Biointerphases
A Journal of Biomaterials and Biological Interfaces

Surface grafting of electrospun fibers using ATRP and RAFT for the control of biointerfacial interactions

Cite as: Biointerphases **8**, 16 (2013); https://doi.org/10.1186/1559-4106-8-16
Submitted: 30 May 2013 . Accepted: 20 June 2013 . Published Online: 05 July 2013

Thomas Ameringer, Francesca Ercole, Kelly M Tsang, Bryan R Coad, Xueliang Hou, Andrew Rodda, David R Nisbet, Helmut Thissen, Richard A Evans, Laurence Meagher, and John S Forsythe

Biointerphases
a SpringerOpen Journal

ORIGINAL ARTICLE Open Access

Surface grafting of electrospun fibers using ATRP and RAFT for the control of biointerfacial interactions

Thomas Ameringer[1,2,4], Francesca Ercole[3], Kelly M Tsang[1,2,3], Bryan R Coad[1,2,5], Xueliang Hou[3,6], Andrew Rodda[1,2,3], David R Nisbet[3,7], Helmut Thissen[1,2], Richard A Evans[1,2], Laurence Meagher[1,2*] and John S Forsythe[3*]

Abstract

Background: The ability to present signalling molecules within a low fouling 3D environment that mimics the extracellular matrix is an important goal for a range of biomedical applications, both *in vitro* and *in vivo*. Cell responses can be triggered by non-specific protein interactions occurring on the surface of a biomaterial, which is an undesirable process when studying specific receptor-ligand interactions. It is therefore useful to present specific ligands of interest to cell surface receptors in a 3D environment that minimizes non-specific interactions with biomolecules, such as proteins.

Method: In this study, surface-initiated atom transfer radical polymerization (SI-ATRP) of poly(ethylene glycol)-based monomers was carried out from the surface of electrospun fibers composed of a styrene/vinylbenzyl chloride copolymer. Surface initiated radical addition-fragmentation chain transfer (SI-RAFT) polymerisation was also carried out to generate bottle brush copolymer coatings consisting of poly(acrylic acid) and poly(acrylamide). These were grown from surface trithiocarbonate groups generated from the chloromethyl styrene moieties existing in the original synthesised polymer. XPS was used to characterise the surface composition of the fibers after grafting and after coupling with fluorine functional XPS labels.

Results: Bottle brush type coatings were able to be produced by ATRP which consisted of poly(ethylene glycol) methacrylate and a terminal alkyne-functionalised monomer. The ATRP coatings showed reduced non-specific protein adsorption, as a result of effective PEG incorporation and pendant alkynes groups existing as part of the brushes allowed for further conjugation of via azide-alkyne Huisgen 1,3-dipolar cycloaddition. In the case of RAFT, carboxylic acid moieties were effectively coupled to an amine label via amide bond formation. In each case XPS analysis demonstrated that covalent immobilisation had effectively taken place.

Conclusion: Overall, the studies presented an effective platform for the preparation of 3D scaffolds which contain effective conjugation sites for attachment of specific bioactive signals of interest, as well as actively reducing non-specific protein interactions.

Keywords: Electrospun fibers; Polymer brushes; Surface-initiated polymerisation; Surface fouling; Surface immobilisation

* Correspondence: laurence.meagher@csiro.au; john.forsythe@monash.edu
[1]CSIRO, Materials Science and Engineering, Clayton, Australia
[2]Cooperative Research Centre for Polymers (CRCP), Clayton, Australia
Full list of author information is available at the end of the article

Background

Nanostructured scaffolds are increasingly being used in *in vitro* and *in vivo* biomedical applications as a way to influence cell-material interactions that occur at the length scale provided by these substrates. Nanotopography can exert powerful effects on ligand-receptor organisation to amplify or alter intracellular signaling pathways that may be responsible for coordinating cellular function, i.e. survival, proliferation, migration, differentiation and the like [1-3]. One nanofabrication method that has gained particular prominence is electrospinning which can be used to produce scaffolds containing fibers with diameters in the nanometer-length scale. Utilising this technique it is possible to fabricate a wide variety of architectures to provide physical guidance cues, support extracellular matrix deposition (ECM) and activate the innate regenerative cascade to promote tissue formation [4].

To improve cellular interactions with electrospun nanofibers, surface modification is commonly carried out, using chemical [5], plasma [6], physisorption [7] and chemisorption [8] methods. Nanofibers have been functionalised with specific signals such as peptides or proteins that can be recognized by cell surface receptors. For example, covalent attachment of epidermal growth factor (EGF) [9], bone morphogenetic protein (BMP) [10] and brain derived neurotrophic factor (BDNF) [11] on electrospun nanofibers has been successful in providing localised and sustained signaling to cell surface receptors.

A strategy that is used to promote cell attachment onto a surface is protein adsorption, a process which can bring about non-specific interactions and may trigger undesired responses, such as a foreign body response *in vivo*. To gain greater specificity and control over cell receptor binding, it is therefore advantageous to provide a surface that resists non-specific protein adsorption, while concurrently presenting specific biomolecules of interest in a 3-dimensional (3D) setting. For example, nanofibrous scaffolds fabricated by electrospinning of star-poly(ethylene glycol) (PEG) and poly(lactic acid-*co*-glycolic acid) blends, resulted in surface enrichment of the star-PEG under the influence of the electric field present during the electrospinning process. The star-PEG lowered non-specific protein adsorption and was able to be subsequently functionalised with an Arginine-Glycine-Aspartic Acid (RGD) peptide to promote cell adhesion [12].

Grafting polymer chains onto or from the surface of electrospun fibers provides another effective means of changing the chemical and physical properties of the fibers and is potentially more robust over longer periods of time compared to blending. Here, grafting of polymers from the surface of the fiber is preferred due to the fact that grafting densities can be modulated and hence more effective control over biointerfacial interactions can be achieved [13].

Controlled polymerisation techniques such as atom transfer radical polymerisation (ATRP) or reversible addition–fragmentation chain transfer (RAFT) polymerisation are particularly attractive for the preparation of surface tethered polymer brushes as they allow accurate control over brush thickness, composition and architecture [2,14]. With a grafting-from approach the polymerisation is directly initiated from initiator-functionalised or a RAFT-agent functionalised surface.

Most of the polymer brushes produced using this approach are prepared using surface-initiated (SI) controlled radical polymerisation techniques. To yield polymers grafted from a fiber surface, pre-activation followed by covalent attachment of an initiating moiety is generally required [15]. In another approach, ATRP initiators have been introduced on the surface of electrospun fibers by adsorption of the ATRP macroinitiators through polyelectrolyte complexation rather than covalent linking [16]. Fu and coworkers [17,18] eliminated the need to attach the initiating moiety in a separate step by electrospinning the dormant ATRP macroinitiator itself. Using an electric field, surface enrichment of the polarisable initiator moieties was achieved and, in a subsequent step, controlled polymerisation was carried out resulting in a brush coating on the surface of the nanofiber. Since then others have used a similar approach to prepare electrospun fibers with surface initiator sites for subsequent grafting [19-21].

