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FULL ARTICLE

Surface modification of polydopamine coated particles *via* glycopolymer brush synthesis for protein binding and FLIM testing

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Glycopolymer brushes were successfully synthesized on polydopamine coated silicon dioxide particles using a one-step 'grafting from' method to produce high density polymer brushes. This one-step 'grafting from' method uses an azide-terminated RAFT agent that simultaneously grows polymer chains and attaches to the polydopamine coating. The azide group rearranges *via* an *in situ* Curtius rearrangement to form an isocyanate group. This reacts with the hydroxyl and amine groups on the polydopamine coating while simultaneously growing a polymer chain. Poly(pentafluorostyrene) polymer brushes were grown and attached to the polydopamine coating, then converted to glycopolymer brushes using a thiol substitution reaction. This creates a surface that facilitates protein binding. Fluorescently tagged Concanavalin A proteins were bound to the surface and the binding ability was investigated using Fluorescence Lifetime Imaging Microscopy (FLIM). This reports the facile preparation of particles that are biocompatible, and can be used *in vivo* as drug carrier systems. The use of polydopamine coatings, one-step 'grafting from' polymer brush synthesis and 'click' chemistry to create glycopolymers, collectively are an improved and simple way to prepare particles for biomedical applications.

Introduction

In 2007, Messersmith and co-workers reported the self-polymerization of dopamine in pH-buffered aqueous solutions to spontaneously create thin and robust polymer coatings on many different materials[1]. Since then, polydopamine coatings have been used for a wide variety of applications such as nanocapsule generation[2, 3], surfaces for biomimetics[4], and living cell encapsulation[5]. Polydopamine coatings can easily be used as a platform for further modification. The abundance of hydroxyl and amine functional groups on polydopamine coatings facilitate many reactions to allow surface modification to be carried out simply[1].

One such modification is polymer brushes, which are polymer chains tethered at one end to a surface or interface through either a physisorption or covalent attachment method[6]. When these chains are end-tethered at a sufficiently high grafting density, the maximization of the conformational entropy forces the chains to stretch away from the surface, thus forming a polymer brush configuration[7]. Modifying surfaces with polymer brush layers instead of bulk polymers offers enhanced polymer surface properties[7-9] and a higher density of chains in a given surface area compared to bulk polymers[10]. Polymer brushes attached using traditional covalent methods can be tethered to a surface using a 'grafting to' or 'grafting from' method. 'Grafting to' involves reacting end-functionalized pre-made polymer chains to the surface. The long polymer chains can experience steric hindrance which limits the grafting density. This is a one-step process and not preferred where high grafting density polymer brush layers are desirous. 'Grafting from' is traditionally a two-step process where in the case of controlled radical polymerization such as reversible addition-fragmentation chain transfer (RAFT) polymerization the chain transfer agent (RAFT agent) is bound to the surface before completing the polymerization. Once the RAFT agent is bound to the surface, the polymerization is carried out. This is termed 'surface-initiated polymerization' and as the monomer units exhibit far less steric hinderance than long polymer chains, higher grafting densities of polymer brushes can be achieved.

Recently Le-Masurier and co-workers reported the use of an azide-terminated RAFT agent[11] to complete a one-step 'grafting from' synthesis of polymer brushes[12]. The azide-terminated RAFT agent undergoes an *in situ* Curtius rearrangement during the polymerization to form an α-isocyanate terminated RAFT agent. The polymer chain grows and while still oligomeric, can attach to surfaces through the α-isocyanate group. The polymer chain continues to grow, achieving a grafting density as high as traditional two-step 'grafting from' methods. Le-Masurier and co-workers reported this method to grow polystyrene polymer brushes on polydopamine coated silicon dioxide particles at a high grafting density in a one-step 'grafting from' method[12].

This paper expands this one-step 'grafting from' method for use in creating glycopolymer brushes for protein binding on polydopamine coated silicon dioxide particles. These materials can be developed for ligand mediated actively targeting drug carrier systems[13, 14]. Glycoplymers, or carbohydrate-containing polymers exhibit the glycoside cluster effect[15] and enhance interactions between the glycopolymer and proteins[16]. Glycopolymers can be easily created from modifying poly(pentafluorostyrene) (PPFS) polymer chains with 1-thio-β-D-glucose *via* a simple 'click' reaction[17].

Glycopolymer brush coatings are therefore be an excellent protein binding surface. Furthermore by modifying a polydopamine coating with glycopolymer brushes, a surface that facilitates protein binding can be created with ease.

