Surface-Functionalization of Plasma-Treated Polystyrene by Hyperbranched Polymers and Use in Biological Applications

Alya Boulares-Pender,[1] Andrea Prager-Duschke,[1] Christian Elsner,[1] Michael R. Buchmeiser[1,2]

[1]*Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstr. 15, D-04318 Leipzig, Germany*
[2]*Institut für Technische Chemie, Universität Leipzig, Linnéstr. 3, D-04103 Leipzig, Germany*

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ABSTRACT: Nitrogen plasma was used to amino-functionalize polystyrene surfaces, which were further modified via the selective introduction of polyamines suitable for the immobilization of biological compounds. This chemical modification was carried out using a multifunctional amine compound linked to glutaraldehyde, leading to the formation of hyperbranched structures at the surface. Up to three generations of branched polymers at the polystyrene (PS) surface were created by successive addition of the functional compounds. Amine functions introduced at the surface were labeled with 2,3,4,5,6-pentafluorobenzaldehyde and analyzed by X-ray photoelectron spectroscopy (XPS), confirming the successful attachment of each generation of branching. Finally, bovine serum albumin and trypsin were immobilized on N_2-plasma-treated PS modified with different amounts of branched graft polymer and found to remain bioactive after immobilization. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2701–2709, 2009

Key words: polystyrene; nitrogen plasma; functionalization; hyperbranched polymers; XPS; derivatization

INTRODUCTION

In recent years, considerable interest focused on tailoring commercial polymer surfaces such as polyethylene and polystyrene (PS) for specific biological and biomedical applications,[1,2] e.g., aiming on an increase in the performance of biological assays by the immobilization of biomolecules on the test supports. Indeed, the bulk properties of commercial polymers, e.g., the optical properties as well as hardness or conductivity, meet practical requirements for biomedical applications, however, their inert nature makes surface functionalization for the consecutive binding of bioactive compounds inevitable.[1] The immobilization of biomolecules requires either electrostatic interaction, affinity interaction, or covalent bonding. In the latter case, the introduction of hydrophilic functionalities at the surface is often envisaged. In their comprehensive study on surface functionalizations for the attachment of bioactive compounds, Goddard and Hotchkiss[1] review various criteria to be considered in the process of binding biocompounds to the polymer surface, as well as the various techniques in use to this end.

The optimization of the surface functionality, in both nature and quantity, for the optimum covalent attachment of the bio-entities at the surface is of primary importance. In this perspective, multifunctional agents can be used and various spacer lengths between the surface and the functional groups can be considered. This multifunctionality increases the probability of covalent interactions between proteins and the surface, whereas the spacer distances the functional group away from the bulk surface,[1] allowing for a better flexibility of the functional segment, as well as for an improved access of the macromolecule to be attached. As a result, the biomolecule, e.g., a protein, is more likely to interact efficiently with the hydrophilic functional groups and bond covalently, whereas it less likely undergoes surface induced denaturation.

To increase functionality density at the surface, several types of polymeric structures can be added to or built at the surface, e.g., polymer brushes,[3,4] perfectly spherical or hemispherical dendritic, or randomly hyperbranched structures.[5–9] Dendrimers[10–13] are very attractive due to the multifunctionality they can deliver to the surface[14–16] through their perfect geometry. However, this very same geometry can be a drawback since the biomolecule, usually of large dimensions, requires a relative low

Correspondence to: M. R. Buchmeiser (michael.buchmeiser@iom-leipzig.de).

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steric hindrance to fit and bind to the corresponding functionalities. Thus, the immobilization of biomolecules is often hampered by the overcrowding of the multifunctionalized dendritic shell.[1] Kainthan et al.,[17] Gillies and Frechet[18] who have worked on poly-glycerols and their biocompatibility, commented on the usefulness of the hyperbranched polymers flexible structures compared with that of dendrimers, stating that their conformational freedom allows hyperbranched polymers to be an effective alternative to dendrimers in many biomedical applications where perfect dendritic structures are not required. In this regard, hyperbranched structures grown from the surface can serve a much better purpose, the biomolecules being more likely to find several anchor points on hyperbranched segments, rather than at selective points of the dendrimer shell.

