Synthesis and characterization of immobilized PAMAM dendrons†

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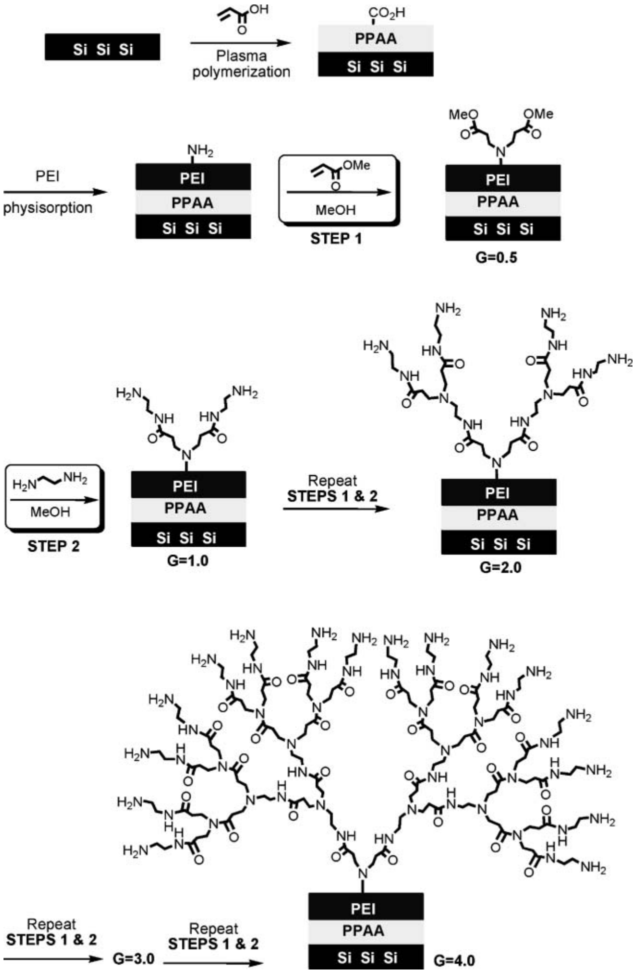
Received (in Cambridge, UK) 1st February 2007, Accepted 28th February 2007
First published as an Advance Article on the web 27th March 2007
DOI: 10.1039/b701550j

This communication describes the synthesis and characterization of immobilized PAMAM dendrons onto a surface modified silicon wafer substrate (functionalized using plasma polymerized PAA) using a "growing from" strategy.

Highly functional dendritic macromolecules have become attractive alternatives to traditional straight chain polymers in a large number of research fields. This is primarily due to their highly ordered, well defined, three-dimensional architectures.[1] These properties have enabled dendrimers to be used successfully in gene delivery,[2] chemical sensing[3] and as drug transport agents.[4] One particularly attractive application is to the areas of sensing, analysis and surface science.[5] Before these applications can be fully realized efficient methods are required, not just for the synthesis of surface immobilized dendrimers, but also for their characterization. Although examples exist in the literature for the immobilization of dendrimers onto solid surfaces, a number of problems remain, particularly with respect to control, final topology and/or characterization.[6] For example, spherical dendrimers that are simply deposited onto a surface can become extremely distorted, taking on flattened disc shapes due to attractive electrostatic interactions between the dendrimer's terminal groups and charged groups on the surface.[7] To improve control in respect of dendron immobilization/synthesis, two general methods can be considered. The first involves taking a dendron and grafting this directly to a suitably functionalized surface (a "grafting to" strategy). The main problems associated with this method are firstly the lack of control with respect to the number of dendritic terminal groups involved in the coupling reaction, and steric crowding, which will influence the size of dendron that can be added and the graft density of the immobilized dendrons. The second general strategy involves a stepwise series of reactions that gradually build up the dendron from a singular focal point on the surface (a "growing from" strategy).[6b] This method should allow larger dendrons to be immobilized onto a surface in a controlled stepwise fashion. Although both methods allow for the controlled synthesis of immobilized dendrons, both require a challenging characterization regime beyond that normally associated with dendrimer synthesis. On reflection, and considering the advantages with respect to control and dendron size, we choose to employ a "growing from" strategy for the synthesis of our initial surface immobilized dendrons. The initial surface selected was an amine functionalized silicon wafer. Specifically, it was proposed that a silicon wafer be coated with a plasma polymerized polyacrylic acid (PPAA) thin film, which could then be treated with polyethyleneimine (PEI) to generate an amine surface suitable for polyamidoamine (PAMAM) dendron synthesis. The plasma polymerization technique[8] was chosen because it is an effective, easy and economical surface modification method used to alter the surface properties of a variety of materials without affecting the material's bulk properties. The materials include metals, polymers, ceramics and composites. Careful manipulation of plasma conditions such as monomer type, power regime and flow rate during a plasma treatment gives control over the chemical composition and the



Scheme 1 Immobilization of PAMAM dendrons to an amine coated surface *via* a growing from regime.

