ACS Combinatorial Science

RESEARCH ARTICLE

pubs.acs.org/acscombsci

Microplates with Adaptive Surfaces

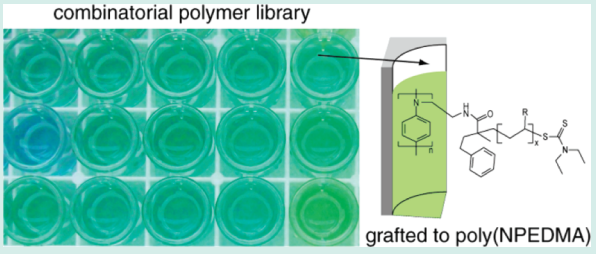
Meshude Akbulut,[†] Dhana Lakshmi,*[,‡] Michael J. Whitcombe,[‡] Elena V. Piletska,[‡] Iva Chianella,[‡] Olgun Güven,[†] and Sergey A. Piletsky[‡]

[†]Hacettepe University, Chemistry Department, Polymer Chemistry Division, 06800, Beytepe, Ankara, Turkey

[‡]Cranfield Health, Vincent Building, Cranfield University, Cranfield, Bedfordshire, MK43 0AL, U.K.

Supporting Information

ABSTRACT: Here we present a new and versatile method for the modification of the well surfaces of polystyrene microtiter plates (microplates) with poly(*N*-phenylethylene diamine methacrylamide), (poly-NPEDMA). The chemical grafting of poly-NPEDMA to the surface of microplates resulted in the formation of thin layers of a polyaniline derivative bearing pendant methacrylamide double bonds. These were used as the attachment point for various functional polymers through photochemical grafting of various, for example, acrylate and methacrylate, polymers with different functionalities. In a model experiment, we have modified poly-NPEDMA-coated microplates with a small library of polymers containing different functional groups using a two-step approach. In the first step, double bonds were activated by UV irradiation in the presence of *N*,*N*-diethyldithiocarbamic acid benzyl ester (iniferter). This enabled grafting of the polymer library in the second step by UV irradiation of solutions of the corresponding monomers in the microplate wells. The uniformity of coatings was confirmed spectrophotometrically, by microscopic imaging and by contact angle measurements (CA). The feasibility of the current technology has been shown by the generation of a small library of polymers grafted to the microplate well surfaces and screening of their affinity to small molecules, such as atrazine, a trio of organic dyes, and a model protein, bovine serum albumin (BSA). The stability of the polymers, reproducibility of measurement, ease of preparation, and cost-effectiveness make this approach suitable for applications in high-throughput screening in the area of materials research.



KEYWORDS: microplates, adaptive surfaces, poly-NPEDMA, photochemical grafting, materials research

INTRODUCTION

Polystyrene microplates have been widely used in immunoassays, for the detection and monitoring of biological or chemical reactions, cell growth, toxicity tests, combinatorial synthesis, and high-throughput screening.[1,2] Typically the immobilization of biological molecules onto the microplate surface is achieved by hydrophobic interactions. The immobilization of small ligands requires microplates with pretreated surface containing amino or carboxylic groups.[3−5] However these plates have a number of drawbacks related to their relatively low surface area, which allows only small quantities of ligands to be immobilized onto their surfaces. There is a need therefore for improved microplates with a higher density of diverse functional groups.

Toward this end, we have developed an efficient and cost-effective method for the modification of microplates with acrylic and methacrylic polymers via grafting to poly(*N*-phenylethylene diamine methacrylamide)-coated microplate wells. The starting material, *N*-phenylethylene diamine methacrylamide (NPEDMA), combines an aniline group and methacrylamide functionality, each capable of polymerization through independent mechanisms.[6] It is possible to prepare a visibly uniform thin coating with this material by electropolymerization or chemical oxidative polymerization through the aniline group, giving rise to a stable polyaniline (PANI) coating, leaving the methacrylamide group exposed for further reaction.[6,7] In 1989, MacDiarmid et al.[8] reported in their article about PANI that it can be deposited by in situ adsorption polymerization as strongly adhering films on a variety of substrates. They suggest that oligomeric radical cations, formed in the early stages of polymerization, are deposited onto hydrophobic surfaces by adsorption, before the reaction proceeds to form high polymer. PANI has been used for the modification of electrode and polymeric (including polystyrene) surfaces through the strong hydrophobic interactions occurring between aromatic rings of the poly-*p*-phenyleneimine segments, formed by the oxidation of aniline monomers, and the substrate surfaces. This has been proposed to account for the formation of stable uniform coatings.[9]

Surface-graft polymerization of unsaturated monomers can then be performed in the poly(NPEDMA)-coated wells to generate surface-anchored diverse polymeric libraries with high densities of functional groups. The activation of poly(NPEDMA) methacrylamide double bonds is achieved using a living initiator, *N*,*N*-diethyldithiocarbamic acid benzyl ester.[7,10] Alternatively, an aniline monomer which incoroporates iniferter[11] could have been used to prepare graftable coatings, however using the

Received: April 21, 2011
Revised: August 17, 2011
Published: September 03, 2011

methacrylamide-derivative may help to stabilize the coatings through the formation of additional cross-links.