Fluorescence lifetime imaging microscopy (FLIM) will be employed to confirm the successful binding of fluorescently tagged proteins to the glycopolymer coated surfaces. The lifetime measurement is different for bound and unbound fluorescently tagged proteins. This differences can be measured and allow the amount of binding to be ascertained. To our knowledge, this is the first report of using FLIM to measure protein binding on glycopolymer brush coated PDA surfaces.

Experimental

Materials

Unless otherwise stated, all materials were obtained from Sigma-Aldrich and used without further purification. Silicon dioxide (SiO_2), dopamine hydrochloride (DA, 98%), tris (hydroxymethyl) aminomethane (TRIS, 99.8%), 4,4'-azobis(4-cyanovaleric acid) (ABCVA, 97%), carbon disulfide (99%), butanethiol (99%), trioctylmethylammonium chloride (Aliquat 336), diphenylphosphoryl azide (DPPPA, 97%), triethylamine (TEA, 99%), fluorobenzene (99%), dibutyl tin dilaurate (DBTDL, 95%), lectin-fluorescein isothiocyanate conjugate from *canavalia ensiformis* (FITC-ConA), 1-thio-β-D-glucose sodium salt (thioglucose), N,N-dimethylformamide (DMF, 99%), N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffer solution (HEPES buffer solution, 99.5%) were all used as received.

Pentafluorostyrene (PFS, 99%) was deinhibited by passing it through basic alumina prior to use. Azobisisobutyronitrile (AIBN) was recrystallized from methanol. A 50% solution of sodium hydroxide was also made up in de-ionized water using sodium hydroxide pellets.

Synthesis of BIAzTC RAFT Agent

The butyl isobutyryl azide trithiocarbonate (BIAzTC) RAFT agent was synthesized from a butyl dimethyl acetic acid trithiocarbonate (BDMAATTC) agent which was prepared according to conditions previously described in the literature by McCormick *et al.*[18] with butanethiol being used instead of ethanethiol. All solvents and liquid reagents were degassed before use and the BDMAATTC was synthesized in a single step.

Butanethiol (18.1 g, 0.200 mol), acetone (96.4 g, 1.66 mol), and trioctylmethylammonium chloride (Aliquat 336; 3.23 g, 8.0×10^{-3} mol) were all added to a round bottom flask and its contents were cooled in an ice bath. A nitrogen atmosphere was provided over the flask. A 50% sodium hydroxide solution (16.8 g, 0.21 mol) was then added over 20 min to the flask which was then allowed to stir for another 20 min. Carbon disulfide (15.2 g, 0.20 mol) was dissolved in acetone (20.3 g, 0.35 mol) and added to the reaction solution. The solution was stirred for an additional 20 min prior to the addition of chloroform (35.8 g, 0.30 mol) to the reaction. Finally, a 50% sodium hydroxide solution (80.0 g, 1.00 mol) was added over 30 min and the reaction was left overnight.

After acetone removal *via* a rotary evaporator, the residue was re-dissolved in water (250 mL) and placed in an ice bath. Concentrated hydrochloric acid was added whilst stirring the reaction vigorously. The aqueous solution was extracted four times with hexane followed by removal under reduced pressure. The residue was washed three times with water prior to column chromatography (ethyl acetate/hexane 2:3 v/v) to isolate the crude product, an orange-brown oil, after solvent removal. Bright yellow crystals of BDMAATTC were formed when the oil, dissolved in a small amount of hexane, was washed with water. This oil was dissolved in a small amount of hexane, and then washed with water three times.

The BDMAATTC agent was converted to butyl isobutyrylazidetrithiocarbonate (BIAzTC) using methods adapted from the literature[11]. In a flask immersed in an ice bath and flooded with a nitrogen atmosphere, BDMAATTC (8 g, 3.36×10^{-2} mol) was dissolved in DCM (25 mL). In a second flask, triethylamine (5 mL, 3.58×10^{-2} mol) and diphenylphosphoryl azide (11 g, 4.00×10^{-2} mol) were dissolved in DCM (30 mL). This solution was added dropwise over 1 hour to the BDMAATTC solution. The mixture was stirred for 2 hours, while kept at 0 °C. The solvents were removed *via* a rotary evaporator and column chromatography (hexane/diethyl ether 98:2 v/v) was used to isolate the crude product. BIAzTC formed a yellow-orange oil that solidified in the freezer and the purity was confirmed *via* ^{1}H NMR.