Common polymer surface treatment techniques used for modification of the surface are often based on standard wet chemistry, e.g., applying strong oxidizing agents, UV irradiation, corona discharge, or plasma treatment. Gas plasma introduces polar functionalities at the surface according to the nature of the gas used, while reducing the use of solvents and the production of chemical waste at the same time. Thus, nitrogen plasma is generally used to introduce amine functionalities at the surface of polymer samples,[1] however, it is well-known that upon contact with ambient atmosphere, oxygen reacts immediately with the plasma-treated surface leading to a consistent amount of oxygen-based functional groups at the sample's surface.[19,20] Then, once the surface is plasma treated and comes in contact with ambient air, different functionalities are present at the surface.[20–23] This makes the careful examination of appropriate systems for the covalent attachment of biomolecules at the surface inevitable.

Subsequent chemical modification of plasma exposed surfaces for the covalent attachment of biomolecules often involves the use of glutaraldehyde[24,25] as a reagent of choice[26–28] because of its high reactivity toward the proteins' amino groups. It is, therefore, used in the immobilization of proteins[29,30] on silicon surfaces after their aminosilanization. Beier and Hoheisel[16] described a procedure for the construction of multibranches at the surface of aminosilanized solid supports for the covalent bonding of DNA using acryloyl chloride with tetraethylenepentamine; however, the acyl chloride requires the use of solvents which affect the polymeric support, e.g., the optical properties of polystyrene.

In this article, we describe the nitrogen plasma-based functionalization of a PS surface followed by the *in situ* building of hyperbranched functional structures using 2-aminoethyl methacrylate hydrochloride (AEMA), glutaraldehyde (GA), and tetraethylene pentamine (TEPA). Up to three generations of GA-TEPA segments were built on the PS surface to increase the amine functionality at the surface. The method described in this article has been chosen for its ease of transfer to the modification of polystyrene-based microtiter plates without alteration of the polystyrene optical properties and its adjustability to biological applications. The final amine functionalities can ultimately be modified to fit any specific biological application.

EXPERIMENTAL

Materials

Polystyrene samples (PS) were cut from Greiner Petri dishes to simulate the surface of PS microtiter plates (in this study NUNC™ Nunclon™ Surface U-96 wells microtiter plates) used as supports in biological assays; both products were made of stabilized PS. Prior to plasma treatment, PS samples were cleaned with ethanol, dried, and then stored in a dust-free container. Tetraethylenepentamine (Tetrene, TEPA) and 2-aminoethyl methacrylate hydrochloride (AEMA) (90%) were obtained from Sigma-Aldrich (Germany). Glutaraldehyde (GA, 25% in aqueous solution), and 2,3,4,5,6-pentafluorobenzaldehyde (PFBA, 98%) were purchased from ABCR GmbH and Co. KG (Karlsruhe, Germany). Their structures are presented in Figure 1. A buffer solution of Na_2CO_3 was prepared using Millipore grade distilled water and by adjusting the pH to 9.2 with hydrochloric acid 1*M*. Ethanol absolute (99%), used to rinse the samples, was purchased from Sigma-Aldrich. Bovine serum albumin (BSA, RIA grade, fraction V, $\geq$96% (GE)) and trypsin from bovine pancreas (EC 3.4.21.4) were from Fluka BioChemika, *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA), sodium phosphate buffer pH 7.4, sodium chloride, and calcium chloride were purchased from Sigma-Aldrich (Germany). HEPES was purchased from Carl Roth GmbH (Germany).

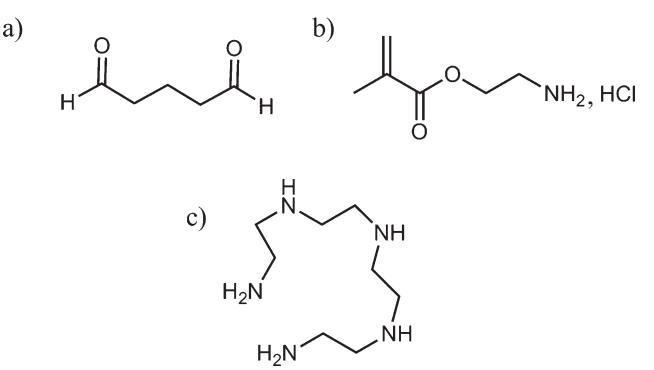

Figure 1 Reagents used in this study a) glutaraldehyde, GA; b) aminoethylmethacrylate AEMA; c) tetraethylenepentamine TEPA.