In this work, we set out to demonstrate the feasibility of this approach by grafting a range of polymers to microplates activated in this manner and to study their affinity for small molecules (atrazine, dyes) and a model protein (BSA).

EXPERIMENTAL PROCEDURES

Oxidative Polymerization of NPEDMA in Microplates. A range of concentrations of NPEDMA and ammonium persulfate were tested to find conditions suitable for producing thin transparent films of poly(NPEDMA) on the microplate well surfaces. The concentration of NPEDMA was varied between 0.027 and 0.05 M and the concentration of APS varied between 0.029 and 0.055 M in a 5 × 5 grid. The concentration of HCl was kept constant at 1 M. In a typical experiment a microplate was washed with methanol and dried prior to use. NPEDMA in acetonitrile (19.3 μL of a 0.3 M solution) was dispensed into the microplate and mixed with 1.5 M HCl (100 μL), water (13.7 μL), and acetonitrile (10.7 μL). APS in water (6.3 μL of a 1 M solution) was added to this mixture to initiate polymerization, which was carried out for 90 min in the dark. Bluish-green films of poly(NPEDMA) were formed on the inner surfaces of the microplate wells. Poly(NPEDMA) films were washed with distilled water (× 3) and the wells filled with HCl (0.01 M) and left to stand for 30 min. To identify concentrations of NPEDMA, APS and HCl that form thin, transparent coatings over microplates, optical densities (ODs) were recorded using a microplate reader at 490, 550, and 650 nm and at various values of pH [(pH 1, 2, 3 (HCl), 5.0 (acetate buffer), 7.4 and 8.0 (phosphate buffers), and in water)].[4,12] These experiments were performed in triplicate. The results of some of these studies are reported in the Supporting Information (see Supporting Information, S1 and S2). The contact angle was measured and optical images taken under a microscope to examine the uniformity of the coated films.

Activation of Pendant Methacrylamide Double Bonds of Poly(NPEDMA) with *N,N*-Diethyl Dithiocarbamic Acid Benzyl Ester. A solution of *N,N*-diethyl dithiocarbamic acid benzyl ester (10 mL, 0.63 mM in hexane) was prepared in a glass vial. After removal of oxygen by purging with nitrogen for 5 min, 200 μL of this solution was added to each well of the modified microplate using a multichannel pipet. The microplate was placed inside a zip-lock plastic bag connected to a nitrogen inlet to maintain an inert atmosphere. The plate was irradiated for 20 min under a nitrogen atmosphere with a Phillip UV lamp (9 W cm^{-2}). After activation the poly(NPEDMA)-coated microplate was washed with distilled water and dried under a stream of nitrogen. The contact angle of the modified surface was measured to confirm the efficiency of the iniferter-grafting process.

Grafting of Various Functional Polymers to the Surface of Iniferter-Activated Poly(NPEDMA)-Coated Microplates. Solutions of various functional monomers (10 mM), dissolved in 20% acetonitrile, were purged with nitrogen for 5 min to remove oxygen and 200 μL of each were placed into the microplate wells. The microplate was placed inside a zip-lock plastic bag connected to nitrogen inlet to maintain an inert atmosphere, as described above. Polymerization was carried out by irradiation for 25 min with a Philips UV lamp. After thorough washing with distilled water, the polymer-grafted microplates were dried and evidence of grafting was obtained by contact angle measurements.

Screening of Synthesized Polymeric Library for Their Ability to Bind Atrazine. A solution of atrazine in water (200 μL of 200 ng mL^{-1}) was added to each well of a polymer-grafted microplate followed by incubation overnight at room temperature. After incubation, aliquots (100 μL) from each well were collected and analyzed for atrazine content by HPLC-MS (mobile phase: methanol with 0.1% formic acid as additive). The run time was 5 min. The flow rate was 0.2 mL min^{-1} and the injection volume was 10 μL). The characteristic fragment of atrazine (m/z 174) was detected by mass-spectrometry using a Micromass Quattro MS, (Waters, UK) equipped with an ESI interface and used in positive ion mode. The MS parameters were as follows: desolvation gas: 850 L h^{-1}; cone gas: 50 L h^{-1}; capillary: 3.5 kV; cone: 22 V; CE: 15 V; source temperature: +120 °C; desolvation temperature: +350 °C; multiplier: 650 V. All experiments were made in triplicate. Percentage binding was calculated according to eq 1 where C_0 represents the concentration of the analyte in the stock solution, C_{Sup} is its concentration in the supernatant following incubation with the polymer library component.