RAFT Polymerization of PFS using BIAzTC

Pentafluorostyrene (PFS, 20 mL. 1.45×10^{-1} mol), BIAzTC (4.02×10^{-1} g, 1.45×10^{-3} mol), and AIBN (4.8×10^{-2} g, 2.9×10^{-4} mol) were added to fluorobenzene (30 mL). This mixture was divided into five vials, which were sealed and placed in an ice bath while being degassed with nitrogen for 20 min. The five vials were heated in an oil bath at 65 °C for various reaction times (5, 10, 15, 20 and 25 h). Gravimetric analysis of the generated poly(pentafluorostyrene) (PPFS) polymers was used to determine monomer conversion while molecular weight was determined using GPC and NMR.

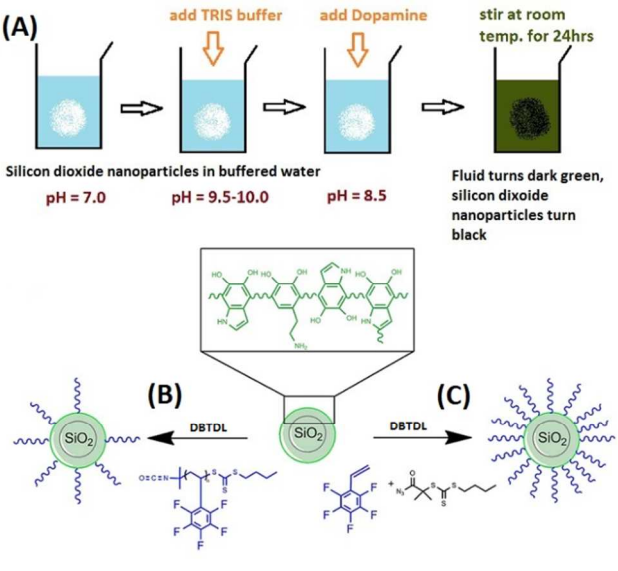

Figure 1. (A) polydopamine coating process on SiO_2 particles, (B) PPFS brush synthesis on PDA@SiO_2 *via* 'grafting to' and (C) 'grafting from'.

Polydopamine coatings on SiO_2 particles

TRIS (9.696×10^{-1} g, 10 mM) was added to de-ionized water (800 mL) to bring the pH to 10. SiO_2 (2.0 g) was added followed by the addition of dopamine hydrochloride (1.6 g, 2 mg/mL) and the pH decreased to 8.5. The solution was stirred overnight at 30 °C (Figure 1A). The particles were filtered and washed with de-

ionized water before being dried in a vacuum oven at 40 °C overnight. The coated particles (PDA@SiO_2) were analyzed using ATR-FTIR, DLS, and TGA.

PPFS 'grafted to' PDA@SiO_2 particles

PPFS taken from the kinetics experiment (15 hour sample, $2.0x10^{-1}$ g, M_n = 5,600 g/mol) and PDA@SiO_2 ($2.0x10^{-1}$ g) were added to fluorobenzene (20 mL) with DBTDL (1 µL). The mixture was sonicated for 10 minutes and then reacted at 65 °C for 15 hours. The PPFS coated PDA@SiO_2 particles were separated by filtration and washed with fluorobenzene and dried in a vacuum oven at 30 °C for 4 hours. The particles were then analyzed using ATR-FTIR, DLS and TGA. (See Figure 1B.)

One-pot 'grafting from' of PFS on PDA@SiO_2

Polydopamine coated SiO_2 particles ($2.0x10^{-1}$ g) were placed in one flask and degassed under vacuum for 20 minutes. PFS (13.5 g, $6.96x10^{-2}$ mol), fluorobenzene (12 mL), BIAzTC ($1.94x10^{-1}$ g, $6.9x10^{-4}$ mol), AIBN ($2.4x10^{-2}$ g, $1.46x10^{-4}$ mol) and DBTDL (1 µL) were added to a second flask. This flask was degassed by bubbling nitrogen through the mixture for 20 minutes prior to cannula transfer to the flask containing the PDA@SiO_2 particles. The solution was reacted at 65 °C for 15 hours, removed from heat, and the particles washed with THF, filtered, and dried in a vacuum oven at 30 °C for 8 hours. The free polymer, collected from the filtered solution after removing the solvent under vacuum, was analyzed using GPC and NMR while the PPFS modified PDA@SiO_2 particles were analyzed using ATR-FTIR, DLS and TGA. (See Figure 1C.)