In this report, we describe a methodology for producing 3D electrospun fibers coated with brushes of (i) protein repellent poly(ethylene glycol) methacrylate (PEGMA) which contained a proportion of end-functionalised alkyne groups using ATRP and (ii) copolymer coatings containing carboxylic acid residues formed using the RAFT methodology to allow the subsequent covalent immobilisation of specific amine functional biomolecules. XPS was used to characterise the surface composition of the fibers after each modification step and fluorine based XPS labels were used to demonstrate that covalent immobilisation of small molecules had been achieved in both cases, either via an azide-alkyne Huisgen 1,3-dipolar cycloaddition reaction or amide bond formation. The coatings, in both cases, were formed using the grafting-from approach, giving polymer brushes with low non-specific interactions with proteins.

Methods

Materials

All chemicals (reagents and solvents) used for synthesis were purchased from Sigma-Aldrich at their highest purity available and used as received unless otherwise

stated. 4-Vinylbenzyl chloride (VBC) and styrene were purified by passing through aluminum oxide 90, activated basic (0.063 - 0.200 nm, Merck) to remove inhibitors prior to use. Poly(ethylene glycol) methacrylate, average MW 529 g/mol, (PEGMA-529) was de-inhibited by running through a column containing inhibitor removing beads (Aldrich). Acrylic acid was distilled at reduced pressure and stored at 4°C for no longer than one month prior use. All other chromatography was performed using silica gel (Kieselgel Merck 60, 0.040 - 0.063 mm) and TLC was performed on Merck Silica 60F254 plates. A DELFIA Eu-Labeling kit, containing Europium labeling reagent, enhancement solution and Europium standard were purchased from Perkin Elmer, Australia. The time-resolved fluorescence assay was measured using opaque fluorescence reading plates which were obtained from Grenier Bio-one, Germany.

General experimental measurements

Gel permeation chromatography (GPC) for p(Sty-*co*-VBC), Polymer A was performed using a Waters 515 HPLC pump, a Waters 717 Plus Autosampler equipped with Waters 2414 refractive index detector, 3 × Mixed-C (7.5 mm × 300 mm, 5 μm particle size, linear molecular weight range 200–2,000,000) and 1 Mixed E PL gel column (7.5 mm × 300 mm, 3 μm particle size, linear molecular weight range up to 30,000) from Polymer Laboratories. Tetrahydrofuran (THF) with a flow rate of 1.0 mL min^{-1} was used as eluent at 22 ± 2°C. Molecular weights were calculated via calibration with narrow polydispersity polystyrene standards (Polymer Laboratories) ranging from 600 to 7.5 × 10^6 g/mol. Number-average (M_n) and weight-average (M_w) molecular weights were evaluated using Waters Millennium/Empower software. A third-order polynomial was used to fit the log M *vs.* time calibration curve, which was linear across the molecular weight ranges.

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained with a Bruker Av400 spectrometer at 25°C. Spectra were recorded for samples dissolved in deuterated solvent and chemical shifts were reported as parts per million from external tetramethylsilane.

Fiber characterisation (TEM and SEM)

Electrospun membranes were mounted on scanning electron microscopy (SEM) stubs for imaging using a JEOL JEM 7001 FEGSEM instrument. Average fiber diameters were calculated from making 20 measurements on each image using ImageJ software. Transmission electron microscopy (TEM) images were obtained by electrospinning fibers onto copper grids and imaging on a Phillips CM20 operated at 200 kV. To avoid any interference during ATRP grafting the copper grids were replaced by holey-carbon coated gold TEM grids (Ted Pella Inc.).

X-ray photoelectron spectroscopy (XPS)

XPS experiments were carried out using an AXIS HSi spectrometer (Kratos Analytical Ltd.) equipped with a monochromatised Al Kα source. The pressure during analysis was typically 5 × 10^{-8} mbar. The elemental composition of surfaces was determined from survey spectra, collected at a pass energy of 320 eV. High-resolution spectra were obtained at a pass energy of 40 eV. Binding energies were referenced to the aliphatic carbon peak at 285.0 eV.

Synthesis of p(Sty-*co*-VBC), polymer A

In a 100 mL round bottom flask 4-vinyl benzyl chloride (VBC) (2.3 g, 90%, 0.014 mol) and styrene (8.8 g, 0.084 mol) were dissolved in 50 mL of toluene. After addition of azobisisobutyronitrile (AIBN) (15 mg, 0.0091 mmol) the flask was capped with a rubber septum and the solution degassed for 10 minutes with a nitrogen stream. The solution was then stirred for 16 hours at 60°C. After cooling, the polymer was precipitated in 300 mL of methanol, re-dissolved in 20 mL of chloroform and re-precipitated in methanol. The white polymer was dried under vacuum in a desiccator and analysed with ^{1}H NMR and GPC. (GPC, THF: M_n 74,000 g/mol, PDI 2.1). Refer to results and discussion section for ^{1}H NMR spectra.

Synthesis of trithiocarbonate (RAFT) modified p(Sty-*co*-VBC), polymer B

To a mixture of 1-butanethiol (2.4 g, 26.6 mmol) and carbon disulfide (2.0 g, 26.2 mmol) in 10 mL of dichloromethane (DCM), triethylamine (TEA) (3.0 g, 29.7 mmol) was added and the solution briefly purged with nitrogen. The yellow solution was left to stir for 1 hour and then added to a solution of p(Sty-*co*-VBC) (2.0 g) in 25 mL of DCM. The solution was stirred for 16 hours at room temperature and then washed with 0.5 M aqueous HCl (3 ×) and brine. The solution was then dialysed against DCM for 5 days with regular exchange of the solvent (Spectrum SpectraPor 1, molecular weight cut off 6–8 kDa). The solvent was then evaporated and the slightly yellow polymeric product analysed with ^{1}H NMR and GPC. M_p 24,450 g/mol; M_w 20,300 g/mol; M_n 7,500 g/mol, PDI 2.70. Refer to results and discussion section for ^{1}H NMR spectra.

Synthesis of ethylene glycol methacrylate succinoyl alkyne (EGMAS-Alk) monomer

The title compound [2] was synthesized in 3 steps as described below:

Step 1: 4-(2-(Methacryloyloxy)ethoxy)-4-oxobutanoic acid. 2-Hydroxyethyl methacrylate (25 g, 0.195 mol) and succinic anhydride (19.5 g, 0.195 mol) were added to DCM (200 mL) under nitrogen. TEA (28.5 mL, 20.72 g. 0.205 mol) was then added dropwise over 20 minutes and the reaction mixture was then refluxed for 1.5 hours. The reaction mixture was then diluted with more DCM (200 mL), washed with 2 M aqueous HCI (150 mL) and then finally with brine (100 mL). The organic phase was then dried with $MgSO_4$ and evaporated to dryness, yielding a viscous, colourless liquid (34.9 g, 77.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.89 (s, 3H, CH_3), 2.59 - 2.67 (m, 4H, 2 × CH_2CO), 4.31 (br. s, 4H, 2 × CH_2OCO), 5.55 (s, 1 H, vinyl CH), 6.08 (s, 1 H, vinyl CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 17.96, 28.64, 28.73, 62.15, 62.29, 126.12, 135.79, 167.11, 171.90, 177.73 ppm.

