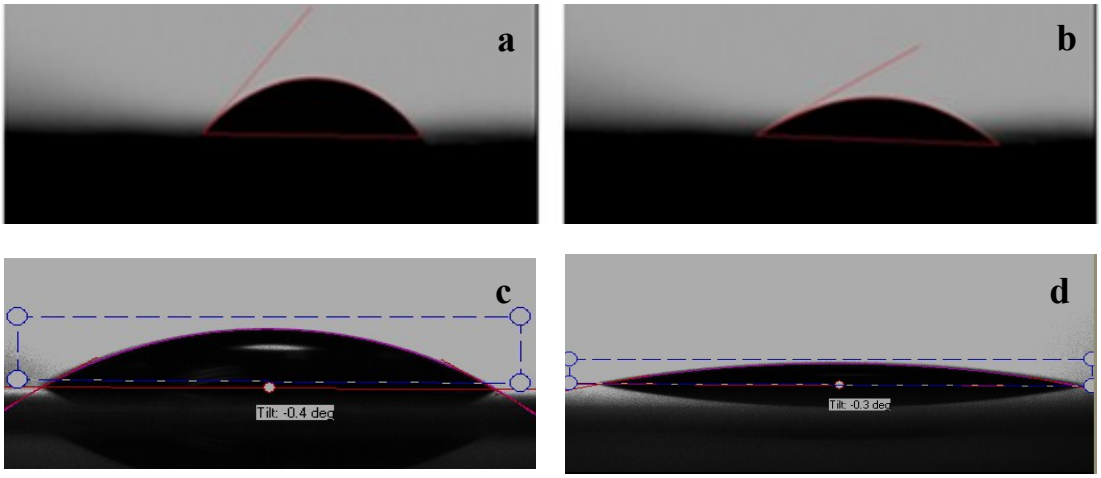

Fig S2 Static water contact angle measurements of electrospun nanofibers of PSB 3 and PSB 4. PSB 3 (WCA – 50.4°, 0 min) (a); PSB 3 (WCA – 30.9°, 2 min) (b); PSB 4 (WCA – 32.5°, 0 min) (c); PSB 4 (WCA – 12.1°) (d).

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