Glycopolymer brush formation on PDA@SiO_2 particles

Thioglucosylation of PPFS chains bound to PDA@SiO_2 particles was carried out using a method described in the literature[17]. 0.1 g of the particles were added to 10 mL DMF. 0.1 g of the 1-thio-β-D-glucose sodium salt and 0.1 g triethylamine were added. The vials were stirred in an orbital shaker at 25 °C for 30 hours. When the reaction had finished, cold methanol was added to the vial, stirred quickly and then filtered. The particles were rinsed with more cold methanol, collected and then dried in a vacuum oven for 6 hours at 25 °C. The same 'click' method was used for samples with PPFS chains bound via 'grafting to' and 'grafting from' techniques. All particles were analyzed via ATR-FTIR, DLS and TGA.

Concanavalin A binding to glycopolymer coated particles

1 mg of thioglucose and 1 mg of PDA@SiO_2 with glycopolymers (PPFS grown via the one-pot 'grafting from' technique with DBTDL catalyst and then thioglucosylated for 30 hours) were both added to 1 mL each of HEPES buffer solution. 10 mg of FITC-ConA was dissolved in 1 mL of HEPES buffer solution and then $2.5x10^{-1}$ mL of the FITC-ConA solution was added to each of the dissolved particle solutions. All three samples were placed in an orbital shaker at 30 °C, 150 rpm overnight. The concentration of each type of particle was thus $8.0x10^{-1}$ mg/mL and the concentration of the FITC-ConA was 10 mg/mL. All three samples were analyzed using Fluorescence Lifetime Imaging Microscopy. Binding of the FITC-ConA to the glycopolymer coated particles was imaged with a fluorescence microscope.

Characterization

Attenuated Total Reflectance - FTIR spectroscopy (ATR-FTIR). ATR-FTIR spectra were obtained using a Bruker Spectrum BX FT-IR system, using diffuse reflectance sampling accessories and a resolution of 4 cm^{-1}. Each sample was analyzed using 128 scans.

NMR Spectroscopy. NMR analysis was carried out using a Bruker 300 MHz spectrometer at room temperature. All samples were dissolved in deuterated solvents such as deuterated DMSO or deuterated chloroform. ^1H, ^{13}C and ^{19}F NMR spectra were all recorded as required.

Gel Permeation Chromatography (GPC). Molecular weight analysis was performed using a Shimandzu modular system containing a DGU-12A degasser, an LC-10AT pump, a SIL-10AD automatic injector, a CTO-10A column oven and a RID-10A refractive index detector. Four Phenomenex columns (100, 103, 104, 106 Å pore size, 5 µm particle size) were used for the analyses. THF (HPLC grade) or DMAc (HPLC grade) with a flow-rate of 1 mL min^{-1} was used as the mobile phase. The injection volume was 50 µL. The samples were prepared at standard concentrations and filtered through 0.45 µm filters. The unit was calibrated using commercially available linear polystyrene standards (0.5-1000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Thermogravimetric Analysis (TGA). TGA was carried out using a Perkin Elmer STA6000 instrument. All samples were analyzed under a nitrogen environment with the following heating profile: heat from 30 °C to 100 °C at 40 °C per min, hold at an isotherm of 100 °C for 28 min, heat from 100 °C to 900 °C at 10 °C per min and finally hold at an isotherm of 900 °C for 30 min.

Dynamic Light Scattering (DLS). DLS studies of the particles were conducted using a Malvern Zetasizer Nano Series running DTS software and operating a 4 mW He−Ne laser at 633 nm. Samples were pre-filtered with a 0.45 µm size microfilter to remove dust particles. The particles were dissolved in deionized water at a concentration of 2.0×10^{-1} mg/mL. The deionized water's refractive index and viscosity were taken from known literature values. The size measurements were carried out in quartz cuvettes at 25 °C, and the temperature was allowed to equilibrate for 5 min. The number-average hydrodynamic particle size and dispersity index were determined based on an average of five measurements.

Transmission Electron Microscopy (TEM). TEM images were obtained using a JEOL 1400 transmission electron microscope. Samples were dispersed in deionized water and cast onto a carbon-coated grid by dropping the mixture onto the grid and letting it dry in air for 1 h. Neither the samples, nor the grid were stained.