$$\text{binding } (\%) = 100 \times \frac{(C_0 - C_{Sup})}{C_0} \quad (1)$$

Screening of Synthesized Polymeric Library for Their Ability to Bind the Dyes: Eosin, Methylene Violet (Bernthsen) and Meldola's Blue. Solutions of the respective dye in water (200 μL, 0.01 mg mL^{-1}) were added to each well of a polymer-grafted microplate and incubated for 30 min. Aliquots (100 μL) were withdrawn from each well and transferred to another microplate for measurement of their optical absorbance at 490 (for eosin), 600 (for methylene violet), and 550 nm (for Meldola's blue) using a microplate reader. All experiments were performed in triplicate and percentage binding calculated according to eq 1.

Screening of Synthesized Polymeric Library for Their Ability to Bind BSA. Solutions of BSA (200 μL, 0.2 mg mL^{-1} in PBS, pH 7.4) were added to each well of a polymer-grafted microplate and incubated for 30 min. Aliquots (10 μL) of the solutions from each well were transferred to another microplate and mixed with 200 μL of working reagent (8:1 mixture of Reagent A and Reagent B), according to the manufacturer's instructions (Pierce BCA Protein Assay Kit). After 30 min incubation at 37 °C, the optical density was measured at 550 nm using a microplate reader to calculate the concentration of protein. Measurements were performed in triplicate and the percentage binding calculated according to eq 1.

RESULTS AND DISCUSSION

Optimization of the Condition of Chemical Polymerization of NPEDMA on the Surface of Polystyrene Microplates. The polymerization of aniline has been used previously to coat microplate wells for immobilization and diagnostic purposes.[12] Chemically oxidized aniline forms homogeneous coatings on practically any surface. The coatings are stable in an aqueous environment and only dissolve slowly in organic solvents such as DMF. Polyaniline however is not easily modified with diverse functional groups. To introduce functional diversity it might be possible to choose to polymerize aniline-based monomers with different substituents in the side chain;[13,14] however, these molecules would have different polymerization kinetics