Step 2: Synthesis of 2-(methacryloyloxy)ethyl-4-chloro-4-oxobutanoate. The oil product obtained in step 1 above, (4-(2-(methacryloyloxy)ethoxy)-4-oxobutanoic acid), was refluxed with thionyl chloride (54 g, 33 mL, 0.454 mol) in DCM (200 mL) for 2 h. The reaction mixture was evaporated to dryness to yield a clear, pale yellow liquid (37.5 g, 99.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.92 (s, 3H, CH_3), 2.68 (t, J = 6.6 Hz, 2H, CH_2COO), 3.20 (t, J = 6.6 Hz, 2H, CH_2COCl), 4.33 (br. s, 4H, 2 × CH_2O), 5.58 (s, 1H, vinyl CH), 6.10 (s, 1H, vinyl CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.12, 29.16, 41.60, 62.11, 62.74, 126.06, 135.79, 166.96, 170.59, 172.82 ppm.

Step 3: Synthesis of EGMAS-Alk. Propargyl alcohol (0.903 g, 0.937 mL, 0.016 mol) was dissolved in DCM (30 mL) and then TEA (1.80 g, 2.47 mL, 0.018 mol) was added to the solution. The solution was cooled to < 0°C and 2-(methacryloyloxy)ethyl-4-chloro-4-oxobutanoate, from the previous step (4.0 g, 0.016 mol) in DCM (10 mL) was then added dropwise to the solution. The reaction mixture was stirred overnight at room temperature and the progress of the reaction was monitored using thin layer chromatography. The crude reaction mixture obtained was then filtered and the filtrate was evaporated to dryness. The resulting dark yellow oil was dissolved in DCM, washed with water (2 × 20 mL), diluted HCI (2 × 20 mL), and brine (2 × 20 mL), dried (Na_2SO_4) and evaporated to dryness to give a clear colourless oil. This oil was further purified via radial chromatography (silica gel) to give the desired product as a clear, colourless oil (2.5 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.91 (s, 3H, CH_3), 2.46 (t, J = 2.4 Hz, 1H, alkyne CH), 2.65 (s, 4H, 2 × (CH_2CO), 4.32 (s, 4H, 2 × CH_2OCO), 4.66 (d, J = 2.4 Hz, 2H, OCH_2CCH), 5.56 (br.s, 1H, vinyl CH), 6.09 (s, 1H, vinyl CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.19, 28.76, 28.78, 52.16, 62.26, 62.41, 74.99, 77.40, 126.01, 135.87, 167.02, 171.31, 171.74 ppm.

Synthesis of trifluoro-4-(azidomethyl)benzoate (TFAB)

Trifluoroethanol (1.01 g, 0.72 mL, 0.010 mol) was dissolved in DCM (20 mL) under a nitrogen atmosphere. The solution was cooled to 0°C and TEA (1.07 g, 0.011 mol) was added. 4-(Chloromethyl)benzoyl chloride (2.0 g, 0.011 mol) in DCM (10 mL) was then added dropwise and the reaction was stirred overnight. The reaction mixture was then washed with water (20 mL) and brine (20 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated to dryness to give a white solid (2.40 g) which was determined to be 95% pure by ^{1}H NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.62 (s, 2H, CH_2Cl), 4.70 (q, J = 8.4 Hz, 2H, CH_2O), 7.50 (d, J = 8.4 Hz, 2H, aromatic), 8.07 (d, J = 8.4 Hz, 2H, aromatic) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 45.41 (CH_2Cl), 61.12 (q, JCF = 37.23 Hz, CH_2O), 123.33 (q, JCF = 276.72 Hz, CF_3), 128.57 (aromatic), 128.94 (aromatic), 130.70 (aromatic), 143.59 (aromatic), 164.68 (–COO) ppm.

The white solid obtained above was then dissolved in DMSO (30 mL) and KI (0.005 g, 0.030 mmol) was added. The reaction was stirred at room temperature and sodium azide (2.18 g, 0.0336 mol) was added portion-wise. The reaction was then stirred overnight. The reaction was worked up by the addition of water (200 mL). The organic components were extracted with DCM (3 × 50 mL), dried ($MgSO_4$) and the solvent was evaporated to give a clear, colourless oil. This oil was further purified by radial chromatography (silica gel, petroleum spirits 40–60:DCM, 1:1) to yield a clear, colourless oil. This oil was finally purified via radial chromatography (solvent gradient, starting with petroleum spirit 40-60°C:DCM 1:1 and finishing with DCM (100%). The product obtained was a clear, colourless liquid (2.05 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.44 (s, 2H, CH_2N_3), 4.71 (q, J = 8.4 Hz, 2H, CH_2O), 7.43 (d, J = 8.4 Hz, 2H, aromatic), 8.10 (d, J = 8.4 Hz, 2H, aromatic) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 54.16 (CH_2N_3), 60.83 (q, JCF = 36.74, CH_2O), 123.04 (q, JCF = 277.31, CF_3), 128.06 (aromatic), 128.24 (aromatic), 130.52 (aromatic), 141.82 (aromatic), 164.74 (–COO) ppm.

Electrospinning of polymer A (ATRP) and polymer B (RAFT)

Polymer A was dissolved in a mixture of DMF and chloroform (1:1) which contained 1 μM dodecyl trimethyl

ammonium bromide (DTAB). The final solution contained 20 wt % polymer. The solution was loaded into a plastic syringe fitted with a copper electrode, which was connected to a high voltage generator of a custom built electrospinning unit. The syringe was kept at a distance of 15 cm from the aluminium cathode maintained at −5 kV. The electrospinning was carried out at an anode voltage of 25 kV, using a flow rate of 0.48 mL/hour. The average fiber diameter determined using a SEM S570 (Hitachi) was found to be 960 ± 280 nm.

In the case of RAFT polymer B, the electrospinner was purged with nitrogen and a steady flow maintained throughout the process. Polymer B was dissolved in chloroform (20 wt %) and the solution was loaded into a plastic syringe fitted with a copper electrode, which was connected to a high voltage generator of a custom built electrospinning unit. The syringe was kept at a distance of 10 cm from the aluminium cathode maintained at −5 kV. The electrospinning was carried out at an anode voltage of 25 kV, using a flow rate of 0.48 mL/hour. The average fiber diameter determined using a SEM S570 (Hitachi) was determined to be 600 ± 200 nm.

ATRP grafting from polymer A electrospun fibers

A 6 mm diameter biopsy punch was used to cut samples from the electrospun mat. The samples were weighed and then placed into the wells of a 96 well TCPS (tissue culture polystyrene) plate. TEM grids coated with electrospun fibers were also placed into some of the wells of the plate. The plate was placed into the vacuum chamber of a glove box and evacuated over night before being transferred into the glove box. The ATRP catalyst system used for the grafting was composed of activating and deactivating copper catalysts (CuCl and $CuCl_2$, respectively), with 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as the chelating ligand. The molar proportions of monomer:CuCl:$CuCl_2$:HMTETA used was 200:1:0.15:2. Solutions of PEGMA-529 and EGMAS-Alk monomers were prepared in the following monomer ratios: 100% PEGMA (**P1**), 95/5 PEGMA/EGMAS-Alk (**P2**) and 90/10 PEGMA/EGMAS-Alk (**P3**). A mixture of 70% ethanol and 30% MilliQ water was used as solvent with the total monomer concentration varied from 0.05-1.0 M. The solutions were purged for 15 minutes with nitrogen, the above catalyst system was then added and the solutions purged with nitrogen for another 5 minutes. 200 μL of the respective solution was added into each well containing electrospun samples. The plate was kept under a nitrogen atmosphere in a glove box for 24 hours and gently agitated using a shaker. The reaction was stopped by exposure to air. The substrates were washed with water, 50 mM EDTA and 50 mM $NaHSO_3$ aqueous solutions. As a final wash the substrates were extensively washed with water. The samples were then stored in purified water (MilliQ) until required for further experiments.