Fluorescence Lifetime Imaging Microscopy (FLIM). FLIM was performed on a Picoquant Microtime200 inverted confocal microscope with a 60X, 1.2 NA water-immersion objective (Olympus). Excitation was *via* a fiber-coupled, pulsed laser diode operating at 470 nm (40 MHz) with a pulse width below 200 ps. The emission was collected using a 550 nm long-pass filter and a single-photon avalanche diode (SPAD) (PDM, MicroPhoton Devices) connected to time-correlated single-photon counting (TCSPC) electronics (Picoharp300, Picoquant). Fluorescein was used to calibrate the phasor plot to a mono-exponential lifetime of 4 ns. The data was acquired and analyzed using SymphoTime software (Picoquant).

Fluorescence Microscopy. Fluorescence images were acquired on a Leica DM2500 M stereo-microscope (Leica Microsystems). Images were visualized with Motic Live Imaging Module software. The fluorescence source was provided by a Leica EL6000 Kubler CODIX illuminator. The excitation filter used was a Leica I3 cube giving a source in the range 450-490 nm. A Moticam 10 camera was used for image acquisition with Moticam Images Plus 2.0 ML software. Images were processed using Image J software (National Institutes of Health).

Results and discussion

RAFT Polymerization of PFS using BIAzTC

An azide functionalized RAFT agent, dodecyl isobutyryl azide trithiocarbonate (DIAzTC), has previously been used in the controlled polymerization of styrene[11, 12]. However, fluorinated monomers have never been employed using azide RAFT agents, and the use of pentafluorostyrene (PFS) is critical to the glycopolymer brush synthesis. As such, an initial kinetic study on the controlled polymerization of PFS using the butyl isobutyryl azide trithiocarbonate (BIAzTC) RAFT agent was investigated. BIAzTC and DIAzTC differ only in that BIAzTC has a shorter four carbon Z-group as opposed to the twelve carbon Z-group for DIAzTC. The functionality and kinetics of each RAFT agent is not affected by this change to the Z-group.

As can be seen in Figure 2, the controlled polymerization of PFS using the BIAzTC RAFT agent was observed. A linear pseudo-first order kinetic plot was obtained with a maximum conversion occurring at about 20 h. This observation is consistent with data generated by Perrier and co-workers[11] with DIAzTC and styrene polymerization. In addition to the linear kinetic plots, the GPC traces all yielded monomodal peaks with dispersities ($Đ$) below 1.13 (See Figures S3 and S4, *Supporting Information*).

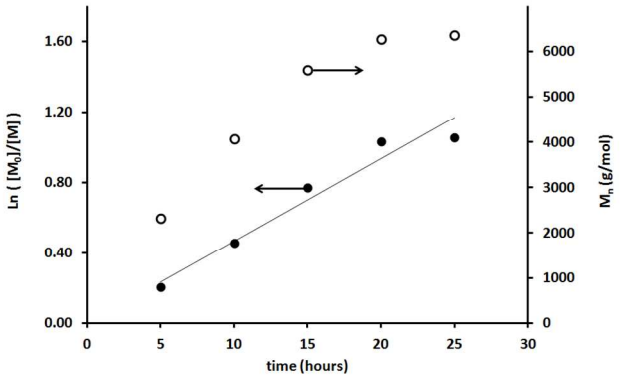

Figure 2. Pseudo-first order kinetic plot and molecular weight evolution with time for PPFS polymerized with BIAzTC.

This data shows that the carbonyl azide functionalized RAFT agent, BIAzTC, can be employed for the controlled polymerization of PFS. Additionally, the isocyanate end group, generated *in situ* during the Curtius rearrangement reaction, is also present during the polymerization (Figure S5, *Supporting Information*). This is also critical, as the isocyanate groups will be employed to perform 'grafting to' and 'grafting from' brush synthesis reactions from the PDA particle scaffolds, similar to work done previously in our labs[12]. Based on the kinetic plot in Figure 2, and the fact that relatively low molecular weight brushes are desired to ensure a high binding affinity with proteins, when glycopolymer brushes are generated, it was determined that PPFS polymerizations with BIAzTC would be conducted at 65 °C for 15 h to generate the required brushes.