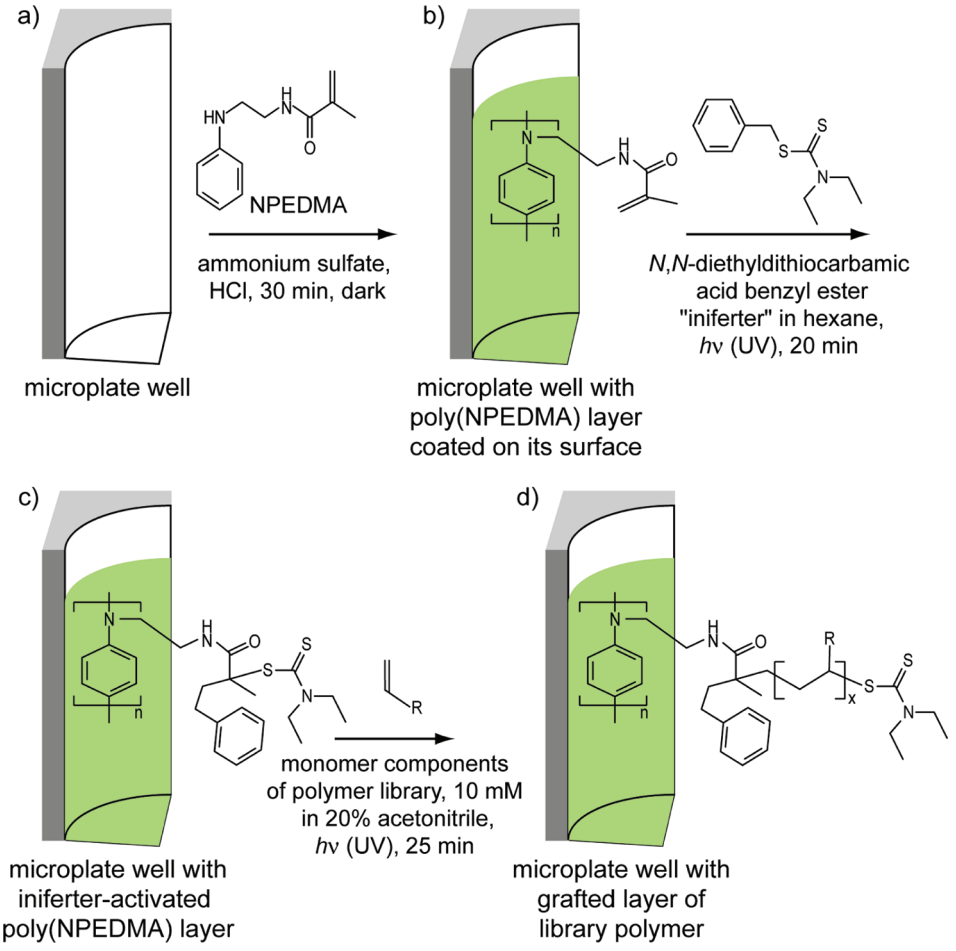


Figure 1. Schematic representation of the preparation of a polymer library grafted to microplate wells via (a) polymerization of NPEDMA, (b) iniferter-activation of double bonds from methacryamide group of poly(NPEDMA), and (c) photopolymerization of the library components from solutions of their monomers in 20% acetonitrile to form (d) the grafted polymer library.

and different physical properties, which would make consistent derivatization of the microplates difficult. As an alternative strategy for microplate functionalization we propose using the aniline derivative, *N*-phenylethylene diamine methacrylamide (NPEDMA),[6] which features a methacrylamide group as a pendant side-chain in its polymers.

Polymerization of NPEDMA can be carried out in situ to coat the well surfaces of polystyrene microplates with thin films of poly(NPEDMA). The justification for using NPEDMA lies in the presence of a methacrylamide group in its molecular structure, making it suitable to act as the anchoring-point for the grafting of other (vinyl) polymers. Our previous studies have shown that the methacrylamide double bond is largely unaffected by oxidative polymerization of the aniline moiety with persulfate.[6] This monomer has the ability to polymerize by electrochemical and chemical oxidative methods to form a polyaniline material with a free radical-reactive surface that can used for further grafting of additional polymer layers (Figure 1). This ability has been used in the present work to generate a small polymer library localized in the wells of microplates, suitable for combinatorial screening studies.

To optimize the conditions of polymerization for the creation of thin, optically transparent and visibly uniform polymer layers, poly(NPEDMA) films were prepared using a range of NPEDMA and APS concentration in the presence of 1 M HCl. After polymerization, the optical densities of the corresponding coatings were measured in water and at various values of pH (1.0, 7.4, and 8.0) at a series of wavelengths (490, 550, 650 nm, being

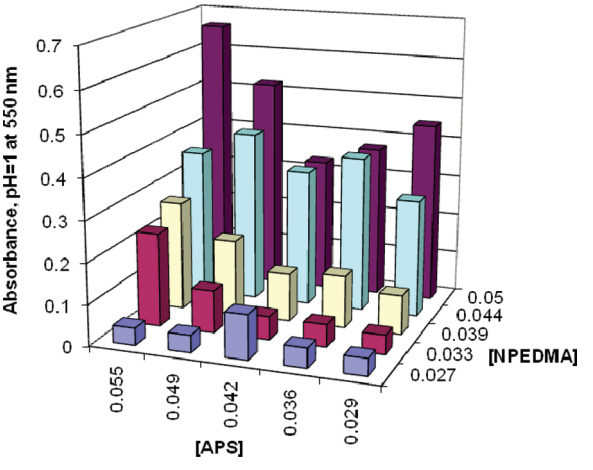


Figure 2. Optical density of poly(NPEDMA)-coated microplate wells prepared at a range of concentration of NPEDMA and APS in 1 M HCl, measured at 550 nm in a microplate reader.