Coupling of TFAB to ATRP grafted electrospun fibers

In a glove box, 10 mg of sodium ascorbate (5 mmol), 4.2 μL of *N,N,N′,N′,N′′*-pentamethyldiethylenetriamine (2×10^{-5} mol), 40 μL (2×10^{-5} mol) of an aqueous $CuSO_4.5H_2O$ solution (0.5M) and 5 mg of TFAB (1.9×10^{-5} mol) were dissolved in 2.5 mL of degassed phosphate buffered saline. Aliquots of this solution (100 μL) were pipetted into the wells of a 96 well plate containing the nanofiber samples from the ATRP grafting experiment. The plate was gently shaken for 20 hours and the fibers were then washed 3 × each with water, 0.1 M EDTA solution and again with water. The samples were then transferred onto a sample holder and dried for subsequent XPS analysis.

RAFT grafting from polymer B electrospun fibers

The fiber mat composed of polymer B was cut into ~1 cm^2 pieces and placed into the wells of a 24 well TCPS plate. To each well 1 mL of ethanol was added to detach the fiber mat from the aluminium foil. The ethanol was removed and the flattened fiber mesh was allowed to dry. Thereby, the fiber meshes adhered slightly to the bottom of the wells allowing for easier handling.

The plate was then transferred into a nitrogen containing glove box. In the glove box 5% (w/v) aqueous solutions of acrylamide (AAm) and acrylic acid (AAc) containing the thermal initiator 2,2'-azobis(2-amidinopropane)-dihydrochloride (0.5 mg/mL) were prepared and degassed by purging with nitrogen for 15 minutes. The AAm solution was mixed with the AAc solution to give molar proportions of 10 mol% AAc and 90% AAm. The mixtures, as well as the pure AAm solution were then added to the wells containing the electrospun fibers. The covered plate was then vacuum-sealed into plastic bags and transferred to a N_2 purged vacuum oven and incubated at 60°C for 72 hours. The fibers were rinsed thoroughly with MilliQ water and then soaked for two days with daily exchanges of water. The fibers were carefully rinsed again 3 × with water before drying.

Coupling of 2,2,2-trifluoroethylamine (TFEA) to RAFT grafted fibers

AAm and AAc/AAm grafted fibers were placed into the wells of a 24 well TCPS plate. 2 mL aliquots containing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) (both 0.125 M in water) were individually added to each well. After 20 minutes the mixed solution was removed and the fibers washed 3 × with MilliQ water. The wells were then filled with a 0.1 M 2,2,2-trifluoroethylamine (TFEA) aqueous solution and incubated for 16 hours at 4°C. The substrates were then washed with MilliQ water and air dried prior to XPS analysis.

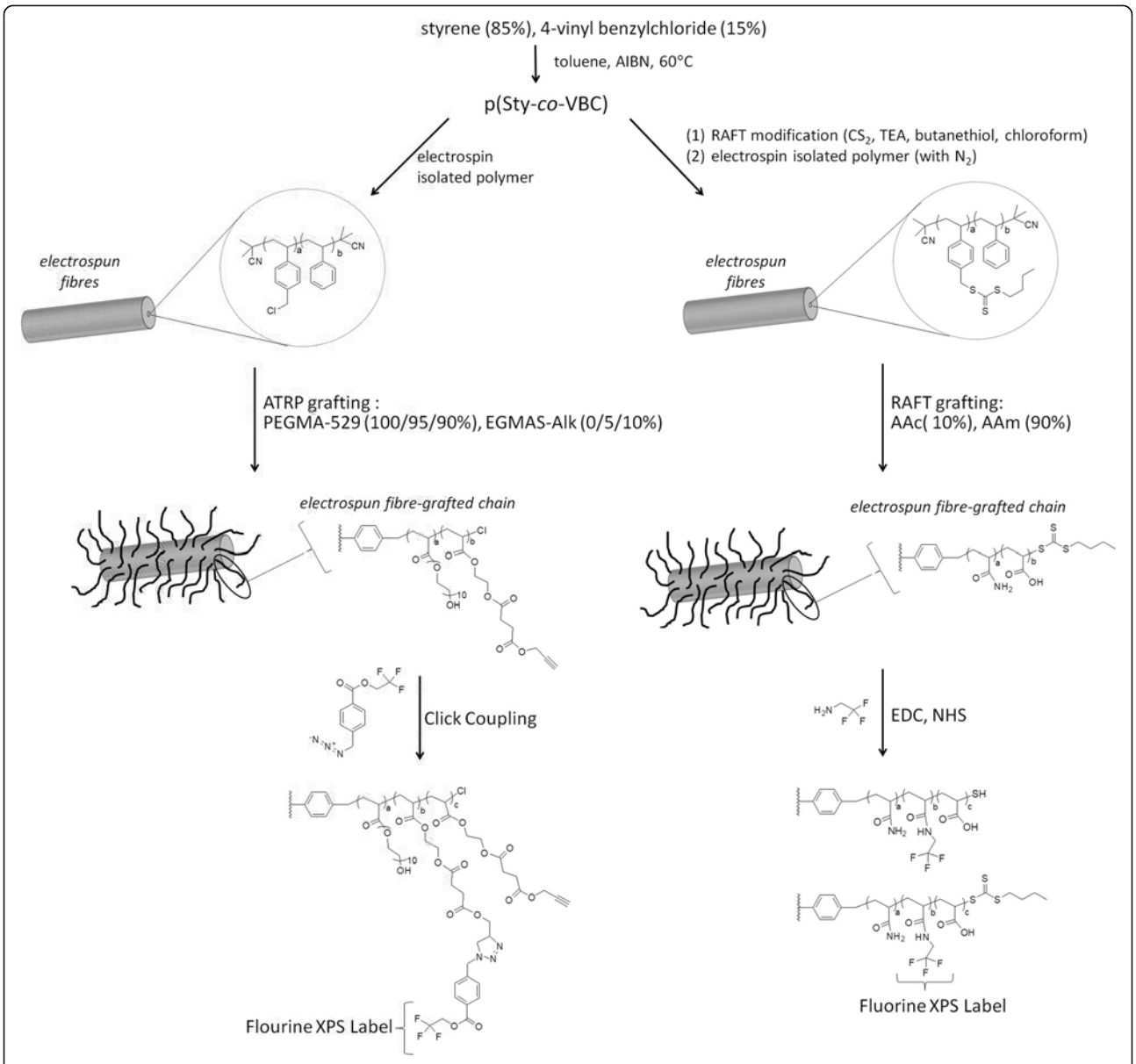

Figure 1 Polymer synthesis, electrospinning, grafting and XPS labelling experiments carried out in this study.