Glycopolymer brush synthesis on PDA@SiO_2 particles

In this study, glycopolymer brushes will be synthesized from PDA@SiO_2 coated surfaces by means of employing a 'grafting to' and a 'grafting from' approach to investigate the difference in grafting densities. When brushes of the same molecular weight are compared, the 'grafting from' technique generates higher grafting densies than the 'grafting to' technique. Furthermore, the 'grafting from' approach will utilise a one-pot polymerization and surface attachment technique previously studied in our labs[12]. The glycopolymer brushes were generated by performing a 'click' chemistry reaction between the thioglucose units and *para*-fluoro units of PPFS brushes. In each instance, the PPFS-PDA@SiO_2 brush particles were generated by their respective technique prior to reacting with thioglucose to yield the glycopolymer brush coated particles. Glucosylating the PPFS polymers before they are bound to the PDA@SiO_2 particles may result in the terminal-isocyanate ends reacting with the hydroxyls from the thioglucose units. This is a slow reaction at room temperature but should be avoided to ensure that the polymer chains bound solely to the PDA@SiO_2 surfaces. It should be noted that the unprotected thioglucose salt was employed as to avoid the need for a deprotecting step. The direct thioglucosylation is possible, rather than coupling through the alcohol, when employing polar solvents and base catalysts[19].

The PPFS polymer used for the 'grafting to' approach was a sample from the kinetic studies using BIAzTC with a reaction time of 15 h (M_n of 5,600 g/mol). ATR-FTIR analysis of this polymer confirmed the existence of the isocyanate moiety on the end of the PPFS polymer chains. To facilitate the 'grafting to' reaction of these chains to the PDA@SiO_2 particle surface, a tin catalyst (DBTDL) was used. The coupling of tertiary isocyanates, such as the end group for our RAFT generated polymers, to both hydroxyl and amine functionalities, such as those on polydopamine coatings, only occur when tin catalysts are present[11, 12]. After tin-catalyzed 'grafting to' reactions were performed, the polymer brush coated particles were filtered and dried. ATR-FTIR analysis as shown in Figure 3B confirmed the successful binding of PPFS polymer brushes to the PDA@SiO_2 surface.

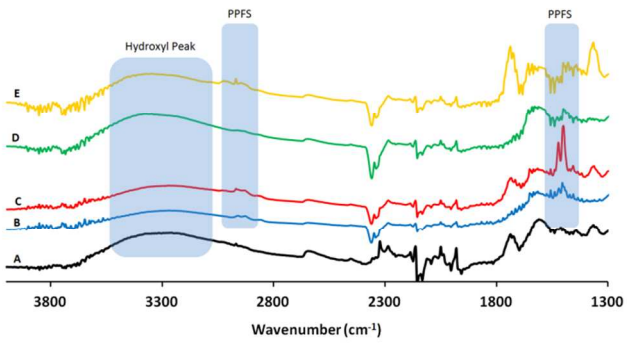

Figure 3. ATR-FTIR spectra showing (A) PDA@SiO_2, (B) PPFS-PDA@SiO_2 *via* 'grafting to', (C) PPFS-PDA@SiO_2 *via* 'grafting from', (D) glycopolymer-PDA@SiO_2 *via* 'grafting to', and (E) glycopolymer-PDA@SiO_2 *via* 'grafting from'.

The DBTDL catalyst was also employed during the one-pot

'grafting from' RAFT polymerizations of PFS in the presence of PDA@SiO_2 particles. As in the kinetic studies, the polymerizations were performed at 65 °C for 15 h so as to generate comparable polymer brush molecular weights, and brush lengths, to those in the 'grafting to' experiments. As with the 'grafting to' process, the 'grafting from' PPFS polymer brush coated particles were washed, filtered, and collected. However, the free PPFS polymer generated during the one-pot 'grafting from' reaction was separated and analyzed by GPC to determine polymer brush characteristics. The PPFS polymer chains grown during the 'grafting from' synthesis on PDA@SiO_2 particles had a dispersity of 1.19 with an M_n of 3,500 g/mol, slightly lower than that used during the 'grafting to' testing. However, NMR analysis was completed to calculate the M_n of the generated PPFS and was determined to be 4,900 g/mol, which is very comparable to the 'grafting to' tests. ATR-FTIR analysis for the PPFS-PDA@SiO_2 brush particles attached using the one-pot 'grafting from' method is shown in Figure 3C. As with the 'grafting to' method, strong signals are shown confirming the successful deposition of the PPFS polymer brushes. The twin peaks characteristic of the fluorinated aromatic ring of PPFS are evident in the spectrum, and these peaks are stronger and more prevalent for the one-pot 'grafting from' method than the 'grafting to' method. This is expected, in that the one-pot 'grafting from' method will produce a higher polymer brush grafting density than the 'grafting to' method.