Sample preparation

PS samples were washed with distilled water and ethanol and carefully dried at room temperature prior to use. After plasma exposure, the samples were immersed in a 1 wt % solution of AEMA in a pH 9.2 Na_2CO_3 buffer solution for 2 h, then rinsed three times with Millipore grade distilled water and ethanol. Samples were then immersed for 2 h in 2 mL of a 2 wt % solution of glutaraldehyde in a pH 9.2 Na_2CO_3 buffer, followed by a 2 wt % solution of TEPA in a pH 9.2 Na_2CO_3 buffer for 2 h. To build up several generations of branching at the surface, the samples were successively immersed in solutions of glutaradehyde and polyfunctional amines in sodium carbonate buffer. After each treatment, the samples were twice carefully rinsed with both Millipore grade distilled water and ethanol. Finally, all samples were dried overnight at 60°C.

The immobilization of BSA and trypsin was carried out by treatment of the sample surfaces with a 2 wt % solution of GA in a pH 9.2 Na_2CO_3 buffer followed by a 2 h treatment with a 3 mg mL^{-1} solution of either BSA in a pH 7.4 sodium phosphate buffer or trypsin in 50 m*M* HEPES buffer solution at pH 8 (100 m*M* NaCl, 10 m*M* $CaCl_2$). All samples were finally rinsed several times with the phosphate buffer solution containing 0.01 wt % of Tween® 20 and with the phosphate buffer solution. After immobilization, the samples were either used immediately or stored at 2–4°C.

Plasma treatment

PS samples were submitted to nitrogen plasma using a FEMTO 2 L laboratory plasma system (40 kHz, 100 W) from Diener Electronic GmbH + Co. KG (Germany) for 5 min.

Materials characterization

The surface composition of the samples was measured using a SAGE 100 system (Specs GmbH, Berlin, Germany) X-ray photoelectron spectrometer (XPS), equipped with nonmonochromatized Al and Mg Kα radiations at 300 W (10 kV, 20 mA) as excitation sources. Binding energies were corrected for static charging of the samples by referring them to the C(1s) peak set at a binding energy of 285.0 eV. XPS-derivatization was carried out as follows: 2,3,4,5,6-pentafluorobenzaldehyde (PFBA, concentration range 8 mmol L^{-1} to 0.1 mol L^{-1} in absolute ethanol) was reacted with the sample surfaces for 2 h, the samples were then three times thoroughly washed with absolute ethanol, and then dried overnight at 60°C prior to analysis. Static contact angles with water and diiodomethane were measured using the sessile drop method on a Krüss contact angle instrument (Krüss GmbH Hamburg, Germany) using a Drop Shape Analysis 2.1 software. All contact angles are the mean value of five measurements on different parts of the sample's surface. Surface tension, polar, and disperse contributions to the surface tension were calculated using the Owens-Wendt-Rabel and Kaelble method.[31–34]

The amounts of proteins immobilized onto the modified microtiter plates were determined by a bicinchoninic acid protein assay (PIERCE, Pierce® Thermo-Scientific. Rockford, IL, BCA Protein Assay Kit) at 37°C, as reported in the literature.[35] Absorptions at 562 nm were measured on an Infinite® 200 microplates reader (TECAN Trading AG, Switzerland).

Monitoring of the trypsin activity through the release of *p*-nitroaniline

A solution of 0.6 m*M* *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA) in 50 m*M* HEPES buffer solution at pH 8 (100 m*M* NaCl, 10 m*M* $CaCl_2$) was added to the trypsin-modified wells of a microtiter plate (280 μL per well). The release of *p*-nitroaniline from BApNA was immediately followed by measuring the absorption at 405 nm at 37°C[1] on an Infinite® 200 microplate reader (TECAN Trading AG, Switzerland). Absorptions were measured every 5 min and values were corrected by subtraction of the first measurement; the values presented correspond to the median values measured on eight wells, with the calculated standard errors shown.

RESULTS AND DISCUSSION

Plasma treatment of PS

Polystyrene (PS) samples were exposed to nitrogen plasma, then the atomic composition at the surface was measured by XPS and compared with that of unexposed PS and oxygen plasma-treated samples, respectively, (Table I). About 10 atom % of nitrogen was found at the PS surface after nitrogen plasma treatment, suggesting an amino functionalization of the surface. The 18 atom % of oxygen at the surface is attributed to the reaction of atmospheric oxygen with the PS surface after plasma treatment. The concomitant increase of the wettability of PS due to the formation of polar functional groups, i.e., hydroxyl and carboxylic acid groups during plasma treatment was confirmed by contact angle measurements (Table II). These findings are in agreement with published results.[37]

Functionalization of plasma-treated PS surfaces

Based on the above-described measurements, we assumed that roughly 10 atom % of amino

TABLE I
Atomic Concentrations at the Surface of Pristine PS, Nitrogen Plasma, and Oxygen Plasma-Treated PS