Protein adsorption studies on PEGMA grafted polymer A electrospun fibers

Europium (Eu)-labelled HSA adsorption studies were conducted using a procedure already reported in literature [22] and a DELFIA Eu-Labeling kit (Perkin Elmer). Briefly, washed and dried electrospun fiber circular discs (6 mm diameter) were transferred to a Corning Ultra Low Protein Binding plate. They were then wet with a small amount of ethanol, and then fully hydrated using water. In these experiments the total human serum albumin (HSA) concentration used was 0.100 mg/mL. A small proportion of HSA was pre-labelled with a Eu-chelate, according to the published procedure [22]. For the protein adsorption step, solutions were used which contained unlabelled HSA and Eu-labelled HSA in a ratio of 500:1 by mass. The washing solution was extracted and then 100 μL of HSA in PBS was added to the grafted and control electrospun fiber discs. The control was composed of p(Sty-*co*-VBC), polymer A, fibers which had undergone the same handling and washing protocols except that no monomer or catalyst had been used in the grafting step. HSA solutions were also added to empty wells as an extra control to quantify protein binding to the plate itself. Samples were incubated with HSA solutions overnight, in the dark, at 4°C. The HSA solutions were then carefully extracted and the discs washed with PBS (5 × for approximately 20 minutes in-between exchanges) and water (3 × for 5 minutes in-

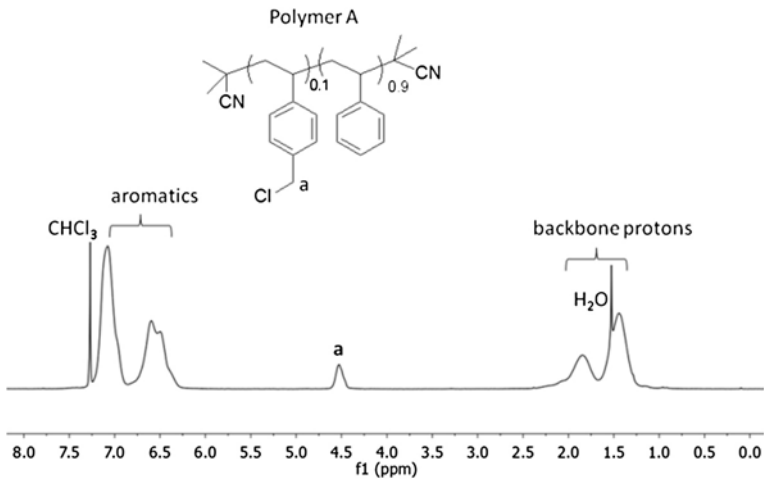

Figure 2 ^{1}H NMR ($CDCl_3$) of polymer A, p(Sty-*co*-VBC), showing main peak assignments.

between exchanges). The Eu tag was dissociated using 120 μL of enhancement solution (Perkin Elmer) (35 minute incubation), 100 μL of which was transferred to a black plate and the adsorbed HSA quantified using a time-resolved fluorescence assay [22]. The Eu concentration in the samples was interpolated from a calibration curve prepared using Europium standards of known concentration. The labelling ratio (mol Eu bound:HSA: mol HSA) was determined on diluted samples of known concentration. The protein concentration was determined using amino acid analysis. The reported mass of adsorbed protein was scaled to the substrate weight to account for differences in amount of scaffold available for grafting. Replicate conditions were then averaged.

Results and discussion

As mentioned previously, the ability to control biomolecule and cell–material interactions in an environment that mimics properties of the extracellular matrix is an important goal for a range of biomedical applications, *in vitro* and *in vivo* [23]. Undesired cell responses can be triggered by non-specific protein adsorption onto the surface which can be problematic in the control of specific receptor-ligand interactions, such those involved in cell signalling pathways [24]. It is therefore advantageous to present specific ligands of interest to cell surface receptors on a surface with inherently low non-specific interactions with biomolecules such as proteins [12,25,26]. Methodologies that are commonly employed have been applied to 2D surfaces but far less to 3D scaffolds such as those used in tissue engineering. This work was aimed at introducing a platform that could be applied for the production of electrospun scaffolds, and which would ultimately allow one to study and control cell-material interactions in a 3D environment, with minimal confounding factors. Our direct approach was to produce a surface coating onto the fibers of a 3D scaffold. This would provide bio-conjugation sites, ultimately for attachment of specific bio-signals, but also in a low fouling environment, so as to reduce non-specific interactions.

Table 1 Elemental composition of ungrafted fibers (P0) vs. ATRP grafted fibers (P1-P3)[a] as determined by XPS analysis

	P0	P1	P2	P3
C 1s	98.2	75.4	77.1	73.1
O 1s	0.4	24.2	22.4	26.7
Cl 2p	1.4	0.4	0.5	0.2

a) P1 grafted with PEGMA-529 (100%); P2 with PEGMA-529:EGMAS-Alk (95:5); P3 with PEGMA-529:EGMAS-Alk (90:10).

Presented as Figure 1 is the experimental map for our study, including the polymer synthesis; electrospinning; grafting and labelling experiments that were carried out. To begin with, we synthesised our base polymer, p(Sty-*co*-VBC) polymer A, using conventional free radical polymerisation. ^{1}H NMR analysis (Figure 2) showed that the isolated polymer contained approximately 10 mol % chlorobenzyl moieties with the remaining material being styrene. This polymer was electrospun directly to yield fibers containing chlorobenzyl initiating sites for SI-ATRP.

One of the aims of this work was to produce PEG polymer brush coated fibers, with a view to reducing non-specific interactions with proteins. To achieve this, we carried out SI-ATRP from electrospun fibers composed of p(Sty-*co*-VBC) polymer A and confirmed the presence of the grafted polymer brush using XPS. The feed solutions used for grafting contained various ratios of PEGMA and EGMAS-Alk monomer, the latter allowing Click chemistry (azide-alkyne Huisgen 1,3-dipolar cycloaddition) to be carried out in a subsequent

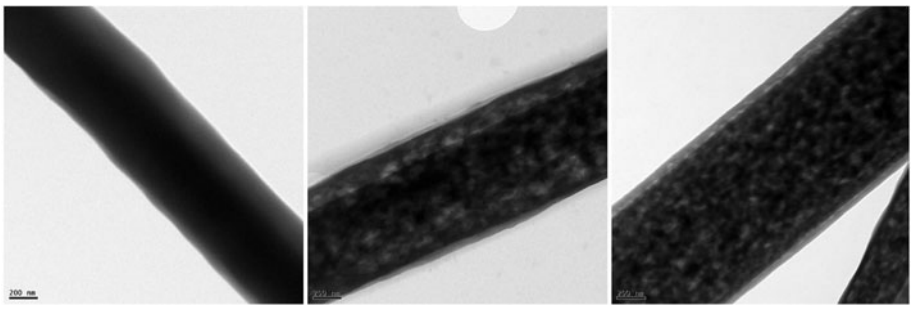

Figure 3 TEM images electrospun polymer fibers; left: non-grafted p(Sty-co-VBC) (P0); middle: fiber grafted with PEGMA-529 100% (P1); right: fiber grafted with PEGMA-529:EGMAS-Alk (95:5, P2).

step. Table 1 displays the XPS-determined elemental composition detected on ungrafted electrospun fibers P0 *vs.* P1, P2 and P3 fibers, grafted with 100%, 95% and 90% PEGMA-529 respectively. The strong increase in oxygen content and reduction in chlorine, which were seen for P1-P3, confirmed the formation of a PEG based coating around these fibers.