The PPFS polymer brushes on the PDA@SiO_2 particles were converted to glycopolymer brushes. Both the 'grafting to' and one-pot 'grafting from' PPFS polymer brushes were reacted with thioglucose to generate a fluorinated glycopolymer particle. This was accomplished *via* 'click' reactions of the thioglucose units, as previously described, for 30 h at room temperature. ATR-FTIR analysis was used to confirm the successful conversion of the PPFS polymer brushes to glycopolymers as shown in Figure 3D and E. In addition to the reduction in the aromatic fluorine peak region at 1450 cm^{-1}, a broad hydroxyl peak is clearly visible due to the addition of the thioglucose units on the PFS moieties along the polymer backbone. The hydroxyl peak is greater for the one-pot 'grafting from' sample (Figure 3E), as would be expected for a polymer brush layer with a higher grafting density, as compared to that for the 'grafting to' generated brushes. All of the samples, both pre- and post- thioglycosylation, were then analyzed using thermogravimetric analysis and dynamic light scattering to quantify the particle size of the grafted PDA particles as well as the grafting density of the polymer brush chains generated. The results of these analysis techniques are shown in Table 1.

Table 1. DLS and TGA results for PPFS and glycopolymer coated PDA@SiO_2 particles.

Sample	*Particle diameter (nm)*	*Percent Weight Loss (grafted chains only)*	*Grafting Density (chains · nm^{-2})*
PDA@SiO_2	52	----	----
PPFS-PDA@SiO_2, 'grafting to'	106	5.01	0.20
Glycopolymer-PDA@SiO_2, 'grafting to'	103	13.58	0.20
PPFS-PDA@SiO_2, 'grafting from'	89	12.77	0.83
Glycopolymer-PDA@SiO_2, 'grafting from'	103	28.28	0.83

As shown previously in the literature[12, 20], Equation 1 can be used, in conjunction with the TGA and GPC results, to calculate the grafting density of the polymer chains to compare polymer brush coverage of the particles. All of the results were first converted to a dry basis before proceeding with grafting density calculations presented in Table 1. It should be noted that the grafting density does not change upon thioglucosylation of the PPFS polymer brushes because the reaction only affects the steric crowding of the moieties and not the density of brush attachment sites.

$$\sigma = \frac{\delta \times V \times W_{poly} \times N_A}{W_{silica} \times M_n \times SA} \qquad (1)$$

These results show reasonable agreement with those found by Le-Masurier and co-workers where polystyrene polymer brushes were synthesized using a 'grafting to' approach[12]. As in this previous study, the one-pot 'grafting from' samples exhibit a much higher grafting density than the samples generated by the 'grafting to' method. However, the 'grafting from' approach for PFS is significantly higher than previously investigated using styrene monomer. This could be due to the use of fluorobenzene for the PFS polymerisation. It is possible that the solvent could be swelling the PDA coated silica particles allowing for greater accessibility to the internal hydroxyl and amine moieties in the PDA layer. This occurrence would also explain the particle diameter increase for the 'grafting from' process being smaller than that for the 'grafting to' process, even though a higher weight loss was observed for the 'grafting from' process (Table 1). TEM images of the glycopolymer-PDA@SiO_2 particles, using the one-pot 'grafting from' technique, are depicted in the supporting information (Figure S9).

Concanavalin A binding

Concanavalin A is the most commonly used protein for investigating carbohydrate-lectin interactions using glycopolymers[21, 22]. As such, this was used as the target lectin for preliminary binding tests on glycopolymer-PDA@SiO_2 particles. While analysis has shown that the glycopolymer brushes are in the brush regime, what is not known is if the high grafting density will adversely affect the binding affinity. For exceedingly high grafting density glycopolymer brushes, only the terminal carbohydrate moieties would be involved in binding due to the inability for the lectin to diffuse down through the brush layer and would essentially behave as a flat surface[23]. Protein binding for densely packed glycopolymer brushes would not therefore exhibit the glycocluster effect, the ability for a lectin to present an enhanced binding affinity by interacting with multiple carbohydrate moieties on a polymer backbone[24, 25].

Fluorescent lifetime imaging microscopy (FLIM) was employed to investigate the binding of fluorescently tagged Concanavalin A (FITC-ConA) to the glycopolymer brush coated PDA@SiO_2 particles. This technique has been extensively used by our group to prove the release of drugs from nanoparticles.[26-30]. It is expected that the dependence of the fluorescence lifetime of FITC

on its local microenvinronment allows us to correlate the binding profile of ConA to the glycopolymer particles. The glycopolymer-PDA@SiO_2 particle sample generated during the one-pot 'grafting from' reactions was chosen for FLIM analysis as it possesses a significantly higher brush grafting density (Table 1).