dx.doi.org/10.1021/co200073w |*ACS Comb. Sci.* 2011, 13, 646–652

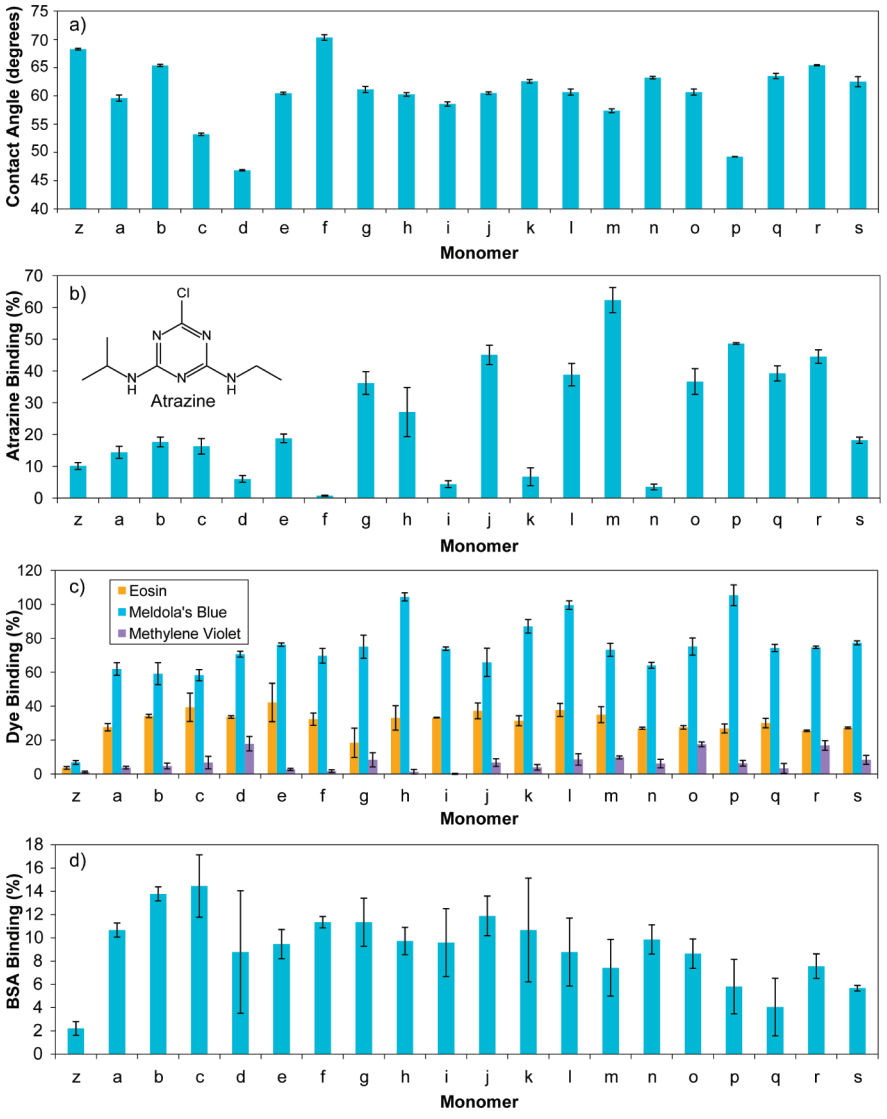


Figure 3. (a) Sessile water contact angles of microplate-grafted polymer library members (in water at pH 7.0); binding of (b) atrazine (200 ng mL^{-1} in water), (c) dyes (0.01 mg mL^{-1} in water), and (d) BSA (0.2 mg mL^{-1} in PBS, pH 7.4) to the polymer library components. Key to library components: z, poly(NPEDMA); a,1-vinylimidazole; b, 2-vinylpyridine; c, AMPSA; d, HEMA; e, 4-vinylpyridine; f, acrolein; g, acrylic acid; h, acrylonitrile; i, allylamine; j, EGDMA; k, itaconic acid; l, *N*,*N*-methylene-*bis*-acrylamide; m, methacrylic acid; n, styrene; o, DEAEM; p, EGMP; q, NPEDMA; r, acrylamide; s, TRIM. All measurements were made in triplicate; error bars represent ±1 standard deviation.

characteristic absorbances of polyaniline)[4,12] (Figure 2 and Supporting Information Figures S1 and S2). Selection of the optimum conditions for well coating was made on the basis of the formation of thin, transparent and visibly homogeneous layers with optical density (OD) $0.2 \leq OD \geq 0.6$. At all wavelengths, we obtained similar trends of absorbance for polyaniline-coated polystyrene.

Although the highest ODs were obtained at 55 mM APS, 50 mM NPEDMA, and 1 M HCl, the coatings were very thick and not sufficiently transparent. On the basis of the previously stated criteria, conditions judged to give the required thin, transparent films were obtained by the polymerization of NPEDMA (39 mM) with APS (42 mM) at an HCl concentration of 1 M. Coating of entire microplates were performed for 90 min in the dark. Modified microplates were subsequently washed with 0.01 M HCl for 30 min. This treatment produced thin and transparent green layers of poly (NPEDMA) deposited on the inner surfaces of the microplates. Repeat experiments resulted in reproducible and good optical quality coatings. Even after many washes the decrease in the optical absorbance was negligible because of strong hydrophobic binding between Poly (NPEDMA) and polystyrene microplates.

A library was constructed by grafting polymers to the inner surfaces of the wells of poly(NPEDMA)-coated microplates via iniferter-mediated photoactivation of the methacrylamide double bonds of poly(NPEDMA). *N*,*N*-Dialkyldithiocarbamate esters can act as iniferters (*ini*-tiator, chain trans-*fer* agent, chain *ter*-minator), imparting living polymerization characteristics when used as photochemical initiators, due to photochemical cleavage of the C−S bond.[15] This leads to the formation of two radicals with very different reactivities. The carbon-centered radical is active and initiates polymerization, which propagates by addition of unsaturated monomers. The second, sulfur-centered radical, is a much less reactive (dormant radical) which does not react with