As a further proof of successful grafting reactions under the conditions used in this study, TEM images of the different fibers were also obtained (Figure 3). The lighter electron density of the PEG coatings can be clearly seen as a grey shade surrounding the core fiber. Based on these the images, the dehydrated graft thickness of the PEG coatings was determined to be ~25 nm (±9 nm) for P1 and 40 nm (±20 nm) for P2.

To show that the presence of a PEGMA polymer brush layer on the surface of the nanofibers could effectively reduce protein adsorption, cut-out electrospun fiber membrane samples of these fibers were weighed and then exposed to a solution containing europium-tagged human serum albumin (Eu-HSA). After incubation any non-adsorbed Eu-HSA was washed off and the residual amount quantified with a time resolved fluorescence assay. The amount of adsorbed Eu-HSA in relation to the fiber mass was determined. Figure 4 displays the protein adsorption after coating with PEGMA (P1), versus the uncoated fibers. A larger difference was seen for the two samples indicating that a protein repellant coating had been formed.

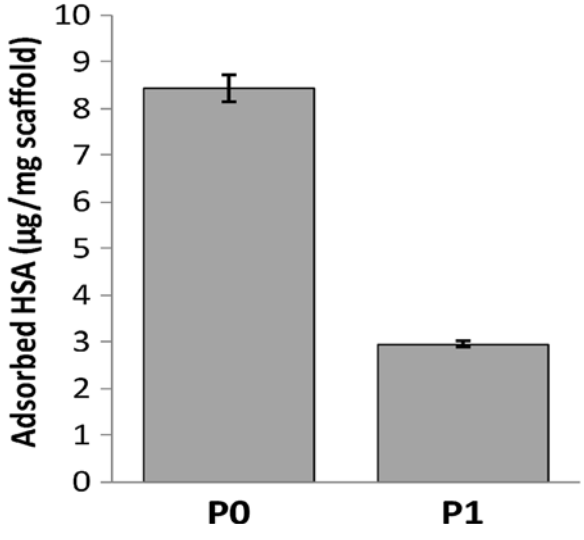

Figure 4 Eu-HSA adsorption on with non-grafted electrospun fibers (P0) and those grafted with PEGMA (P1).

Pendant alkyne side groups, introduced by co-grafting with a monomer containing terminal alkyne side-chains, EGMAS-Alk, were able to be reacted, via copper (I)-mediated 1,3-dipolar cycloaddition [27], with the azide group of a fluorine-functionalised XPS label, TFAB (refer to Figure 1 for structure). The XPS-determined elemental composition for P1-P3 grafted fibers showed that the alkyne-containing fibers were able to be coupled with the fluorine-azido compound TFAB using this Click coupling chemistry (Table 2). This is illustrated by the increase in the fluorine atomic %, concurrent with the increase in alkyne concentration used for SI-ATRP grafting of the fibers. Furthermore, only very low levels of fluorine were observed on P1 fibers (100% PEGMA) suggesting that the degree of non-specific adsorption of the TBAF molecule was low.

Overall SI-ATRP proved effective both as a surface modification strategy that allowed for the covalent coupling of a specific molecule as well as providing a surface

Table 2 Elemental composition of ATRP grafted fibers with TBAF label[a] as determined by XPS analysis

	P1	P2	P3
C 1s	91.1	76.0	75.5
O 1s	7.9	20.6	19.5
N 1s	0.9	1.6	2.2
F 1s	0.1	1.8	2.9

a) P1 grafted with PEGMA-529 (100%); P2 with PEGMA-529:EGMAS-Alk (95:5); P3 with PEGMA-529:EGMAS-Alk (90:10) P3.

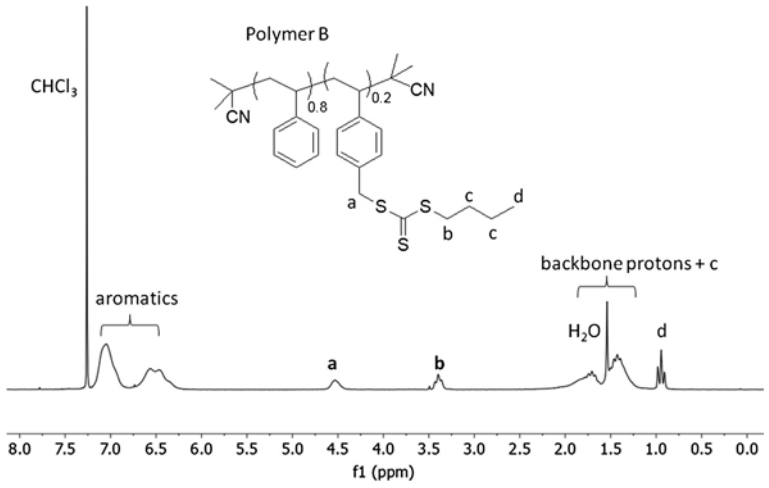

Figure 5 ^{1}H NMR ($CDCl_3$) of RAFT polymer B, showing main peak assignments.

that resists non-specific protein adsorption. Future work will be aimed at attaching biologically relevant molecules, as a way of controlling cell receptor - ligand interactions of interest.

Whilst SI-ATRP has proven itself to be a versatile and easy to apply technique to prepare polymer brushes, chemically useful monomers such as acrylic acid can present problems for ATRP due to complexation that can occur between acid moieties and the metal-ligand catalyst system. Furthermore, the copper catalyst system can be difficult to remove completely from the final polymer brushes, at least to a level that is acceptable for biomedical applications. RAFT is an attractive alternative for producing polymer brushes that we wanted to investigate in this work, in particular because of its compatibility with a wide range of functionality in monomers and solvents, especially those relavant to many biological applications [28]. In an additional step we therefore derivatised the benzyl chloride groups of poly(Sty-*co*-VBC) polymer A, with butyltrithiocarbonate groups to produce polymer B (Figure 1). The goal here was to introduce RAFT-tethered polymerization sites onto the electrospun fibers, from which surface grafting using RAFT could be carried out.

^{1}H NMR analysis of sample (Figure 5) showed full conversion of benzyl chloride groups to RAFT groups. A diminished proportion of styrene residues compared to polymer A, may simply be the result of the work-up.

Electrospinning of RAFT polymer B was able to be performed under similar conditions as for polymer A. However, the oxygen in the system was replaced by purging the electrospinning apparatus with nitrogen as a way to reduce the likelihood of oxidising potentially sensitive RAFT groups, especially in the presence of the high voltage used for the electrospinning process. We found that carrying out the spinning in the presence of oxygen produced fibers which were intact, however, XPS analysis (Table 3) showed more than a 100% increase in the O/S elemental ratio from 0.68/1.70 (=0.40) to 1.96/2.16 (=0.90) for fibers electrospun in the presence of O_2 versus N_2. This decrease in sulfur content indicated that some of the RAFT groups may have been compromised during electrospinning in the presence of oxygen. RAFT fibers electrospun in the prescence of N_2 and grafted with 100% AAm also showed a greater increase in N % (4.44 versus 3.34) after grafting. This also indicated that RAFT groups may have been less affected when electrospun in an inert atmosphere.