Fluorescein isothiocyanate (FITC) is an amine-reactive derivative of fluorescein dye that has an intrinsic fluorescence lifetime of approximately 4 ns at a neutral pH.[31] As shown in Table 2, it is evident that fluorescence lifetime data illustrates that FITC-ConA has two different lifetime values of 3.70 and 2.28 ns with a ratio of relative amplitudes of 2:1. A double exponential fitting was used in this experiment as the χ^2 value was 1.05 which is close to 1, yielding the best fit of the data. The two values for the fluorescence lifetime of FITC-ConA revealed the two distinct microenvironments around the FITC molecules which are chemically attached to ConA. The shorter lifetime (2.28 ns) can be explained by energy transfer of FITC molecules due to the close proximity between molecules, known as self-quenching phenomenon.

Table 2. FLIM analysis results.

Sample	*Lifetime (ns : ns)*	*Signal Intensity Ratio*	*χ^2 value*
FITC-ConA	2.28 : 3.70	2 : 1	1.05
FITC-ConA : thioglucose	2.28 : 3.70	2 : 1	1.12
FITC-ConA : glycopolymer-PDA@SiO_2	2.35 : 4.21	7 : 1	1.38

After incubation with thioglucose, there was no observable difference in the lifetimes or the relative amplitude ratio, as seen in Table 2. However, after incubation of ConA with glycopolymer coated PDA@SiO_2, a significant change in the relative amplitude ratio was noted for the shorter lifetime. This sizeable increase of shorter lifetime can be attributed to the formation of clusters between ConA and the glycopolymer coated PDA@SiO_2. While the glycocluster effect[17] would improve the binding of the FITC-ConA with the glycopolymer coated PDA@SiO_2, there is a far lower molar amount of particles in our binding experiment than the analogus thioglucose experiment, which could account for the long lifetime signal presence. Furthermore, the glycocluster effect can enhance the self-quenching due to the close proximity of FITC molecules which leads to the decrease in lifetime value as mentioned above. The decrease in fluorescence lifetime value of FITC-dextran from 3.9 ns to 1.7 ns upon binding with ConA was also observed in a previous study[32]. The FLIM analysis showed that binding of FITC-ConA is occurring as there is an increase in the shorter lifetime fraction. The binding of ConA to carbohydrates is well known, and FLIM clearly is a useful technique to prove the binding. Fluorescence microscopy was also used to show the FITC-ConA binding to the glycopolymer brush coated PDA@SiO_2 particles and images of the binding can be seen in Figure 4.

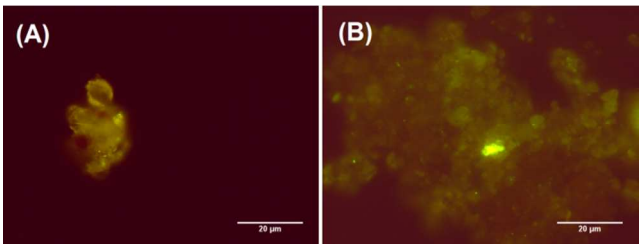

Figure 4. Fluorescence microscopy images of Concanavalin A binding to glycopolymer coated PDA@SiO_2 particles- (A) small cluster of particles as well as (B) large agglomeration.

Conclusions

Glycopolymer brushes were successfully synthesised on polydopamine surfaces in the form of PDA@SiO_2 particles. Using the recently developed one-step 'grafting from' synthesis method, high density polymer brushes were grown and attached to the polydopamine surface. In only three simple steps, the SiO_2 particles were coated with polydopamine, had high density PPFS polymer brushes attached and these polymer chains converted to glycopolymer brushes. The BIAzTC RAFT agent has been proven to work well with pentafluorostyrene monomers. The polydopamine coatings were confirmed to be excellent platforms for attaching the polymer brushes. FLIM analysis confirmed the protein binding, showing a distinct difference in the fluorescence lifetimes of the bound and unbound FITC-ConA proteins. As expected thermogravimetric analysis showed that a 'grafting to' method will produce a lower grafting density of polymer brushes than a 'grafting from' method. Given that polydopamine is biocompatible[33], it can be hollowed out[2, 3] and loaded with a drug. Polymer brushes can act as ligands for actively targeted drug delivery[13, 14] and we have described a simple method for achieving the successful attachment of high density polymer brushes. Through the sequential use of polydopamine coating technology, one-step 'grafting from' polymer brush synthesis and 'click' chemistry, a facile method of creating glycopolymer coated polydopamine particles has been reported.

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Notes and references

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