Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

SUPPLEMENTARY INFORMATION SECTION

A. EXPERIMENTAL

1. Chemicals

Dichloromethane 98% (Kimix), sodium chloride 98% (Sigma-Aldrich), acetone (Protea Chemicals), glucosamine hydrochloride 99.5% (Calbiochem), mannosamine hydrochloride 99.5% (Calbiochem), dimethyl sulfoxide (Kimix), styrene 99.5% (Fluka Chemika), maleic anhydride 99% (Acros organics), methyl ethyl ketone 99.7% (Sigma-Aldrich), iso-propanol (Kimix), n-hexane (Kimix), horseradish peroxidase (Sigma), anti-HRP antibodies (Sigma), anti-rabbit IgG (Sigma), 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt (Sigma), nitro blue tetrazolium (Sigma), concanavalin A (Sigma), ninhydrin (Sigma), Tween 20 (Sigma), phenol (Sigma), potassium cyanide 98% (Fluka), casein (Sigma), thimerosal (Sigma), N_{α},N_{α}-bis(carboxymethyl)-L-lysine hydrate 97% (Aldrich), 1-amino-3-chloropropane hydrochloride 98% (Aldrich), 4-dimethylaminopyridine 99% (Sigma), dichloromethane (Kimix), 1,2-dichloroethane (Sigma), chloroform (Kimix), di-*tert*-butyl-dicarbonate 97% (Aldrich), acetonitrile (Sigma), 1-propylamine 99% (Sigma), 1-bromododecane 97% (Aldrich), 1-bromohexadecane 97% (Aldrich), ammonia solution (Merck), dodecylamine 99% (Aldrich), hexadecylamine 98% (Aldrich), 3-(*N*,*N*-dimethylamino)-1-propylamine 99% (Aldrich), bromoethane 99% (Merck) and diethyl ether (Kimix) were used without further purification. 2,2' Azobis(isobutyronitrile) (AIBN) (Riedel de Haen) was recrystallized twice from methanol and dried under vacuum before use. *N*,*N*-dimethylformamide 97% (Fluka), triethylamine 99.5% (Sigma-Aldrich), ethanol (Sasol), pyridine 99% (Sigma), ethyl acetate (Sasol) and methanol (Sasol) were distilled and kept on 4 Å molecular sieve before use. Sodium acetate 99% (Sigma), manganese(II) chloride 98% (Sigma), calcium chloride (Merck), potassium chloride (Merck), sodium hydrogen phosphate 99% (Sigma), potassium dihydrogen phosphate 99% (Holpro) and potassium carbonate 99.5% (Unilab), received in a moisture-free state, was further dried at 120 °C for 24 hours just before use.

2. Analysis techniques

a) Nuclear magnetic resonance spectroscopy (NMR)

^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Varian VXR 400 MHz instrument equipped with a Varian magnet (7.0 T). Depending on the solubility of the synthesized compounds, deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as solvents. All chemical shifts are reported in ppm downfield from tetramethylsilane (TMS), used as an internal standard (δ = 0 ppm).

b) Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy

Infrared spectra were recorded using a Nicolet FTIR spectrometer (model Nexus) from Thermo-Fischer equipped with a Smart Golden Gate ATR accessory with a diamond/ZnSe internal reflection crystal. The spectra were recorded from 4000 cm^{-1} to 600 cm^{-1} with a spectral resolution of 4 cm^{-1} and were the sum of 64 individual scans. No sample preparation was necessary and samples were in solid state. Omnic software was used for data acquisition and processing.

Conversion Calculation: The calculation of the conversion percentage when SMA was functionalized with a surface-functionalization agent to yield the corresponding functionalized SMI was done as follows. The mode of each relevant IR spectrum was changed from transmission to absorbance and the baseline was corrected. The absorbance band from the aromatic styrene unit at 703 cm^{-1} was used as internal standard as the styrene group did not partake in the surface-functionalization reaction and was deemed a constant. The absorbance band from the carbonyl group of the anhydride unit at 1774 cm^{-1} was used to determine the extent of the functionalization reaction as the anhydride unit reacted with the primary amine of the surface-functionalization agent to form an imide, thus giving rise to a new band at ~ 1700 cm^{-1} with a corresponding decrease in the peak at 1774 cm^{-1}.

The heights of the styrene peak at 703 cm^{-1} and the carbonyl peak at 1774 cm^{-1} were taken, before and after functionalization, as measure of the fraction of carbonyls converted to imides by calculating the decrease in the height of the carbonyl peak of the functionalized SMI in relation to that of pristine SMA where the styrene peak at 703 cm^{-1} is constant.

c) Size exclusion chromatography (SEC)

Molar mass and dispersity (*Đ*) were obtained using size exclusion chromatography (SEC). Size exclusion chromatography (SEC) analysis was carried out on a DMAc solvent system using a flow rate of 1.0 mL/min. The instrument setup consisted of a Shimadzu LC-10AD pump, a Waters 717Plus autosampler, a column system fitted with a 50x8 mm guard column in series with three 300x8 mm, 10 µm particle size GRAM columns (2 x 3000Å and 100Å) obtained from PSS, a Waters 2487 dual wavelength UV detector and a Waters 410 differential refractive index (DRI) detector all in series. 100 µL injection volumes are sampled individually with the oven temperature of the column and DRI detector kept at 40 °C. The solvent was stabilized with 0.05% BHT (*w/v*) and 0.03% LiCl (*w/v*), and samples were filtered through a 0.45 µm GHP filter to prevent any impurities entering the system. Calibration was done using PMMA standards (Polymer Laboratories) ranging from 690 to 1.2 x 10^6 g/mol. Data acquisition was done using Millennium software, version 4.

3. Methods

3.1 Synthesis of poly(styrene-*co*-maleic anhydride) (SMA)

Conventional radical copolymerization was used to synthesize an alternating copolymer of styrene and maleic anhydride in a 1:1 molar ratio styrene:maleic anhydride.[1]

Maleic anhydride (MAnh) (14 g, 0.14 mol), styrene monomer (15 g, 0.14 mol) and 2,2' azobis(isobutyronitrile) (AIBN, 0.1182 g, 7.20*10^{-4} mol) were dissolved in 200 mL methyl ethyl ketone (MEK). The reaction mixture was degassed with N_2 for 30 minutes and stirred overnight (16 hours) at 60 °C. The reaction mixture was cooled to room temperature, precipitated in 500 mL iso-propanol and washed with n-hexane. The polymer was dried under vacuum at room temperature to remove any unreacted monomer and residual solvent and then analyzed using SEC. M_n = 128 000 g/mol, *Đ* = 2.7

Major IR absorptions: 3061, 2929, 1854, 1774, 1602, 1494, 1453, 1219, 1079, 953, 922, 763, 703 cm^{-1}.

^{1}H-NMR (acetone-d_6): δ (ppm) = 6.9-7.7 (s broad, 5H aromatic), 3.66 (s broad, 2H, C**H**-C**H**-), 2.29 (s broad, 3H, -C**H$_2$**-C**H**-)

^{13}C-NMR (acetone-d_6): δ (ppm) = 172.55 (-O-**C**=O), 137.61 (aromatic -**C**-), 129.05 (aromatic -**C**-), 52.04 (-**C**H-), 42.13 (-**C**H-), 35.65 (-**C**H$_2$-)

3.2 Electrospinning of SMA

SMA was dissolved in a 1:2 DMF:acetone solution (15 wt. %).[2] The prepared solution was placed in a 1 mL plastic syringe connected to a syringe pump (Harvard, Model 33 Twin Syringe Pump). An electrode lead of a high voltage power supply capable of generating positive DC voltages from 0 to 25 kV was connected to the blunt metal needle of the syringe. The positive potential was set at + 7.5 kV. The flow rate was set at 0.01 mL/min and the needle diameter was 21 gauge. A stationary foil covered collector was placed 15 cm from the needle tip and connected to a negative electrode. The negative potential was set at - 7.5 kV. The collected electrospun fibers were placed under vacuum at 50 °C to remove any residual solvents.

3.3 Surface-modification of SMA nanofibers

a) Surface-modification of SMA nanofibers with amino sugars (glucosamine and mannosamine) to yield 1

i) Synthesis of SMI-Gluc

Glucosamine hydrochloride (344 mg, 1.6 mmol)) and triethylamine (TEA, 162 mg, 1.6 mmol) in 2.5 mL iso-propanol was stirred at 60 °C until dissolved.[15] The glucosamine solution was cooled to room temperature. The electrospun SMA fibers (215 mg) were placed in a petri dish and covered with the prepared glucosamine solution.[3,4] The petri dish was closed and rotated on a belly dancer laboratory shaker for 3 hours at ambient conditions. The functionalized fibers were removed and washed with iso-propanol three times. The fibers were air dried, followed by heating under vacuum at 60 °C for 24 hours. The dried fibers were washed with distilled water three times and dried at room temperature.