Polymer B, electrospun in the presence of N_2, gave 600 nm fibers and these were used as substrates for poly (acrylic acid)/poly(acrylamide) brush coatings.

The fibers were grafted with aqueous solutions of either 100% AAm or a feed composition containing 10% AAc. We

Table 3 Elemental composition as determined with XPS of polymer B, electrospun with and without N_2, before and after acrylamide (AAm) grafting

	Electrospinning no N_2		Electrospinning with N_2	
	Bare fiber	100% AAm grafted	Bare fiber	100% AAm grafted
C 1s	95.9	90.0	97.6	89.0
O 1s	2.0	5.5	0.7	5.5
N 1s	-	3.3	-	4.4
S 2p	2.2	1.1	1.7	1.0

included the acrylic acid into the monomer feed so that the resultant coatings could then be coupled to amine functionalised molecules via NHS activated esters, which is a very convenient and well known method for coupling in an aqueous environment. On the other hand, polyacrylamide coatings are inherently low fouling [29] and generally speaking we have found that a significant molar percentage of acrylic acid can be incorporated into copolymer coatings, along with acrylamide, without compromising the low-fouling nature of the resultant copolymer coating (unpublished data). Overall, these factors make the RAFT approach a rational platform, providing bio-conjugation sites which are conveniently accessed, and as part of a low-fouling substrate.

The grafting was carried out in a glove box, where the fibers were immersed into aqueous solutions of either 100% AAm or a mixture of 10% AAc and 90% AAm. Heating the mixtures at 60°C produced chemically grafted chains on the surface of the fibers as well as polymers which formed in solution. Solution polymer is produced as a result of the initiator being present in solution. After three days any unbound polymers produced during the process were washed away by immersing the fibers in MilliQ water and carefully rinsing them several times with water. The fibers were then briefly immersed into a solution of EDC and NHS to form the NHS-activated ester of the carboxylic groups in the polymer coating, which is capable of reacting with amines via amide bond formation. After washing away the excess EDC and NHS coupling agents the fibers were immersed into a solution of 2,2,2-trifluoroethylamine (TFEA). After washing and drying the fibers the elemental fluorine of the CF_3 groups could be easily detected and quantified by XPS. Indeed it is worth noting that some conversion of trithiocarbonate end-groups to thiols (as in Figure 1), could be the result of aminolysis occurring in the presence of amines during the coupling reaction. However, we don't believe that the reaction conditions used would promote a significant level of this. And further, the side reaction would not result in coupling of the fluorinated marker to the surfaces to be detected by XPS.

To investigate the specificity of the amide reaction and demonstrate that the fluorine signal in the XPS spectra was not due to adsorbed molecules on the surface, several controls were run: fibers grafted with AAm only (i.e. no acid groups for conjugation); fibers grafted with 1:9 AAc/AAm but not activated; 1:9 AAc/AAm grafted fibers activated but not treated with amine and 1:9 AAc/AAm grafted fibers treated with amine but no activation agents. Table 4 displays the elemental composition as detected by XPS analysis of the fibers. The first thing to note is that the 100% AAm coated fibers had a higher nitrogen value compared to the 1:9 AAc/AAm copolymer coatings, indicating that a coating was successfully formed. The 100% AAm coated fibers displayed zero fluorine % showing that no adsorption had taken place. The strong fluorine % for the activated and TFEA-incubated fibers, along with the negligible amount seen for the unactivated fibers indicates that successful covalent binding of TFEA had taken place.

In this work we demonstrated that the RAFT approach holds great promise for producing surface grafted electrospun fibers with carboxylic acid functionality that can be utilised further for conjugation. In order to extend the RAFT platform, future work will be directed at grafting non-fouling PEG monomers, as was carried out using the ATRP grafting approach.

Conclusion

In this work we have reported both the electrospinning of polystyrene based copolymers and surface initiated ATRP and RAFT grafting from these materials to produce robust hydrophilic polymer brush coatings. PEGMA brushes, produced via SI-ATRP imparted protein resistance to the electrospun fibers. Grafting in the presence of an alkyne-functional monomer introduced moieties which could be utilised in subsequent immobilisation reactions using click chemistry. Carboxylic acid groups were successfully introduced on the surface of fibers using the RAFT grafting technique and these groups could also be used for the covalent immobilisation of amine compounds. For the RAFT approach, the electrospinning process was carried out in the presence of nitrogen as there was some evidence of deterioration of RAFT groups.

Overall, this study has presented a viable route to prepare electrospun scaffolds that have low background protein adsorption and that can also provide bioactive signals if required, such as proteins, peptides or small molecules. These strategies will continue to be investigated as effective methods for presenting cell-signalling molecules within a biomimetic, low fouling 3D environment which is of relevance to cell culture applications.

Table 4 Elemental composition of RAFT-grafted electrospun fibers as determined by XPS analysis

	AAm (100%)	AAc:AAm (1:9)	AAc:AAm (1:9) NHS/EDC[a]	AAc:AAm (1:9) TFEA[b]	AAc:AAm (1:9) TFEA+NHS/EDC[c]
C 1s	69.7	77.2	81.9	73.3	71.0
O 1s	15.6	13.7	10.9	16.7	15.5
N 1s	14.7	9.2	7.1	10.0	10.9
F 1s	-	-	-	-	2.5

a) NHS/EDC activated only; b) TFEA treated only; c) NHS/EDC activated and TFEA treated.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
TA: carried out synthesis and electrospinning of polymers A and B, ATRP grafting from polymer A fibers, coupling of XPS marker off ATRP grafted and RAFT grafted fibers, XPS analysis and compilation of experimental section; FE: drafted manuscript including figures, tables, graphs; KMT: synthesis of polymers A and B, RAFT grafting from polymer B fibers and subsequent XPS analysis, analysis of europium labelled HSA and subsequent adsorption experiments; BRC: conceived and optimized procedure for ATRP grafting and Eu-HSA protein adsorption study; XH: carried out SEM and TEM analysis of fibers; AR: set up of nitrogen purging equipment for electrospinner, electrospinning of RAFT fibers; DRN: significant intellectual input regarding electrospinning experiments; LM: co-development and conception for the breadth of the studies, scientific leadership of polymer synthesis and polymer grafting work, development and supervision of protein adsorption assay experiments, review of data acquired during project, co-supervision of TA and AR; JSF, HT and RAE: significant intellectual inputs into design of study and conception of ideas. All authors read and approved the final manuscript.

Acknowledgements
This research was supported by the Cooperative Research Centre for Polymers (CRCP). The authors are grateful to Suzanne Pereira who synthesized both the EGMAS-Alk monomer and the TFAB XPS label.

Author details
[1]CSIRO, Materials Science and Engineering, Clayton, Australia. [2]Cooperative Research Centre for Polymers (CRCP), Clayton, Australia. [3]Department of Materials Engineering, Monash University, Clayton, Australia. [4]Present Affiliation : Faculty of Engineering & Industrial Sciences, Swinburne University of Technology, PO Box 218, Hawthorn, Victoria 3122, Australia. [5]Present Affiliation: Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Australia. [6]Present Affiliation: Institute for Frontier Materials, Geelong Technology Precinct, Geelong Waurn Ponds Campus, Deakin University, Victoria 3216, Australia. [7]Present Affiliation: ANU College of Engineering and Computer Science, Australian National University, Canberra ACT 0200, Australia.