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† Electronic supplementary information (ESI) available: XPS analysis detail, and materials. See DOI: 10.1039/b701550j

surface properties of the deposited thin films, including surface wettability, chemical inertness, adhesion, hardness and biocompatibility.[9] It is for these reasons the plasma technique has been utilised successfully in the fields of biomaterials, tissue engineering and cell culture, sterilization and in the immobilization of biomolecules at the interface.[10] The specific strategy is illustrated in Scheme 1 and begins with the deposition of a PPAA layer onto a silicon wafer in a custom built reactor. Deposition power, time and monomer flow rate were fixed at 20 W, 20 minutes and 2.5 standard cubic centimetres per minute respectively during the plasma treatment. The amine surface required to initiate the PAMAM synthesis was added by physisorbing poly-cationic PEI by immersing the freshly prepared PPAA thin films into a 3 mg mL^{-1} PEI solution which had been dissolved in pH 7 phosphate buffered saline (PBS). After 2 hours the substrates were washed several times rigorously with distilled water to remove any unbound PEI and buffer salts. Having obtained the necessary amine surface the iterative PAMAM synthesis, involving sequential Michael addition and amidation reactions, could begin.[1] The amine terminated substrate was subsequently immersed in a (3 mL) 1/1 (v/v) methyl acrylate–methanol solution for 2 hours, whereby the surface underwent Michael addition to yield an immobilized ester terminated dendron (G0.5). Immersing the ester terminated substrate in a 1/1 (v/v) ethylenediamine–methanol solution for 2 hours rendered an amine terminated dendron (G1). Repetition of both Michael addition and amidation steps yielded higher generation dendrons. After each step the substrates were washed several times with methanol to ensure that no unreacted reagents remained.

Characterization of immobilized dendrons presented a considerable challenge. Conventional dendrimers (*i.e.* solution soluble) can be readily characterized using traditional methods such as NMR and mass spectrometry techniques.[1] However, these methods are unsuitable for analysing surface immobilized molecules and an alternative method is required. One such technique is X-ray photoelectron spectroscopy (XPS), which is a surface sensitive technique which possesses the appropriate sensitivity to monitor each stage in the growing from process. XPS spectra are obtained by irradiating the material with monochromatic radiation, a process that results in the emission of core level electrons that are specific to each element. Therefore, quantitative information regarding the chemical composition of the material and the chemical or electronic state of each element at the surface can be obtained. XPS carbon core line spectra show changes in the binding energy of the ejected photoelectrons, which correspond with changes in the carbon environment. Specifically, information about precise carbon containing functional groups can be obtained. As a result each stage in the stepwise growth of the PAMAM dendrons can be monitored. Hydrocarbons give rise to strong peaks around 285 eV (CHx). When oxygen containing functional groups are introduced, characteristic shifts in the binding energy occur. For example, peaks corresponding to amides (N–C=O) occur around 288 eV, whilst peaks associated with esters and acids (O=C–O) can be seen at 289.5 eV.[11] These properties can therefore be used to characterize our synthesis. For example, immobilization of PEI resulted in a reduction of the acid associated shift (O=C–O) at 289.5 eV and a broadening of the lower energy peak associated with C–N amine bonds (286 eV). The peak at 286 eV illustrates the complex nature of the surface due to the overlapping of peaks afforded to C–O and C–N bonds, Fig. 1. The initial immobilization of the first generation dendrons (amine terminated) produces only slight variations in the carbons XPS profile, with some evidence of an increase in the peak shift associated with the introduction of amide groups at 288 eV. However, as higher generation dendrons are created at the surface it is apparent from Fig. 1 that the relative intensity of the amide-associated peak shift increases with each generation, indicating that the surface density of amide groups is increasing with each completed cycle of growth. The appearance of a small peak at ~289 eV associated with the O=C–O group increases slightly as dendrons G1–G3 are generated. One reason for this peak could be incomplete reaction of the terminal esters, or undesired hydrolysis of the terminal ester groups, which are then unable to react during the amidation step. If either of these possibilities was occurring then we would have lost control of the synthesis and the growing from strategy would be flawed. A second possibility was that the excess methyl acrylate reagent was not being removed. If this was the case then the problem could be overcome by careful (but rigorous) washing.[12] As such, the precursor to the 4th generation immobilized dendron (the ester terminated G3.5 dendron) was carefully washed with excess methanol before subjecting the wafer to the final amidation step. XPS analysis of the G4 dendron showed that the peak at ~289 eV, associated with the O–C=O group, was no longer present. This confirmed that previous generations had not undergone undesirable hydrolysis or incomplete reaction, but had indeed been contaminated with excess methyl acrylate (which was removed after rigorous washing).