Major IR absorptions: 3281, 2944, 1699, 1645, 1538, 1454, 1396, 1176, 1030, 950, 838, 766, 703 cm^{-1}.

ii) Synthesis of SMI-Man

The same method was followed as 3.3 (a)(i) using 344 mg mannosamine hydrochloride (1.6 mmol) and 162 mg TEA (1.6 mmol).

Major IR absorptions: 3383, 2925, 1704, 1553, 1494, 1454, 1360, 1175, 1030, 762, 700 cm^{-1}.

Major IR absorptions: 3377, 2926, 1777, 1712, 1602, 1538, 1494, 1453, 1217, 1071, 1029, 915, 758, 698 cm^{-1}.

b) Surface-modification of SMA nanofibers with amino acid derivative (BCML) to yield 2

N_{α},N_{α}-bis(carboxymethyl)-L-lysine hydrate (BCML, 330 mg, 1.26 mmol) and TEA (128 mg, 1.28 mmol) were dissolved in 2 mL iso-propanol. The electrospun SMA fibers (170 mg) were placed in a petri dish and covered with the prepared BCML solution.[3,4] The petri dish was closed and rotated on a belly dancer laboratory shaker for 2 hours at ambient conditions. The functionalized fibers were removed and washed with iso-propanol three times. The fibers were air dried, followed by heating under vacuum at 130 °C for 24 hours. The dried fibers were washed with distilled water three times and dried at room temperature.

Major IR absorptions: 3402, 2943, 1704, 1495, 1454, 1178, 1103, 917, 762, 701 cm^{-1}.

c) Surface-modification of SMA nanofibers with protein (Concanavalin A) to yield 3

A 4 mg/mL solution of Concanavalin A in PBS buffer (8 g NaCl, 0.2 g KCl, 11.5 g Na_2HPO_4, 0.2 g KH_2PO_4, pH 7.4) was prepared. $MnCl_2$ (0.013 mg/mL) and $CaCl_2$ (0.011 mg/mL) were added to the solution.[5-8] The electrospun SMA fibers (36 mg) were placed in a petri dish and covered with the Con A/PBS solution.[3,4] It was incubated for one hour at 37 °C on a belly dancer laboratory shaker. The fibers were subsequently washed three times, for 10 min at a time, with PBS-Tween buffer (PBS, 0.01% Tween 20) to remove any unreacted protein adsorbed on the nanofibrous surface. The fibers were air dried, followed by heating under vacuum at 80 °C for 24 hours.

Major IR absorptions: 3350, 2930, 2649, 1774, 1699, 1655, 1539, 1495, 1456, 1393, 1068, 1034, 766, 702 cm^{-1}.

i) Ninhydrin test

A ninhydrin test was performed to verify that the protein, Concanavalin A, was immobilized on the electrospun SMA fibers in experiment 3.3c).[9]

An 0.5 mg sample of SMI-Con A was placed in a polytop. To the polytop was added 50 µL ninhydrin in ethanol solution (500 mg ninhydrin in 10 mL 95% absolute ethanol), 50 µL phenol in ethanol solution (40 g phenol in 10 mL 95% absolute ethanol) and 50 µL KCN in pyridine solution (2 mL 0.001M KCN solution in distilled water, diluted to 100 mL with distilled pyridine). The polytop was heated at 90 °C for 5 minutes and a colour change was observed from colourless to dark blue. This colour change is indicative of a positive test result for the presence of protein.

Pristine electrospun SMA fibers and ninhydrin reagents (without SMA) were used as negative control and Con A was used as positive control.

ii) Immobilization of Horseradish peroxidase (HRP) on SMI-Con A

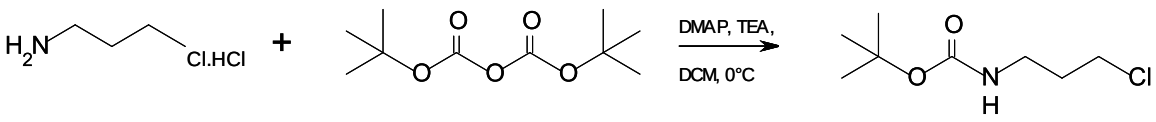
This test was performed to verify that the protein, Concanavalin A, was immobilized on the electrospun SMA fibers in experiment 3.3c) with its biological activity still intact.[10,11]

The SMI-Con A nanofibers were incubated in casein buffer (10 mM TRIS, pH 7.6, 0.15 M NaCl, 0.5% Casein, 0.02% Thimerosal) for 20 minutes at 37 °C in order to block all non-specific sites. The fibers were subsequently washed three times, for 10 min at a time, with PBS-Tween buffer (PBS, 0.01% Tween 20). The casein blocked SMI-Con A nanofibers were now incubated with HRP (4 mg/mL solution of HRP in PBS buffer) for 60 minutes at 37 °C followed by wash steps as previously described. The fibers were now incubated with anti-HRP antibodies (1:10 000 in casein buffer) for 2 hours at 37 °C followed by wash steps as previously described. A secondary antibody (anti-rabbit IgG, 1:20 000) was then added to the fibers and incubated for 2 hours at 37 °C followed by wash steps as previously described. The substrate was now added to the washed fibers, consisting of 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt/nitro blue tetrazolium (BCIP-T/NBT): 33 µL of BCIP-T(50 mg/mL in DMF) and 44 µL of NBT (75 mg/mL in 70% DMF) in 10 mL of alkaline phosphatase buffer (100 mM TRIS, pH 9.5, 100 mM NaCl, 10 mM $MgCl_2$) and incubated at room temperature. A colour change was observed from light green to dark blue. This colour change is indicative of a positive test result for the presence of biologically active Con A.

Pristine electrospun SMA fibers were used as negative control and Con A as positive control.

d) Surface-modification of SMA nanofibers with *N*-dodecyl-*N*-propyl-propane-1,3-diamine to yield 6

i) Boc protection of 1-amino-3-chloropropane



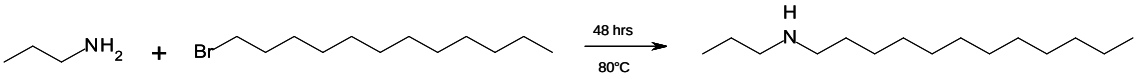
Scheme 1 Synthesis of N-tert-butoxycarbonyl-1-amino-3-chloropropane.

1-Amino-3-chloropropane hydrochloride (5 g, 38 mmol), 4-dimethylaminopyridine (DMAP, 0.24 g, 19 mmol) and triethylamine (TEA, 8.44 g, 84 mmol) in 7.5 mL dichloromethane (DCM) was stirred at room temperature until dissolved. To this solution was added dropwise a solution of di-*tert*-butyl-dicarbonate (t-Boc, 12.4 g, 57 mmol) in 7.5 mL DCM at 0 °C over 1 hour. The reaction was stirred for 24 hours at room temperature whereafter it was concentrated under vacuum. The residue was taken up in ethyl acetate and washed twice with saturated NaCl. The organic phase was dried over K_2CO_3, filtered and concentrated under vacuum to give the *N-tert*-butoxycarbonyl derivative in 79% yield.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.65 (s broad, 1H, -CO-N**H**-CH_2-), 3.53 (t, 2H, Cl-C**H$_2$**-CH_2-), 3.22 (quartet, 2H, -NH-C**H$_2$**-CH_2-), 1.91 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.38 (s, 9H, -C(C**H$_3$**)$_3$)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 155.98 (-**C**=O), 79.25 (-**C**(CH_3)$_3$), 42.42 (-**C**H_2-Cl), 37.97 (-**C**H_2-NH-), 32.63 (-CH_2-**C**H_2-CH_2-), 28.44 (-C(**C**H_3)$_3$)

ii) Synthesis of N-propyldodecyl-1-amine



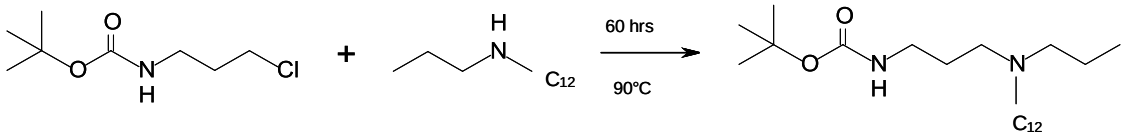
Scheme 2 Synthesis of N-propyldodecyl-1-amine.