	C atom %	N atom %	O atom %	C/N	C/O	O/N
PS	98.4	0	1.6	–	61.5	–
N_2 plasma-treated PS	71.1	10.6	18.3	6.7	3.8	1.7
O_2 plasma-treated PS	79.4	1.2	19.4	66.2	4.1	16.2
GI.1 PS-(GA-TEPA)$_1$	73.6	5.5	20.7	13.4	3.5	3.8
GI.2 PS-(GA-TEPA)$_2$	73.3	9.1	17.6	8.0	4.2	1.9
GII.1 PS-AEMA(GA-TEPA)$_1$	73.8	6.4	19.8	11.5	3.7	3.1
GII.2 PS-AEMA(GA-TEPA)$_2$	71.8	8.1	20.1	8.8	3.6	2.5
GII.3 PS-AEMA(GA-TEPA)$_3$	74.1	10.2	15.7	7.3	4.7	1.5

Nitrogen plasma exposed PS treated with (GA-TEPA)$_n$ and with AEMA(GA-TEPA)$_n$, respectively, as determined by XPS.

functionalities were available at the surface of PS samples exposed to N_2-plasma. To demonstrate the proof of concept, we treated the plasma-modified samples with an aqueous solution of GA followed by a 2 wt % solution of TEPA in Na_2CO_3 buffer at pH 9.2. TEPA, a multifunctional compound having both primary and secondary amine groups, is susceptible to react with GA forming both imine and enamine bonds[38,39] (Fig. 2). In view of the chemistry of aldehydes, one may well assume that a GA aqueous solution of pH 3.1 contains mostly the free glutaraldehyde and only minor amounts of acetalic and oligomeric products produced by self condensation of free GA, which is more favored at a basic pH.[26,27,40,41] Established biological protocols[42] suggest the dilution of the GA solution in a Na_2CO_3 buffer of pH 9.2. This actually lowers the overall pH of the solution, which was not further adjusted. According to Okuda et al.[43] these pH conditions are optimal for the attachment of the free GA to the amine surface functionalities and the reaction is then irreversible.

Reaction of the plasma treated surface, with GA and TEPA solutions, respectively, produced a polyamine functionalized sample termed **GI.1** (PS-(GA-TEPA)$_1$), another cycle of the same treatment resulted in the formation of a branched graft polymer called **GI.2** (PS-(GA-TEPA)$_2$). Table I presents the compositions at the surfaces of **GI.1** and **GI.2** as determined by XPS. These confirm the increase of the nitrogen content from one generation to another. Water and diiodomethane contact angles measurements were collected (Table II) and compared with those of the PS starting material, as well as to a PS

TABLE II
Water (*W*) and diiodomethane (*D*) contact angle values obtained at the surface of pristine PS, N_2-plasma-functionalized PS, N_2-plasma-functionalized PS-treated with GA, and N_2-plasma-functionalized PS-treated with (GA-TEPA)$_n$, and PS-AEMA-(GA-TEPA)$_n$

	Contact angle		Surface tension	Dispersive	Polar
	W (°)	*D* (°)	(m*N* m^{-1})	(m*N* m^{-1})	(m*N* m^{-1})
PS	80.8	53,8	35.9	35.9	0
PS after N_2 plasma	wets	wets	N/A	–	–
Treated with GA	31.3	44.7	65.5	37.1	28.3
GI.1 (GA-TEPA)$_1$					
1st meas. after 2 days	27.5	41.4	68.0	38.9	29.1
2nd meas. after 14 days	41.8	44.1	59.7	37.5	22.2
3rd meas. after 2 months	39.6	50.8	59.7	33.8	25.9
GI.2 (GA-TEPA)$_2$					
1st meas. after 3 days	27.5	42.2	67.8	38.5	29.3
2nd meas. after 14 days	39.7	49.8	59.8	34.4	25.4
3rd meas. after 2 months	42.4	44.9	59.3	37.1	22.2
GII.1 PS-AEMA(GA+TEPA)$_1$	55.4	47.0	51.0	35.9	15.0
GII.2 PS-AEMA(GA+TEPA)$_2$	46.9	42.3	57.2	38.4	18.8
GII.3 PS-AEMA(GA+TEPA)$_3$	42.0	18.5	65.0	48.2	16.8