dx.doi.org/10.1021/co200073w |*ACS Comb. Sci.* 2011, 13, 646–652

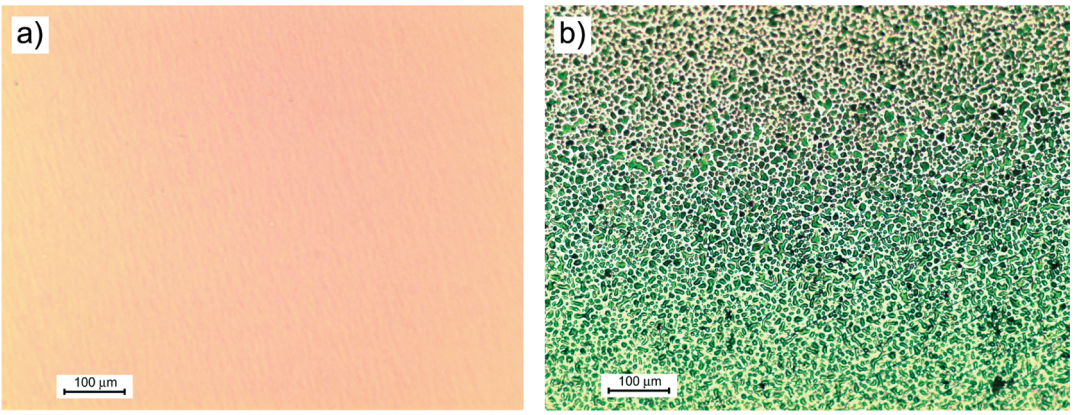


Figure 4. Optical images (10 × magnification) of (a) uncoated PS microplate well and (b) after coating with poly (NPEDMA). Scale bar =100 μm.

monomers, buts acts uniquely as a recapping moiety for the macro-radical growing chain, forming a new iniferter species. This allows polymerization to proceed in a controlled manner and for the reinitiation of polymer growth in the presence of a new monomer solution (allowing block copolymer formation and facilitating grafting). Poly(NPEDMA) bears additional methacrylamide groups which contains double bonds, therefore UV exposure in the presence of *N*,*N*-diethyldithiocarbamate benzyl ester, results in addition of the inferter species to the poly-(NPEDMA) double bonds from the methacrylamide groups. Surface confinement and lack of mobility limits the extent of cross-linking at the surface, producing instead many adducts between iniferter and NPEDMA methacrylamide residues. Using the above method, it was possible to prepare surface-confined macro-iniferters, localized at the walls of the microplate wells.

These were used in the preparation of the polymer library by filling wells with degassed solutions of different monomers and reinitiating polymerization by UV irradiation. The effectiveness of grafting was confirmed by contact angle measurements (Figure 3a). All coatings were hydrophilic (CA < 90°) and, as expected, the most hydrophilic monomers showed the lowest contact angle values: 41.6°, 46.8°, and 49.2° for AMPSA, HEMA, and EGMP, respectively (measurements were done at pH 7.0).

The optical densities of the microplate coatings were compared at each stage of the grafting process: coating with poly-(NPEDMA); iniferter immobilization and grafting of various polymers (see Supporting Information, Figure S5). The optical densities of the coatings were very similar for each well with only small variations between experiments. The standard deviation in the measurements for wells modified with the same polymer was below 5% in all cases. After iniferter immobilization a slight decrease in optical density was observed. Grafting of the different members of the polymer library gave rise to marginally different effects depending on the quantity and type of grafted polymer.

In addition to measurement of optical densities and contact angle, the coating and grafting were also characterized by optical microscopy (Figure 4). Film homogeneity of Poly(NPEDMA) coatings was checked at the microscopic level (10 × magnification). Under close inspection the coatings of poly(NPEDMA) on the surface of polystyrene appeared to have a granular structure, which is typical for polyaniline films. Weighing of individual microplate wells before and after grafting showed that between 3 and 4 mg of modified polyaniline was grafted per well.

Since it was difficult to quantify the degree of grafting within the microplate wells, it proved impossible to tell how efficient the grafting procedure was for each of the monomers tested here. Because of this consideration, comparisons based on the analyses provided below should be regarded as qualitative rather than quantitative in value, being a function both of the strength of interaction between the analytes and the grafted polymer and of the amount of polymer grafted to the respective wells.