Propylamine (2 g, 33 mmol) was added dropwise to a solution of 1-bromododecane (10.13 g, 40 mmol) in 11 mL 1:2 methanol:acetonitrile at room temperature. The reaction was refluxed at 80 °C for 48 hours whereafter it was concentrated under vacuum. The ammonium salt obtained was basified with ammonia and extracted with diethyl ether. The ether layer was washed with saturated NaCl solution to neutrality, dried over $CaCl_2$, filtered and concentrated under vacuum to yield 8.57 g of product.[28]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.7 (quartet, 2H, -CH_2-C**H$_2$**-NH-), 2.53 (m, 3H, -N**H**-C**H$_2$**-CH_2-), 1.67 (sextet, 2H, CH_3-C**H$_2$**-CH_2-), 1.54 (sextet, 2H, -CH_2-C**H$_2$**-CH_3), 1.25 (s, 18H, -(C**H$_2$**)$_9$-), 0.94 (t, 3H, C**H$_3$**-CH_2-), 0.87 (t, 3H, CH_2-C**H$_3$**)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 50.92 (-**C**H_2-NH-), 49.16 (-**C**H_2-NH-), 31.97 (-**C**H_2-), 29.71 (-**C**H_2-), 29.69 (-**C**H_2-), 29.66 (-**C**H_2-), 29.58 (-**C**H_2-), 29.38 (-**C**H_2-), 27.52 (-**C**H_2-), 22.74 (-**C**H_2-CH_3), 14.16 (-CH_2-**C**H_3), 11.90 (-CH_2-**C**H_3)

iii) Synthesis of N-tert-butoxycarbonyl-N'-dodecyl-N'-propyl-propane-1,3-diamine



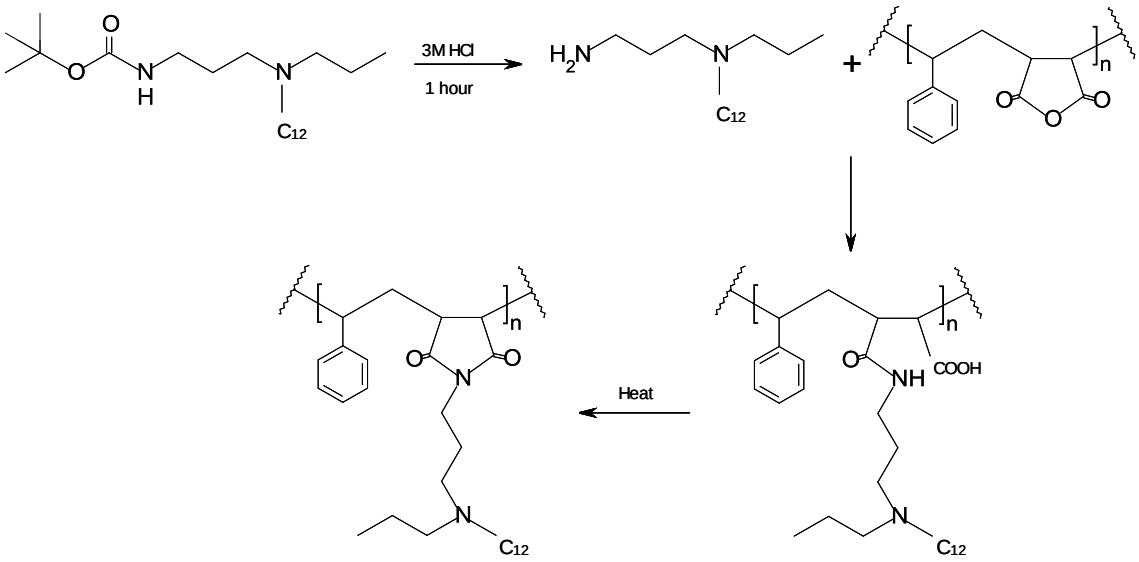
Scheme 3 Synthesis of N-tert-butoxycarbonyl-N'-dodecyl-N'-propyl-propane-1,3-diamine.

To a solution of *N*-propyldodecyl-1-amine (2 g, 8.8 mmol) in 10 mL 1,2-dichloroethane was added dropwise a solution of *N-tert*-butoxycarbonyl-1-amino-3-chloropropane (1.9 g, 10.6 mmol) in 5 mL 1,2-dichloroethane at room temperature. The reaction was refluxed at 90 °C for 60 hours whereafter it was concentrated under vacuum. The ammonium salt obtained was basified with ammonia and extracted with chloroform. The chloroform layer was washed with saturated NaCl solution to neutrality, dried over $CaCl_2$, filtered and concentrated under vacuum to yield 3.09 g of *N-tert*-butoxycarbonyl-*N'*-dodecyl-*N'*-propyl-propane-1,3-diamine.[12]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.67 (s broad, 1H, -CO-N**H**-CH_2-), 3.56 (t, 2H, -CO-NH-C**H$_2$**-), 3.24 (m, 2H, -N-C**H$_2$**-CH_2-), 2.57 (m, 2Hx2, -CH_2-C**H$_2$**-N-), 1.92 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.56 (m, 2H, C**H$_2$**-CH_3), 1.4 (s, 9H, -C(C**H$_3$**)$_3$), 1.22 (s, 20H, -(C**H$_2$**)$_{10}$-), 0.88 (t, 3H, C**H$_3$**-CH_2-), 0.83 (t, 3H, CH_2-C**H$_3$**)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 156.23 (-**C**=O), 79.40 (-**C**(CH_3)$_3$), 55.56 (-**C**H_2-N-), 53.72 (-**C**H_2-N-x2), 38.14 (-**C**H_2-NH-), 32.08 (-**C**H_2-), 29.82 (-**C**H_2-x2), 29.77 (-**C**H_2-x2), 29.61 (-**C**H_2-), 29.53 (-**C**H_2-), 28.58 (-C(**C**H_3)$_3$), 27.68 (-**C**H_2-), 27.57 (-**C**H_2-), 25.71 (-**C**H_2-), 22.85 (-**C**H_2-CH_3x2), 14.30 (-CH_2-**C**H_3), 11.90 (-CH_2-**C**H_3)

iv) Surface-modification of SMA nanofibers with N-dodecyl-N-propyl-propane-1,3-diamine



Scheme 4 Surface-functionalization of SMA nanofibers with N-dodecyl-N-propyl-propane-1,3-diamine.

N-tert-butoxycarbonyl-*N'*-dodecyl-*N'*-propyl-propane-1,3-diamine (1 g, 2.7 mmol) was dissolved in 5 mL 3 M HCl-EtOAc (1:1).[13] After stirring the reaction mixture for 1 hour, the solvent was removed under vacuum and diethyl ether was added to the remaining water layer. The organic phase was extracted, dried over $CaCl_2$, filtered and concentrated under vacuum to yield the unprotected primary amine.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.8 (s broad, 2H, N**H$_2$**-CH_2-), 3.51 (t, 2H, NH_2-C**H$_2$**-), 3.35 (m, 2H, -N-C**H$_2$**-CH_2-), 2.58 (m, 2H, -CH_2-C**H$_2$**-N-), 2.45 (m, 2H, -CH_2-C**H$_2$**-N-), 1.75 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.54 (m, 2H, -CH_2-C**H$_2$**-CH_3), 1.43 (m, 2H, -CH_2-C**H$_2$**-CH_3), 1.21 (s, 18H, -(C**H$_2$**)$_9$-), 0.86 (t, 3H, C**H$_3$**-CH_2-), 0.83 (t, 3H, CH_2-C**H$_3$**)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 54.16 (-**C**H_2-N-), 52.48 (-**C**H_2-N-x2), 45.27 (-**C**H_2-NH_2), 31.95 (-**C**H_2-), 29.64 (-**C**H_2-), 29.63 (-**C**H_2-), 29.53 (-**C**H_2-), 29.48 (-**C**H_2-), 29.37 (-**C**H_2-), 29.14 (-**C**H_2-), 26.99 (-**C**H_2-x3), 22.72 (-**C**H_2-CH_3), 14.17 (-CH_2-**C**H_3), 11.36 (-CH_2-**C**H_3)

The electrospun SMA fibers (100 mg) were placed in a petri dish and covered with a solution of *N*-dodecyl-*N*-propyl-propane-1,3-diamine (140 mg, 0.5 mmol) in 5 mL iso-propanol.[3,4] The petri dish was closed and rotated on a belly dancer laboratory shaker for 2 hours at ambient conditions. The functionalized fibers were removed and washed with iso-propanol three times. The fibers were air dried, followed by heating under vacuum at 130 °C for 24 hours.