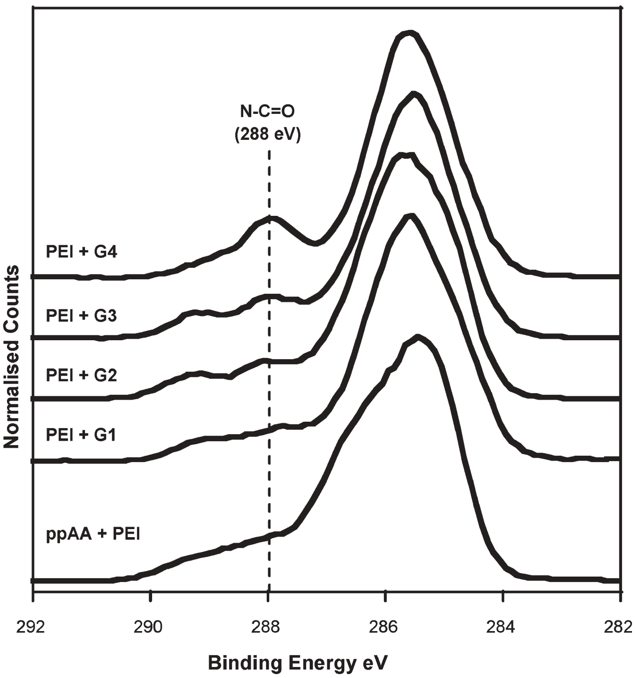

Fig. 1 XPS carbon profile monitoring the immobilization of PAMAM dendrons at the interface. The growing peak at 288 eV confirms an increase in amide bonds as the dendron grows.

The unique 3-D highly branched architectures of dendrimers and dendrons also provide multiple sites for metal coordination not only at the periphery but also within the dendritic construct as highlighted by Tran.[13] Previous studies have monitored the

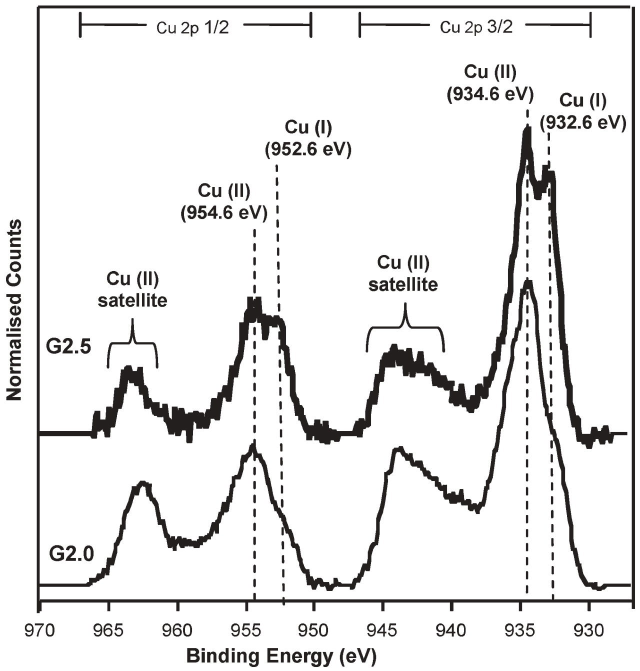

Fig. 2 XPS copper profile describing the coordination behaviour of copper with immobilized dendrons G2 and G2.5.

coordination state of copper using EPR,[14] however, XPS is also well suited to the task of identifying the coordination state of metals.[15] Freeman[16] previously carried out XPS analyses on coordinated copper systems and reported copper in both the Cu^{2+} state indicative of copper coordination at ~934 eV and in the Cu^{+} state at ~932 eV. In this study, immobilized dendron copper complexes were prepared by immersing dendron immobilized substrates into 1 mL of a copper sulfate solution (0.284 g copper(II) sulfate in 10 mL PBS) at 4 °C and leaving them to react overnight. Thorough washing with distilled water to remove any excess copper followed. Analysis of XPS spectra for immobilized dendron copper complexes compared favourably with Freeman's work[16] and highlights the presence of a peak at 934.6 eV for both full and half generation immobilized dendrons (Fig. 2). Interestingly, full generation immobilized dendrons appear to coordinate copper to a greater extent compared to their half generation analogues and this can be attributed to differences in terminal group functionality for each generation, which is in agreement with Zhao[17] who compared ethylenediamine core G4-NH_2 and G4-OH dendrimers in solution and observed a two-fold increase in copper coordination for the former. This suggests that dendritic macromolecules, whether in solution or immobilized at the surface, exhibit similar behaviour largely related to the nature of their terminal functional groups. Thus, for immobilized dendrons at the interface it can be assumed that the extent of copper coordination is dendron generation dependent due to the differences in functional group termini. Copper(I) has been observed in both spectra at 932.6 eV for full and half generation immobilized dendrons and this can be ascribed to X-ray damage[16] causing reduction of copper(II).

The work described in this communication clearly shows that dendrons can be immobilized onto surfaces avoiding problems associated with cooperative electrostatic interactions between dendrimers and surfaces. The synthesis and immobilization of dendrons onto a plasma polymerized surface can be achieved with a high degree of control. XPS was successfully used to qualitatively characterize the surface immobilized dendrons, which allowed us to overcome the problems associated with previous attempts to characterize immobilized dendrons.[6] Furthermore, the immobilized PAMAM dendrons synthesized were used to coordinate copper, the extent of which was shown to be dependent on dendron generation (as demonstrated by XPS). These systems have potential for applications in surface catalysis (*i.e.* lab on a chip technology), bio-sensing and as protein binding surfaces. This work is currently being developed in our laboratory.

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