Screening of Polymer Library for Its Affinity to Atrazine. Coated plates were tested for their ability to bind atrazine from water. The concentration of free atrazine in the microplate wells before and after incubation was analyzed using HPLC-MS. The purpose of screening was not to assess quantitatively the absolute value of bound atrazine per gram of grafted polymer but to give indication which polymers show preferential binding (Figure 3b). The highest affinity to atrazine was shown by grafted poly-methacrylic acid, poly(NPEDMA) and polymerized EGDMA. Interestingly, methacrylic acid has been previously identified earlier as a functional monomer for the preparation of atrazine-imprinted polymers.[16−19]

Screening of Polymer Library for Its Affinity to Water-Soluble Dyes. In the previous experiment, the analysis of binding was performed using HPLC-MS. In the case of colored compounds, the binding could be accessed directly using an optical microplate reader. To demonstrate this ability, the binding of three chemically diverse dyes: eosin (acidic, negatively charged), methylene violet (Bernthsen) (basic, uncharged), and Meldola's blue (cationic, hydrophobic) (see Supporting Information, Figure S4, for the structures of the dyes used) was measured. As expected, the binding pattern differed depending on whether the dye functionality was basic or acidic (Figure 3c). Another contributing factor was hydrophobic binding which, in the case of Meldola's blue, was responsible for high levels of nonspecific binding. Hydrophobic interactions also contributed to nonspecific binding of other dyes, which was noticeably strongest in the case of the relatively hydrophobic polymers, polyacrylonitrile, and poly-NPEDMA.

Screening of Polymer Library for Its Affinity to BSA. Lastly, the ability of the polymer library to bind proteins was screened, using albumin as the model target. The highest affinity to BSA was observed for poly(AMPSA) (Figure 3d). Since AMPSA is very strong acid, the explanation for the high level of binding seen could lie in its ability to ionize basic functional groups on the protein, forming strong ionic interactions with amines in the protein structure. As in the previous case, an important contributing factor in the interactions between polymers and BSA is hydrophobic binding, as shown in the case of monomers such as

acrolein, 2-vinylpyridine and EGDMA. Since there is no clear correlation between hydrophobicity of the grafted polymers and their affinity for targets, we can conclude that there is no single factor that dominates binding. It seems, as expected that the interactions between target molecules and grafted polymers are complex phenomena, which cannot easily be predicted. This makes combinatorial screening very important for the identification of candidate materials for the development of affinity matrices and functional coatings. Thus the screening tool developed here may have value because of its generic nature, flexibility, easy fabrication, and straightforward application.

CONCLUSION

In conclusion, the results presented here demonstrate the efficiency of a surface-confined grafting procedure for the generation of diverse polymeric libraries in microplates for screening of the corresponding materials for their affinity to analytical targets, such as proteins, various dyes, and herbicide molecules.

The grafting process is easy, reproducible,[7,10,11] and cost-efficient and produces versatile and stable polymer coatings. The screening approach can be applied for small or large analyte molecules, which makes it suitable for clinical, environmental, pharmaceutical, and biotechnological applications.

ASSOCIATED CONTENT

Supporting Information. Materials and equipment used in the Experimental Procedure; the results of optimization experiments on the polyaniline formation; the effect of pH on the polyanilines; mass spectrum of atrazine used in the LC-MS quantitation of this analyte; chemical structures of the analytes (atrazine and dyes) used in this work; optical densities of coatings before and after iniferter activation and following grafting of polymer library components. This information is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*Tel: +44 1234 758328, Fax +44 1234 758380, Email: d.lakshmi.s06@cranfield.ac.uk.

ACKNOWLEDGMENT

Authors acknowledge with gratitude financially support from International Atomic Agency and British Council (MA) and European Commission (FP7 WATERMIM).

ABBREVIATIONS

AMPSA, acrylamido-2-methyl-1-propansulfonic acid; APS, ammonium persulfate; BAAm, *N,N′*-methylene-*bis*-acrylamide; BSA, bovine serum albumin; CA, contact angle; DEAEM, *N,N*-diethylaminoethyl methacrylate; DEDTC, *N,N*-diethyl dithiocarbamic acid benzyl ester; EGDMA, ethylene glycol dimethacrylate; EGMP, ethyleneglycol methacrylate phosphate; HCl, hydrochloric acid; HEMA, 2-hydroxyethyl methacrylate; IA, itaconic acid; MAA, methacrylic acid; NPEDMA, *N*-phenylethylene diamine methacrylamide; OD, optical density; TRIM, trimethylolpropane trimethacrylate

REFERENCES

(1) Hornbeck, P., Winston, S. E., Fuller, S. A. UNIT 11.2 Enzyme-Linked Immunosorbent Assays (ELISA). In *Current Protocols in Molecular Biology*; Ausubel, F. M., Brent, R., Kingston, R. K., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Eds.; John Wiley & Sons, Inc.: New York, 2001.

(2) Jeon, B. J.; Kim, M. H.; Pyun, J. C. Parylene-A coated microplate for covalent immobilization of proteins and peptides. *J. Immunol. Methods* **2010**, *353*, 44–48.

(3) Schellekens, G. A.; Visser, H.; de Jong, B. A. W.; Van den Hoogen, F. H. J.; Hazes, J. M. W.; Breedveld, F. C.; van Venrooij, W. J. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum.* **2000**, *43*, 155–163.