Major IR absorptions: 2926, 2854, 1856, 1778, 1699, 1558, 1495, 1454, 1217, 1077, 920, 761, 700 cm^{-1}.

e) Surface-modification of SMA nanofibers with *N*-hexadecyl-*N*-propyl-propane-1,3-diamine to yield 10

i) Synthesis of N-propylhexadecyl-1-amine

Propylamine (2 g, 33 mmol) was added dropwise to a solution of 1-bromohexadecane (12.2 g, 40 mmol) in 10 mL 1,2-dichloroethane at room temperature. The reaction was refluxed at 90 °C for 48 hours whereafter it was concentrated under vacuum. The ammonium salt obtained was basified with ammonia and extracted with diethyl ether. The ether layer was washed with saturated NaCl solution to neutrality, dried over $CaCl_2$, filtered and concentrated under vacuum to yield 8.57 g of product.[12]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.6 (quartet, 2H, -CH_2-C**H$_2$**-NH-), 2.36 (m, 3H, -N**H**-C**H$_2$**-CH_2-), 1.53 (sextet, 2H, CH_3-C**H$_2$**-CH_2-), 1.4 (m, 2Hx2, -CH_2-C**H$_2$**-), 1.24 (s, 24H, -(C**H$_2$**)$_{12}$-), 0.9 (t, 3H, C**H$_3$**-CH_2-), 0.86 (t, 3H, CH_2-C**H$_3$**)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 51.60 (-**C**H_2-NH-), 49.75 (-**C**H_2-NH-), 31.89 (-**C**H_2-), 30.90 (-**C**H_2-x2), 29.69 (-**C**H_2-x2), 29.68 (-**C**H_2-x2), 29.65 (-**C**H_2-x2), 29.60 (-**C**H_2-), 29.59 (-**C**H_2-), 29.35 (-**C**H_2-), 27.60 (-**C**H_2-), 22.66 (-**C**H_2-CH_3x2), 14.08 (-CH_2-**C**H_3), 11.73 (-CH_2-**C**H_3)

ii) Synthesis of N-tert-butoxycarbonyl-N'-hexadecyl-N'-propyl-propane-1,3-diamine

To a solution of *N*-propylhexadecyl-1-amine (2 g, 7 mmol) in 10 mL 1,2-dichloroethane was added dropwise a solution of N-*tert*-butoxycarbonyl-1-amino-3-chloropropane (1.62 g, 8.4 mmol) in 5 mL 1,2-dichloroethane at room temperature. The reaction was refluxed at 90 °C for 48 hours whereafter it was concentrated under vacuum. The ammonium salt obtained was basified with ammonia and extracted with chloroform. The chloroform layer was washed with saturated NaCl solution to neutrality, dried over $CaCl_2$, filtered and concentrated under vacuum to yield 2.17 g of *N-tert*-butoxycarbonyl-*N*'-hexadecyl-*N*'-propyl-propane-1,3-diamine.[12]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.67 (s broad, 1H, -CO-N**H**-CH_2-), 3.56 (t, 2H, -CO-NH-C**H$_2$**-), 3.24 (m, 2H, -N-C**H$_2$**-CH_2-), 2.59 (m, 2H, -CH_2-C**H$_2$**-N-), 2.43 (m, 2H, -CH_2-C**H$_2$**-N-), 1.94 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.42 (s, 9H, -C(C**H$_3$**)$_3$), 1.26 (s, 30H, -(C**H$_2$**)$_{15}$-), 0.89 (t, 3H, C**H$_3$**-CH_2-), 0.87 (t, 3H, CH_2-C**H$_3$**)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 155.97 (-**C**=O), 79.50 (-**C**(CH_3)$_3$), 56.95 (-**C**H_2-N-), 54.32 (-**C**H_2-N-x2), 37.98 (-**C**H_2-NH-), 31.97 (-**C**H_2-), 29.72 (-**C**H_2-x9), 28.43 (-**C**H_2-), 28.40 (-C(**C**H_3)$_3$), 27.73 (-**C**H_2-), 26.49 (-**C**H_2-), 22.70 (-**C**H_2-CH_3x2), 14.11 (-CH_2-**C**H_3), 12.03 (-CH_2-**C**H_3)

iii) Surface-modification of SMA nanofibers with N-hexadecyl-N-propyl-propane-1,3-diamine

N-tert-butoxycarbonyl-*N'*-hexadecyl-*N'*-propyl-propane-1,3-diamine (1 g) was deprotected in the same manner as 3.3d)(iv).

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.75 (s broad, 2H, N**H$_2$**-CH_2-), 3.47 (m, 2H, NH_2-C**H$_2$**-CH_2-), 3.37 (m, 2H, -CH_2-C**H$_2$**-N-), 3.05 (m, 2H, -CH_2-C**H$_2$**-N-), 2.94 (m, 2H, -CH_2-C**H$_2$**-N-), 1.86 (m, 2H, -CH_2-C**H$_2$**-CH_2-), 1.76 (m, 2H, -CH_2-C**H$_2$**-CH_2-), 1.24 (s, 26H, -(C**H$_2$**)$_{13}$-), 1.0 (t, 3H, C**H$_3$**-CH_2-), 0.86 (t, 3H, CH_2-C**H$_3$**)

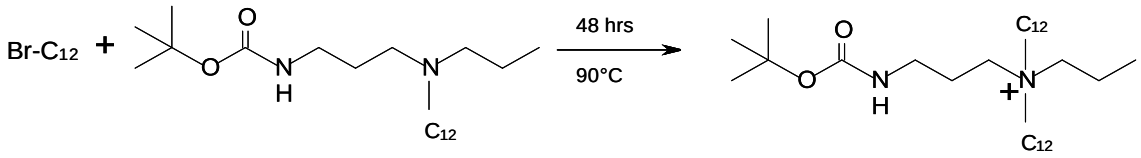
^{13}C-NMR ($CDCl_3$): δ (ppm) = 54.21 (-**C**H_2-N-), 52.58 (-**C**H_2-N-x2), 45.48 (-**C**H_2-NH_2), 32.21 (-**C**H_2-), 29.31 (-**C**H_2-), 29.97 (-**C**H_2-), 29.96 (-**C**H_2-), 29.94 (-**C**H_2-), 29.93 (-**C**H_2-), 29.85 (-**C**H_2-), 29.75 (-**C**H_2-), 29.68 (-**C**H_2-), 29.63 (-**C**H_2-x2), 27.00 (-**C**H_2-), 27.14 (-**C**H_2-), 26.11 (-**C**H_2-), 22.98 (-**C**H_2-CH_3x2), 14.32 (-CH_2-**C**H_3), 11.52 (-CH_2-**C**H_3),

The electrospun SMA fibers (100 mg) were placed in a petri dish and covered with a solution of *N*-hexadecyl-*N*-propyl-propane-1,3-diamine (180 mg, 0.5 mmol) in 5 mL iso-propanol.[3,4] The petri dish was closed and rotated on a belly dancer laboratory shaker for 2 hours at ambient conditions. The modified fibers were removed and washed with iso-propanol three times. The fibers were air dried, followed by heating under vacuum at 130 °C for 24 hours.

Major IR absorptions: 2925, 2853, 1855, 1778, 1698, 1494, 1454, 1367, 1250, 1167, 1077, 923, 761, 700 cm^{-1}.

f) Surface-modification of SMA nanofibers with *N,N*-didodecyl-*N*-propyl-propane-1,3-diamine to yield 8

i) Synthesis of N-tert-butoxycarbonyl-N',N'-didodecyl-N'-propyl-propane-1,3-diamine



Scheme 5 Synthesis of N-tert-butoxycarbonyl-N',N'-didodecyl-N'-propyl-propane-1,3-diamine.

1-Bromododecane (1.88 g, 7.5 mmol) was added dropwise to a solution of *N-tert*-butoxycarbonyl-*N'*-dodecyl-*N'*-propyl-propane-1,3-diamine (1.4 g, 3.77 mmol) in 10 mL 1,2-dichloroethane at room temperature. The reaction mixture was refluxed at 90 °C for 48 hours whereafter it was concentrated under vacuum. The mixture was crystallized from methanol/diethyl ether three times to get a pure product in 83% yield.[12]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.65 (s broad, 1H, -CO-N**H**-CH_2-), 3.51 (t, 2Hx2, -N-C**H$_2$**-CH_2-), 3.26 (m, 2H, -N-C**H$_2$**-CH_2-), 2.83 (m, 2H, -CH_2-C**H$_2$**-N-), 2.62 (m, 2H, -CH_2-C**H$_2$**-N-), 1.94 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.75 (sextet, 2Hx3, -C**H$_2$**-CH_3), 1.42 (s, 9H, -C(C**H$_3$**)$_3$), 1.24 (s, 36H, -(C**H$_2$**)$_{18}$-), 0.87 (t, 3Hx3, C**H$_3$**-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 155.90 (-**C**=O), 79.17 (-**C**(CH_3)$_3$), 56.11 (-**C**H_2-N-), 54.09 (-**C**H_2-N-x3), 37.89 (-**C**H_2-NH-), 32.64 (-**C**H_2-x2), 31.88 (-**C**H_2-x2), 29.61 (-**C**H_2-x2), 29.60 (-**C**H_2-x2), 29.58 (-**C**H_2-x2), 29.53 (-**C**H_2-x2), 29.45 (-**C**H_2-x2),

28.36 (-C(**C**H_3)$_3$), 27.60 (-**C**H_2-x2), 26.86 (-**C**H_2-x2), 25.33 (-**C**H_2-), 22.66 (-**C**H_2-CH_3x3), 14.09 (-CH_2-**C**H_3x2), 11.91 (-CH_2-**C**H_3)

ii) Surface-modification of SMA nanofibers with N,N-didodecyl-N-propyl-propane-1,3-diamine

N-tert-butoxycarbonyl-*N',N'*-didodecyl-*N'*-propyl-propane-1,3-diamine (1 g) was deprotected in the same manner as 3.3d)(iv).