Received: 30 May 2013 Accepted: 20 June 2013
Published: 5 July 2013

References

1. Stevens MM, George JH (2005) Exploring and Engineering the Cell Surface Interface. Science 310:1135–1138
2. Johnson G, Evans RA, Thissen H, Meagher L, Pasic P (2008) Polymeric Coatings and Methods for Forming Them. CSIRO, WO, 2008/019450 A1
3. Koegler P, Clayton A, Thissen H, Santos GNC, Kingshott P (2012) The influence of Nanostructured Materials on Biointerfacial Interactions. Adv Drug Deliver Rev 64:1820–1839
4. Nisbet DR, Forsythe JS, Shen W, Finkelstein DI, Horne MK (2009) A Review of the Cellular Response on Electrospun Nanofibers for Tissue Engineering. J Biomat Appl 24:7–29
5. Nisbet DR, Yu LMY, Zahir T, Forsythe JS, Shoichet MS (2008) Characterization of Neural Stem Cells on Electrospun Poly(epsilon-caprolactone) Submicron Scaffolds: Evaluating their Potential in Neural Tissue Engineering. J Biomat Sci Polym E 19:623–634
6. Baker SC, Atkin N, Gunning PA, Granville N, Wilson K, Wilson D, Southgate J (2006) Characterisation of Electrospun Polystyrene Scaffolds for Three-dimensional in Vitro Biological Studies. Biomaterials 27:3136–3146
7. Polini A, Pagliara S, Stabile R, Netti GS, Roca L, Prattichizzo C, Gesualdo L, Cingolani R, Pisignano D (2010) Collagen-functionalised Electrospun Polymer Fibers for Bioengineering Applications. Soft Matter 6:1668–1674
8. Winblade ND, Nikolic ID, Hoffman AS, Hubbell JA (2000) Blocking Adhesion to Cell and Tissue Surfaces by the Chemisorption of a Poly-L-lysine-graft-(poly(ethylene glycol)phenylboronic acid) Copolymer. Biomacromolecules 1:523–533
9. Choi JS, Leong KW, Yoo HS (2008) In Vivo Wound Healing of Diabetic Ulcers using Electrospun Nanofibers Immobilized with Human Epidermal Growth Factor (EGF). Biomaterials 29:587–596
10. Park YJ, Kim KH, Lee JY, Ku Y, Lee SJ, Min BM, Chung CP (2006) Immobilization of Bone Morphogenetic Protein-2 on a Nanofibrous Chitosan Membrane for Enhanced Guided Bone Regeneration. Biotechnol Appl Bioch 43:17–24
11. Horne MK, Nisbet DR, Forsythe JS, Parish CL (2010) Three-Dimensional Nanofibrous Scaffolds Incorporating Immobilized BDNF to Promote Proliferation and Differentiation of Cortical Neural Stem Cells. Stem Cells Dev 19:843–852
12. Grafahrend D, Heffels K-H, Beer MV, Gasteier P, Moeller M, Boehm G, Dalton PD, Groll J (2011) Degradable Polyester Scaffolds with Controlled Surface Chemistry Combining Minimal Protein Adsorption with Specific Bioactivation. Nat Mater 10:67–73
13. Hook AL, Voelcker NH, Thissen H (2009) Patterned and Switchable Surfaces for Biomolecular Manipulation. Acta Biomater 5:2350–2370
14. Barbey R, Lavanant L, Paripovic D, Schuewer N, Sugnaux C, Tugulu S, Klok HA (2009) Polymer Brushes via Surface-Initiated Controlled Radical Polymerization: Synthesis, Characterization, Properties and Applications. Chem Rev 109:5437–5527
15. Xu FJ, Zhao JP, Kang ET, Neoh KG, Li J (2007) Functionalization of Nylon Membranes via Surface-Initiated Atom-Transfer Radical Polymerization. Langmuir 23:8585–8592
16. Gualandi C, Vo CD, Focarete ML, Scandola M, Pollicino A, Di Silvestro G, Tirelli N (2013) Advantages of Surface-Initiated ATRP (SI-ATRP) for the Functionalization of Electrospun Materials. Macromol Rapid Commun 34:51–56
17. Fu GD, Lei JY, Yao C, Li XS, Yao F, Nie SZ, Kang ET, Neoh KG (2008) Core-sheath Nanofibers from Combined Atom Transfer Radical Polymerization and Electrospinning. Macromolecules 41:6854–6858
18. Yao F, Xu L, Lin B, Fu G-D (2010) Preparation and Applications of Functional Nanofibers based on the Combination of Electrospinning, Controlled Radical Polymerization and Click Chemistry. Nanoscale 2:1348–1357
19. Yoshikawa C, Zhang K, Zawadzak E, Kobayashi H (2011) A Novel Shortened Electrospun Nanofiber Modified with a 'Concentrated' Polymer Brush. Sci Technol Adv Mat 12 015003:1–7
20. Brandl C, Greiner A, Agarwal S (2011) Quick Polymerization from Electrospun Macroinitiators for Making Thermoresponsive Nanofibers. Macromol Mater Eng 296:858–864
21. Yano T, Yah WO, Yamaguchi H, Terayama Y, Nishihara M, Kobayashi M, Takahara A (2011) Precise Control of Surface Physicochemical Properties for Electrospun Fiber Mats by Surface-Initiated Radical Polymerization. Polym J 43:838–848
22. Coad BR, Lu Y, Meagher L (2012) A Substrate-Independent Method for Surface Grafting Polymer Layers by Atom Transfer Radical Polymerization: Reduction of Protein Adsorption. Acta Biomater 8:608–618
23. Meyers SR, Grinstaff MW (2012) Biocompatible and Bioactive Surface Modifications for Prolonged In Vivo Efficacy. Chem Revs 112:1615–1632
24. Salinas CN, Anseth KS (2008) The Influence of the RGD Peptide Motif and its Contextual Presentation in PEG Gels on Human Mesenchymal Stem Cell Viability. J Tissue Eng Regen Med 2:296–304
25. Doran MR, Frith JE, Prowse ABJ, Fitzpatrick J, Wolvetang EJ, Munro TP, Gray PP, Cooper-White JJ (2010) Defined High Protein Content Surfaces for Stem Cell Culture. Biomaterials 31:5137–5142
26. Ameringer T, Fransen P, Bean P, Johnson G, Pereira S, Evans RA, Thissen H, Meagher L (2012) Polymer Coatings that Display Specific Biological Signals While Preventing Nonspecific Interactions. J Biomed Mat Res A 100A:370–379
27. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. Angew Chem Int Ed 41:2596–2599
28. Boyer C, Bulmus V, Davis TP, Ladmiral V, Liu J, Perrier S (2009) Biological Applications of RAFT Polymerization. Chem Rev 109:5402–5436
29. Kochkodan VM, Sharma VK (2012) Graft Polymerization and Plasma Treatment of Polymer Membranes for Fouling Reduction: A Review. J Environ Sci Heal A 47(12):1713–1727

doi:10.1186/1559-4106-8-16
Cite this article as: Ameringer *et al.*: Surface grafting of electrospun fibers using ATRP and RAFT for the control of biointerfacial interactions. *Biointerphases* 2013 **8**:16.