(4) Bossi, A.; Piletsky, S. A.; Piletska, E. V.; Righetti, P. G.; Turner, A. P. F. An assay for ascorbic acid based on polyaniline-coated microplates. *Anal. Chem.* **2000**, *72*, 4296–4300.

(5) Nakanishi, K.; Sakiyama, T.; Kumada, Y.; Imamura, K.; Imanaka, H. Recent advances in controlled immobilization of proteins onto the surface of the solid substrate and its possible application to proteomics. *Curr. Proteomics* **2008**, *5*, 161–175.

(6) Lakshmi, D.; Whitcombe, M. J.; Davis, F.; Chianella, I.; Piletska, E. V.; Guerreiro, A.; Subrahmanyam, S.; Brito, P. S.; Fowler, S. A.; Piletsky, S. A. Chimeric polymers formed from a monomer capable of free-radical, oxidative, and electrochemical polymerisation. *Chem. Commun.* **2009**, 2759–2761.

(7) Lakshmi, D.; Bossi, A.; Whitcombe, M. J.; Chianella, I.; Fowler, S. A.; Subrahmanyam, S.; Piletska, E. V.; Piletsky, S. A. Electrochemical sensor for catechol and dopamine-based on a catalytic molecularly imprinted polymer-conducting polymer hybrid recognition element. *Anal. Chem.* **2009**, *81*, 3576–3584.

(8) MacDiarmid, A. G.; Epstein, A. Polyanilines: A novel class of conducting polymers. *Faraday Discuss. Chem. Soc.* **1989**, *88*, 317–332.

(9) Sreenivasan, K. Detection of creatinine enriched on a surface imprinted polystyrene film using FT-ATR-IR. *J. Mol. Recogn.* **2006**, *19*, 408–412.

(10) Berti, F.; Todros, S.; Lakshmi, D.; Whitcombe, M. J.; Chianella, I.; Ferroni, M.; Piletsky, S. A.; Turner, A. P. F.; Marrazza, G. Quasi-monodimensional polyaniline nanostructures for enhanced molecularly imprinted polymer-based sensing. *Biosens. Bioelectron.* **2010**, *26*, 497–503.

(11) Ivanova-Mitseva, P. K.; Fragkou, V.; Lakshmi, D.; Whitcombe, M. J.; Davis, F.; Guerreiro, A.; Crayston, J. A.; Ivanova, D. K.; Mitsev, P. A.; Piletska, E. V.; Piletsky, S. A. Conjugated polymers with pendant iniferter units: Versatile materials for grafting. *Macromolecules* **2011**, *44*, 1856–1865.

(12) Piletsky, S. A.; Panasyuk, T. L.; Piletskaya, E. V.; Sergeeva, T. A.; El'skaya, A. V.; Pringsheim, E.; Wolfbeis, O. S. Polyaniline-coated microtiter plates for use in longwave optical bioassays. *Fresenius J. Anal. Chem.* **2000**, *366*, 807–810.

(13) Zheng, W. Y.; Levon, K.; Laakso, J.; Oesterholm, J. E. Characterization and solid-state properties of processable *N*-alkylated polyanilines in the neutral state. *Macromolecules* **1994**, *27*, 7754–7768.

(14) Gök, A.; Sari, B.; Talu, M. Synthesis and characterization of conducting substituted polyanilines. *Synth. Met.* **2004**, *142*, 41–48.

(15) Otsu, T. Iniferter concept and living radical polymerization. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 2121–2136.

(16) Sergeyeva, T. A.; Piletsky, S. A.; Brovko, A. A.; Slinchenko, E. A.; Sergeeva, L. M.; Elskaya, A. V. Selective recognition of atrazine by molecularly imprinted polymer membranes. Development of conductometric sensor for herbicides detection. *Anal. Chim. Acta* **1999**, *392*, 105–111.

(17) Sergeyeva, T. A.; Piletsky, S. A.; Brovko, A. A.; Slinchenko, E. A.; Sergeeva, L. M.; Panasyuk, T. L.; Elskaya, A. V. Conductimetric sensor for atrazine detection based on molecularly imprinted polymer membranes. *Analyst* **1999**, *124*, 331–334.

(18) Piletsky, S. A.; Piletska, E. V.; Bossi, A.; Karim, K.; Lowe, P.; Turner, A. P. F. Substitution of antibodies and receptors with molecularly imprinted polymers in enzyme-linked and fluorescent assays. *Biosens. Bioelectron.* **2001**, *16*, 701–707.

(19) Kueseng, P.; Noir, M. L.; Mattiasson, B.; Thavarungkul, P.; Kanatharana, P. Molecularly imprinted polymer for analysis of trace atrazine herbicide in water. *J. Environ. Sci. Health B* **2009**, *44*, 772–780.