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.79 (s broad, 2H, N**H$_2$**-CH_2-), 3.53 (t, 2Hx2, N-C**H$_2$**-CH_2-), 3.4 (t, 2H, -CH_2-C**H$_2$**-N-), 2.94 (m, 2Hx2, -CH_2-C**H$_2$**-N-), 1.86 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.75 (sextet, 2Hx3, -CH_2-C**H$_3$**), 1.25 (s, 36H, -(C**H$_2$**)$_{18}$-), 0.89 (t, 3Hx3, C**H$_3$**-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 53.93 (-**C**H_2-N-), 52.26 (-**C**H_2-N-x3), 45.17 (-**C**H_2-NH_2), 32.62 (-**C**H_2-x2), 31.89 (-**C**H_2-x2), 29.61 (-**C**H_2-x2), 29.60 (-**C**H_2-x2), 29.53 (-**C**H_2-x2), 29.45 (-**C**H_2-x2), 29.32 (-**C**H_2-x2), 28.88 (-**C**H_2-x2), 26.86 (-**C**H_2-x3), 22.53 (-**C**H_2-CH_3x3), 14.09 (-CH_2-**C**H_3x2), 11.20 (-CH_2-**C**H_3)

The electrospun SMA fibers (100 mg) were placed in a petri dish and covered with a solution of *N,N*-didodecyl-*N*-propyl-propane-1,3-diamine (220 mg, 0.5 mmol) in 5 mL diethyl ether.[3,4] The petri dish was closed and rotated on a belly dancer laboratory shaker for 2 hours at ambient conditions. The modified fibers were removed and washed with diethyl ether three times. The fibers were air dried, followed by heating under vacuum at 130 °C for 24 hours.

Major IR absorptions: 3398, 2926, 2854, 1856, 1777, 1698, 1568, 1495, 1454, 1402, 1218, 1078, 920, 761, 701 cm^{-1}.

g) Surface-modification of SMA nanofibers with *N,N*-dihexadecyl-*N*-propyl-propane-1,3-diamine to yield 12

i) Synthesis of N-tert-butoxycarbonyl-N',N'-dihexadecyl-N'-propyl-propane-1,3-diamine

1-Bromohexadecane (2.08 g, 6.8 mmol) was added dropwise to a solution of *N-tert*-butoxycarbonyl-*N*'-hexadecyl-*N*'-propyl-propane-1,3-diamine (1.5 g, 3.4 mmol) in 10 mL 1,2-dichloroethane at room temperature. The reaction was refluxed at 90 °C for 48 hours whereafter it was concentrated under vacuum. The mixture was crystallized from methanol/diethyl ether three times to get a pure product in 78% yield.[12]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.65 (s broad, 1H, -CO-N**H**-CH_2-), 3.51 (t, 2Hx2, -N-C**H$_2$**-CH_2-), 3.36 (m, 2H, -N-C**H$_2$**-CH_2-), 2.81 (m, 2Hx2, -CH_2-C**H$_2$**-N-), 1.95 (quintet, 2H, -CH_2-C**H$_2$**-CH_2-), 1.75 (sextet, 2Hx3, -C**H$_2$**-CH_3), 1.43 (s, 9H, -C(C**H$_3$**)$_3$), 1.24 (s, 52H, -(C**H$_2$**)$_{26}$-), 0.86 (t, 3Hx3, C**H$_3$**-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 155.89 (-**C**=O), 79.43 (-**C**(CH_3)$_3$), 56.83 (-**C**H_2-N-), 54.24 (-**C**H_2-N-x3), 37.85 (-**C**H_2-NH-), 32.61 (-**C**H_2-x2), 31.90 (-**C**H_2-x2), 29.67 (-**C**H_2-x2), 29.66 (-**C**H_2-x2), 29.65 (-**C**H_2-x2), 29.63 (-**C**H_2-x4), 29.60 (-**C**H_2-x2), 29.53 (-**C**H_2-x2), 29.45 (-**C**H_2-x2), 29.34 (-**C**H_2-x2), 28.88 (-C(**C**H_3)$_3$), 28.87 (-**C**H_2-x2), 28.37 (-**C**H_2-x2), 26.87 (-**C**H_2-), 22.68 (-**C**H_2-CH_3x3), 14.09 (-CH_2-**C**H_3x2), 11.94 (-CH_2-**C**H_3)

ii) Surface-modification of SMA nanofibers with N,N-dihexadecyl-N-propyl-propane-1,3-diamine

N-tert-butoxycarbonyl-*N',N'*-dihexadecyl-*N*'-propyl-propane-1,3-diamine (1 g) was deprotected in the same manner as 3.3d)(iv).

^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.76 (s broad, 2H, N**H$_2$**-CH_2-), 3.51 (t, 2Hx2, N-C**H$_2$**-CH_2-), 3.38 (m, 2H, -CH_2-C**H$_2$**-N-), 2.85 (m, 2Hx2, -CH_2-C**H$_2$**-N-), 1.84 (m, 2H, -CH_2-C**H$_2$**-CH_2-), 1.75 (sextet, 2Hx3, -CH_2-C**H$_3$**), 1.24 (s, 52H, -(C**H$_2$**)$_{26}$-), 0.87 (t, 3Hx3, C**H$_3$**-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 56.14 (-**C**H_2-N-), 54.73 (-**C**H_2-N-x3), 45.15 (-**C**H_2-NH_2), 32.62 (-**C**H_2-x2), 31.92 (-**C**H_2-x2), 29.68 (-**C**H_2-x2), 29.67 (-**C**H_2-x4), 29.64 (-**C**H_2-x4), 29.61 (-**C**H_2-x2), 29.54 (-**C**H_2-x2), 29.46 (-**C**H_2-x2), 29.35 (-**C**H_2-x2), 28.89 (-**C**H_2-x2), 28.35 (-**C**H_2-x2), 26.87 (-**C**H_2-), 22.65 (-**C**H_2-CH_3x3), 14.10 (-CH_2-**C**H_3x2), 11.80 (-CH_2-**C**H_3)

The electrospun SMA fibers (100 mg) were placed in a petri dish and covered with a solution of *N',N'*-dihexadecyl-*N'*-propyl-propane-1,3-diamine (268 mg, 0.5 mmol) in 5 mL diethyl ether.[3,4] The petri dish was closed and rotated on a belly

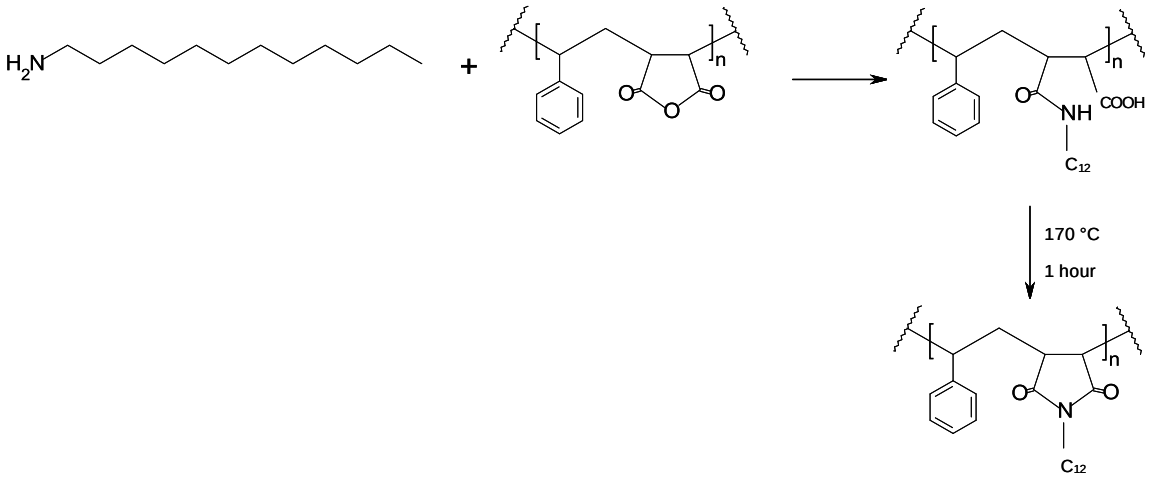
dancer laboratory shaker for 2 hours at ambient conditions. The functionalized fibers were removed and washed with diethyl ether three times. The fibers were air dried, followed by heating under vacuum at 130 °C for 24 hours.

Major IR absorptions: 3390, 2921, 2851, 1777, 1710, 1563, 1494, 1454, 1220, 922, 760, 700 cm^{-1}.

3.4 Bulk-modification of SMA

a) Synthesis of styrene-[*N*-dodecyl-maleimide] copolymer to yield 5

The synthetic work of Vermeesch and Evenson was used as basis for this procedure.[3,4]



Scheme 6 Synthesis of styrene-[N-dodecyl-maleimide] copolymer.

Dodecylamine (3.66 g, 20 mmol) was dissolved in DMF and added dropwise to a solution of SMA (2 g, 10 mmol) in 16 mL DMF at room temperature. The reaction was heated and refluxed at 170 °C for 1 hour whereafter the reaction was cooled, the polymer was precipitated in acetonitrile and filtered. The product was dried under vacuum at 60 °C for 24 hours to remove any residual solvent.

Major IR absorptions: 2924, 2853, 1697, 1584, 1495, 1454, 1401, 1352, 1159, 1079, 760, 702 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.8-7.4 (s broad, 5H, aromatic), 3.29 (s broad, 2H, -C**H**-C**H**-), 3.21 (s broad, 2H, -$(CO)_2$N-C$\mathbf{H_2}$-), 2.32 (s broad, 3H, -C$\mathbf{H_2}$-C**H**-), 1.25 (s, 20H, -(C$\mathbf{H_2}$)$_{\mathbf{10}}$-), 0.88 (s, 3H, C$\mathbf{H_3}$-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 177.58 (-N-**C**=O), 138.13 (aromatic -**C**-), 128.93 (aromatic -**C**-), 50.59 (-**C**H-), 40.37 (-**C**H-), 38.45 ($(CO)_2$N-**C**H_2-), 36.75 (-**C**H-), 32.15 (-**C**H_2-), 31.14 (-**C**H_2-), 29.88 (-**C**H_2-), 27.07 (-**C**H_2-), 22.92 (-**C**H_2-CH_3), 14.35 (-CH_2-**C**H_3)

b) Synthesis of styrene-[*N*-hexadecyl-maleimide] copolymer to yield 9

The same method was followed as 3.4a) using hexadecylamine (4.8 g, 20 mmol).

Major IR absorptions: 2923, 2853, 1696, 1602, 1495, 1455, 1402, 1352, 1161, 1032, 760, 703 cm^{-1}.

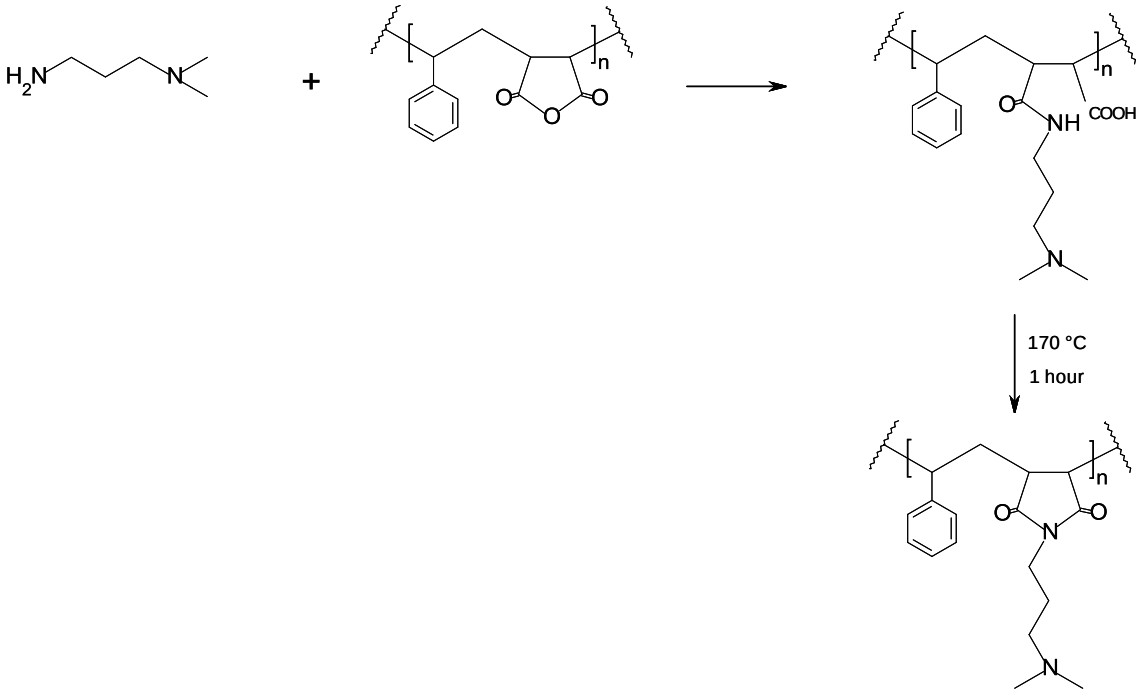
^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.8-7.5 (s broad, 5H, aromatic), 3.21 (s broad, 2H, -C**H**-), 2.95 (s broad, 2H, -$(CO)_2$N-C$\mathbf{H_2}$-), 2.28 (s broad, 3H, -C$\mathbf{H_2}$-C**H**-), 1.26 (s, 28H, -(C$\mathbf{H_2}$)$_{\mathbf{14}}$-), 0.87 (s, 3H, C$\mathbf{H_3}$-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 177.62 (-N-**C**=O), 138.74 (aromatic -**C**-), 128.76 (aromatic -**C**-), 51.62 (-**C**H-), 40.36 (-**C**H-), 38.88 ($(CO)_2$N-**C**H_2-), 32.16 (-**C**H_2-), 29.60 (-**C**H_2-), 29.96 (**C**H_2-), 26.98 (-**C**H_2-), 22.92 (-**C**H_2-CH_3), 14.35 (-CH_2-**C**H_3)

c) Synthesis of styrene-[*N*-3-(*N*'-ethyl-*N*',*N*'-dimethylammonium)propyl maleimide] copolymer to yield 4

i) Synthesis of styrene-[N-3-(N',N'-dimethylamino)propyl maleimide] copolymer (SMI-tC)

The synthetic work of Vermeesch and Evenson was used as basis for this procedure.[3,4]



Scheme 7 Synthesis of styrene-[N-3-(N',N'-dimethylamino)propyl maleimide] copolymer.

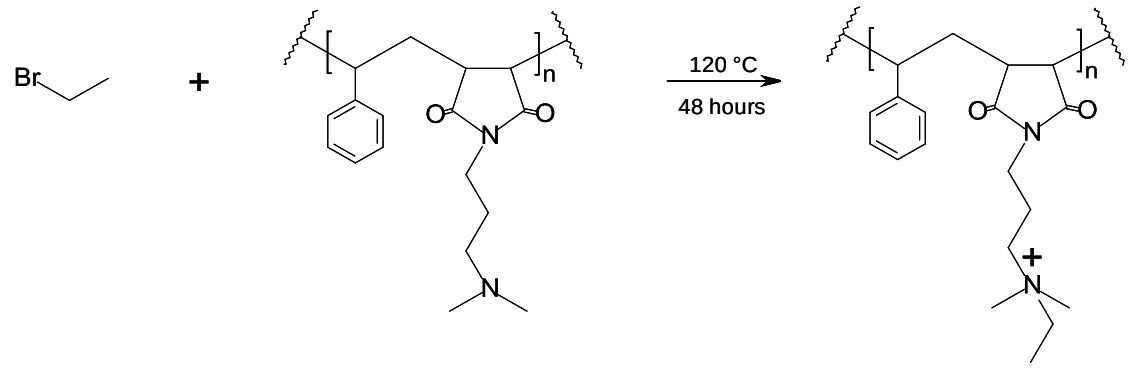
3-(*N*,*N*-dimethylamino)-1-propylamine (3.3 g, 33 mmol) was added dropwise to a solution ofSMA (5 g, 25 mmol) in 25 mL DMF at room temperature. The reaction was heated and refluxed at 170 °C for 1 hour whereafter the reaction was cooled, the polymer was precipitated in diethyl ether and filtered. The product was dissolved in methanol/THF, precipitated into diethyl ether, filtered and dried under vacuum at 60 °C for 24 hours to remove any residual solvent.

Major IR absorptions: 2941, 2768, 1744, 1691, 1495, 1453, 1400, 1345, 1216, 1149, 1036, 758, 703 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.7-7.5 (s broad, 5H, aromatic), 3.33 (s broad, 4H, -C**H**-C**H**-, -$(CO)_2$N-C$\mathbf{H}_2$-), 2.28 (s broad, 3H, -C**H**-C$\mathbf{H}_2$-), 2.17 (s, 8H, -N-C$\mathbf{H}_2$-, -N-C$\mathbf{H}_3$x2,), 1.5 (s, 2H, -CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 177.27 (-N-**C**=O), 136.87 (aromatic -**C**-), 128.67 (aromatic -**C**-), 57.03 (-N-**C**H_2-), 52.84 (-**C**H-), 45.50 (-N-**C**H_3), 37.88 ($(CO)_2$N-**C**H_2-), 37.09 (-**C**H-), 31.13 (-**C**H_2-), 25.38 (-**C**H_2-)

*ii) Synthesis of styrene-[N-3-(N'-ethyl-N',N'-dimethylammonium)propyl maleimide] copolymer (**4**)*



Scheme 8 Synthesis of styrene-[*N*-3-(*N*'-ethyl-*N',N'*-dimethylammonium)propyl maleimide] *copolymer.*

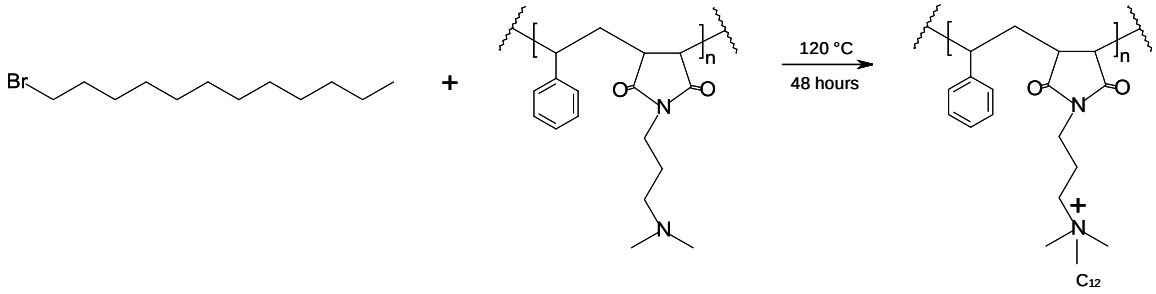
Bromoethane (0.73 g, 6.7 mmol) was added dropwise to a solution of styrene-[*N*-3-(*N',N'*-dimethylamino)propyl maleimide] copolymer (1.5 g, 5.3 mmol) in 12 mL DMF at room temperature. The reaction was heated to 120 °C for 48 hours[12] whereafter the reaction was cooled, the polymer was precipitated in acetone and filtered. The polymer was dried under vacuum at 60 °C for 24 hours to remove any residual solvent.

Major IR absorptions: 3383, 1686, 1495, 1453, 1405, 1356, 1177, 1022, 763, 708 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.7-7.5 (s broad, 5H, aromatic), 3.21 (s broad, 4H, -C**H**-C**H**-, -$(CO)_2$N-C$\mathbf{H_2}$-), 2.84 (s broad, 13H, -N-C$\mathbf{H_3}$x2, -N-C$\mathbf{H_2}$-x2), 1.60 (s, 2H, -C$\mathbf{H_2}$-), 1.12 (s, 3H, C$\mathbf{H_3}$-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 180.97 (-N-**C**=O), 141.01 (aromatic -**C**-), 128.45 (aromatic -**C**-), 64.52 (-N-**C**H_2-), 60.60 (-N-**C**H_2-), 50.80 (-N-**C**H_3), 42.38 (-**C**H-), 35.41 ($(CO)_2$N-**C**H_2-), 22.71 (-**C**H_2-), 8.23 (-**C**H_3)

d) Synthesis of styrene-[*N*-3-(*N*'-dodecyl-*N',N'*-dimethylammonium)propyl maleimide] copolymer to yield 7



Scheme 9 Synthesis of styrene-[N-3-(N'-dodecyl-N',N'-dimethylammonium)propyl maleimide] copolymer.

1-Bromododecane (1.15 g, 4.6 mmol) was added dropwise to a solution of styrene-[*N*-3-(*N',N'*-dimethylamino)propyl maleimide] copolymer (1.0 g, 3.5 mmol) in 18 mL DMF at room temperature. The reaction was heated to 110 °C for 48 hours[12] whereafter the reaction was cooled, the polymer was precipitated in diethyl ether, filtered and washed thoroughly three times with pentane. The polymer was dried under vacuum at 60 °C for 24 hours to remove any residual solvent.

Major IR absorptions: 3419, 2924, 2853, 1692, 1454, 1402, 1356, 1182, 1025, 758, 705 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.8-7.5 (s broad, 5H, aromatic), 3.34 (s, 6H, -N(C$\mathbf{H_3}$)$_2$), 3.23 (s, 4H, -C**H**-C**H**-, -$(CO)_2$N-C$\mathbf{H_2}$-), 2.97 (s broad, 5H, -N-C$\mathbf{H_2}$-x2, -C**H**-), 2.71 (s, 2H, -C$\mathbf{H_2}$-), 1.57 (s, 4H, -C$\mathbf{H_2}$-x2), 1.23 (s, 18H, -(C$\mathbf{H_2}$)$_9$-), 0.83 (s, 3H, C$\mathbf{H_3}$-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 178.09 (-N-**C**=O), 138.98 (aromatic -**C**-), 129.10 (aromatic -**C**-), 64.39 (-N-**C**H_2-), 62.26 (-N-**C**H_2-), 51.28 (-N-**C**H_3), 45.99 (-**C**H-), 35.00 ($(CO)_2$N-**C**H_2-), 32.05 (-**C**H_2-), 29.77 (-**C**H_2-), 26.59 (-**C**H_2-), 22.99 (-**C**H_2-), 22.80 (-**C**H_2-), 14.20 (-**C**H_3)

e) Synthesis of styrene-[*N*-3-(*N*'-hexadecyl-*N',N'*-dimethylammonium)propyl maleimide] copolymer to yield 11

The same method was followed as 3.4d) using 1-bromohexadecane (1.4 g, 4.6 mmol).

Major IR absorptions: 3400, 2926, 2854, 1690, 1455, 1404, 1356, 1183, 766, 706 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.8-7.5 (s broad, 5H, aromatic), 3.34 (s, 6H, -N(C$\mathbf{H_3}$)$_2$), 3.24 (s, 4H, -C**H**-C**H**-, -$(CO)_2$N-C$\mathbf{H_2}$-), 2.98 (s broad, 5H, -N-C$\mathbf{H_2}$-x2, -C**H**-), 2.70 (s, 2H, -C$\mathbf{H_2}$-), 1.56 (s, 4H, -C$\mathbf{H_2}$-x2), 1.21 (s, 16H, -(C$\mathbf{H_2}$)$_{13}$-), 0.82 (s, 3H, C$\mathbf{H_3}$-CH_2-)

^{13}C-NMR ($CDCl_3$): δ (ppm) = 178.61 (-N-**C**=O), 138.48 (aromatic -**C**-), 129.03 (aromatic -**C**-), 64.33 (-N-**C**H_2-), 63.18 (-N-**C**H_2-), 51.48 (-N-**C**H_3), 45.40 (-**C**H-), 36.70 ($(CO)_2$N-**C**H_2-), 32.13 (-**C**H_2-), 29.99 (-**C**H_2-), 26.60 (-**C**H_2-), 23.04 (-**C**H_2-), 22.89 (-**C**H_2-), 14.34 (-**C**H_3)

4. Ziehl-Neelsen staining protocol

The polymers were covered with carbol fuchsin solution, heated from below using a lit cotton swap until steam started to rise from the slide, and left for 5 minutes. The stained polymers were carefully rinsed with water, taking care that the polymer remained on the slide. The stained polymers were subsequently destained with dilute hydrochloric acid for one minute and counter stained with methylene blue for two minutes. The stained polymers were carefully rinsed with water, taking care that the polymer remained on the slide.

B. ANALYSES

1. Affinity studies with BCG at neutral pH

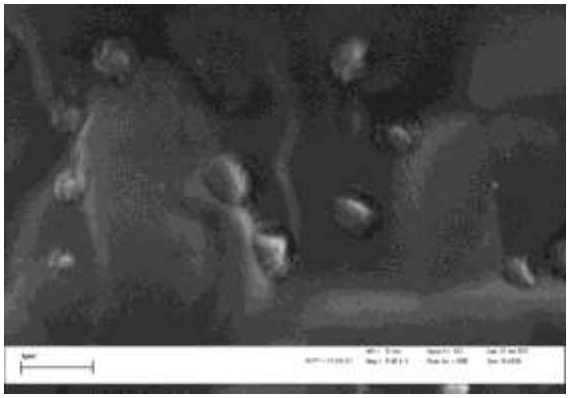


Figure 1 Enlargement of the wart-like structures on the surface of the washed nanofibers of 7.

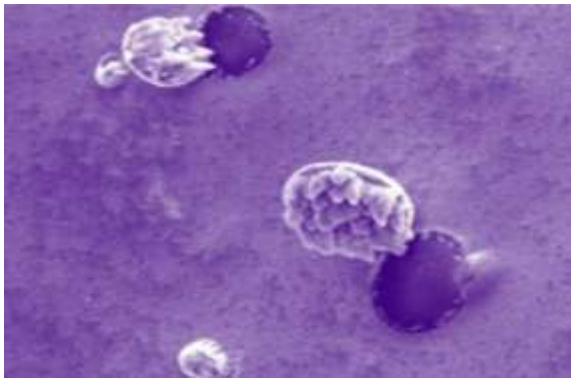


Fig.2 Two wart-like structures that have started to detach from the surface of polymer 7, indicative of aggregated BCG (image was captured without a scale-bar).

2. Affinity studies with BCG at pH 2

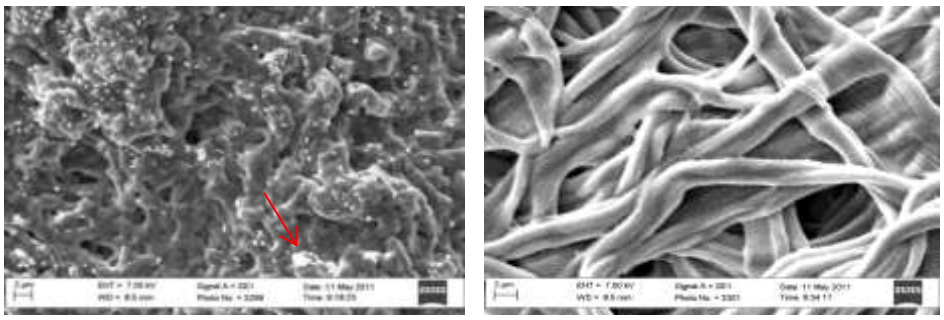


Fig. 3 SEM images of the washed nanofibers of (a) **7** after incubation with BCG at 37 °C and pH 2 for one hour and (b) **7** after incubation in PBS as negative control. Red arrows indicate captured BCG.

Time studies using BCG at pH 2

(a) (b) (c) (d)

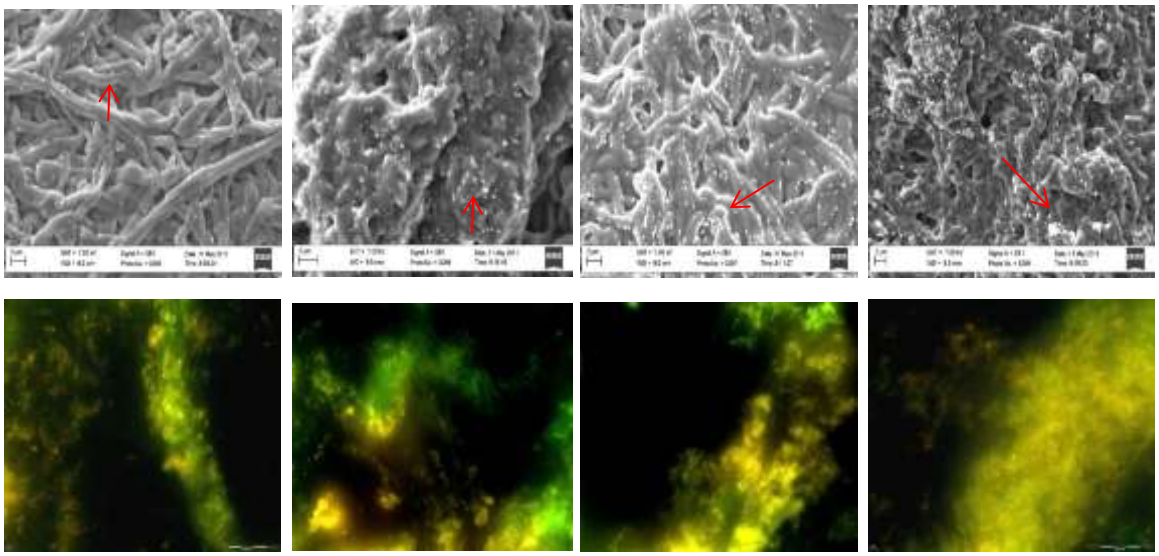
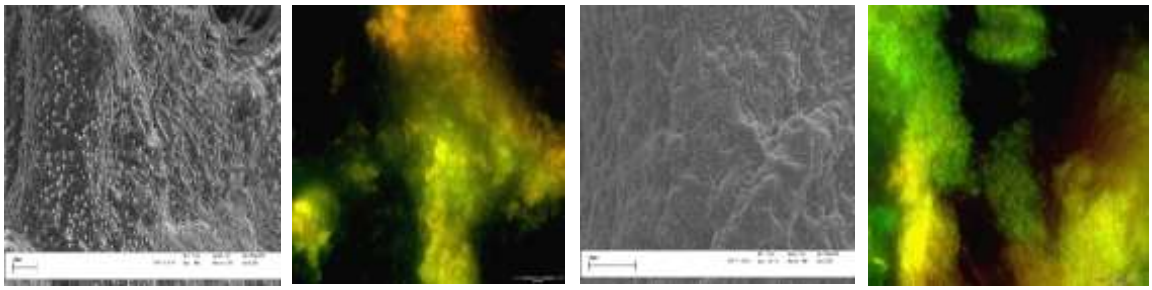


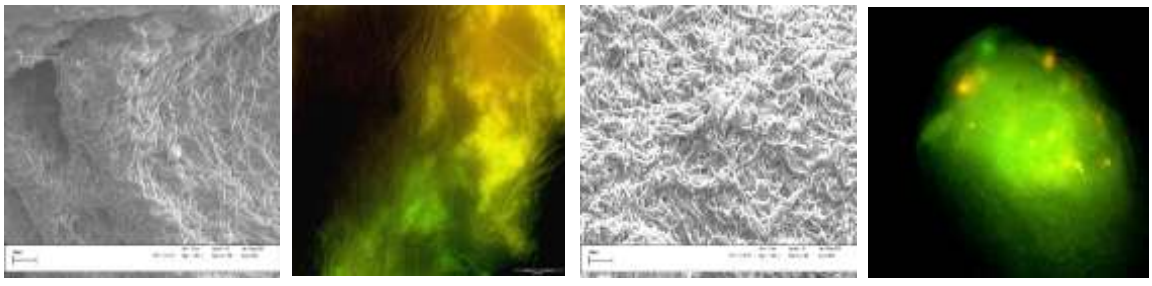
Fig. 4 SEM images (top row) and FM images (bottom row) of the washed nanofibers of 7 after incubation with BCG at pH 2 for (a) 15 min., (b) 30 min., (c) 45 min. and (d) 60 min. Red arrows indicate captured BCG.

Concentration studies using BCG: SEM and FM images:

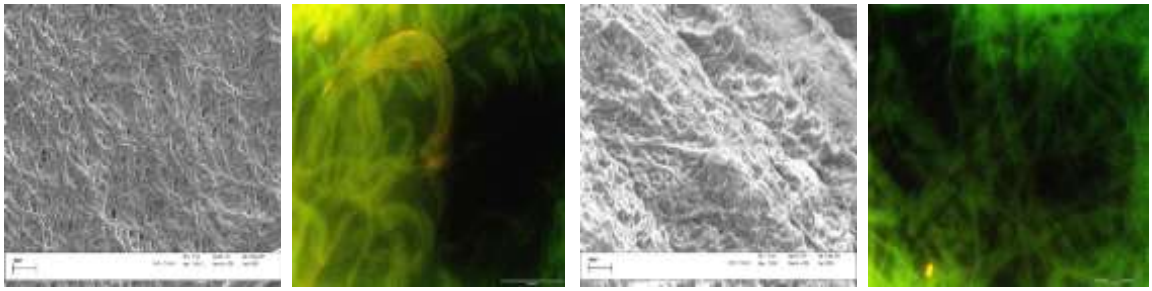
(a) 10^8 BCG/mL (b) 10^7 BCG/mL



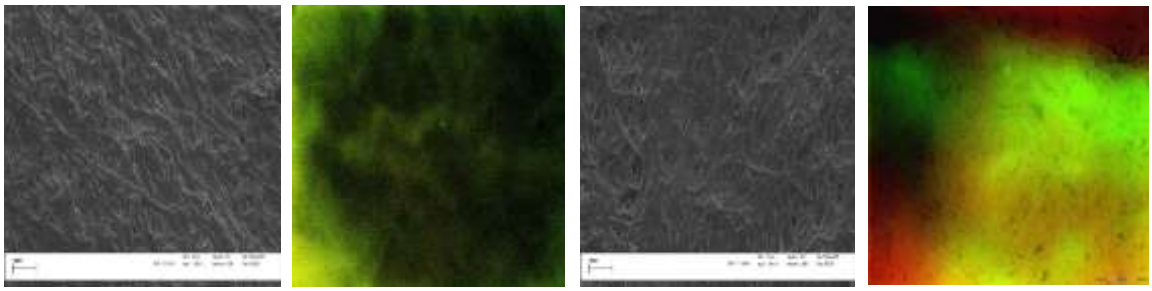
(c) 10^6 BCG/mL (d) 10^5 BCG/mL



(e) 10^4 BCG/mL (f) 10^3 BCG/mL



(g) 10^2 BCG/mL (h) 10 BCG/mL



(i) PBS

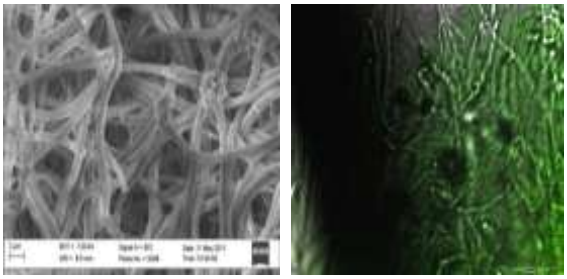


Fig. 4 SEM images (left) and FM images (right) of the washed nanofibers of 7 after incubation with decreasing concentrations of BCG at 37 °C and pH 2 for one hour.

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