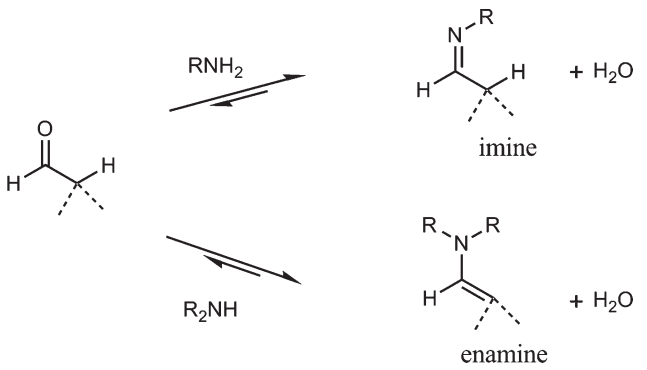

Figure 2 Reaction of an aldehyde with primary and secondary amines.

sample treated with nitrogen plasma and a solution of GA. Measurements on **GI.1** and **GI.2** were carried out at different times as follows: after a couple of days following the chemical modification, to allow for a surface reorganization, and after 3 weeks. We observed changes in the contact angles at the surface of the samples within 2 weeks indicative of further surface reorganization,[44] thereafter, we did not observe further significant changes in contact angle values. We thus believe that the surface had then reached equilibrium after 2 weeks. The surface tension and the polar contribution values indicate that the modified surface holds a certain hydrophilic character suitable for biological applications.

Aminoethylmethacrylate as spacer

In a next step, we introduced a spacer between the polymer and the multifunctional amine. As stated in the introduction, such a spacer distances the functional group away from the bulk surface,[1] allowing for a better flexibility of the functional segment as well as for an improved access of the macromolecule to be attached. A methacrylate function was chosen because of its better reactivity toward the various surface functionalities produced by the plasma treatment and the subsequent contact with ambient air, extending the distance between the surface and the first branching point. This was expected to facilitate functionalization resulting in larger amounts of anchoring groups for protein immobilization. For these purposes, plasma-exposed PS was treated with a solution of AEMA. From AEMA, we built up to three generations of branched graft-polymers designated by **GII.1** to **GII.3** (PS-AEMA-(GA-TEPA)$_n$ with $n = 1$ to 3) (Fig. 3). XPS analysis gave the compositions at the surface of the three generations of samples (Table I), confirming the increase of nitrogen content at the surface with each additional TEPA segment. Contact angle measurements (Table II) showed an increase in surface tension with each generation.

As a means to establish the successful attachment of various generations of branching onto the PS surface, we used pentafluorobenzaldehyde (PFBA), a known chemical derivatization agent for primary amine groups. XPS analysis was carried out after reacting PFBA at different concentrations with the primary amino groups present at the surface (Fig. 4). At this point, it was not clear whether PFBA was able to react also with some secondary amino groups, the more since reactions of aromatic aldehydes with secondary amines have been reported in the literature.[45] The XPS data on both N and F collected after derivatization were used to trace the variation of the ratio of atomic concentration between nitrogen and fluorine versus the concentration of PFBA (Fig. 5). The curve corresponding to the first generation **GII.1** derivatized by PFBA is consistent with the assumption that the PFBA reacts with the primary amines available and that, on average, only one of the two TEPA primary functions is then available. Indeed, the curve reached a plateau for a ratio [N]/[F] of about one indicating that an average of one molecule of PFBA is attached to one **GII.1** segment. (Such an attachment, would in theory result in $\sim$ 17 atom % of fluorine and in 15 atom % of nitrogen, resulting in an [N]/[F] ratio of $\sim$ 1). The two other curves show that the derivatization is limited by the number of amino groups available at the surface and that, as expected, the nitrogen content increases with the number of TEPA branches indicative for a successful attachment of the segments.

To demonstrate the suitability of our systems for the attachment of biomolecules, we first immobilized bovine serum albumin (BSA) on PS modified with different generations of GA-TEPA, i.e., PS-AEMA-(GA-TEPA)$_n$ ($n = 1$–3). The results obtained by XPS following the addition of BSA were inconclusive, indeed the amounts of sulfur that were present in the protein immobilized at the sample surface were within the detection limits of our XPS and could therefore not provide a definite means of quantification of protein attachment on the surface. Thus, we opted for a biochemical quantification of BSA attached at the surface using a bicinchoninic acid protein assay (BCA assay) on PS-AEMA-(GA-TEPA)$_n$ ($n = 1$–3) modified microtiter plates, on which BSA was immobilized as described above. The results obtained for the two types of microtiter plates are illustrated in Figure 6, where only the median values of 12 measurements realized on each generation of GA-TEPA are plotted. As expected, the concentration in BSA at the surface increased steadily with the number of generation in the case of the nitrogen plasma-treated microtiter plates. In

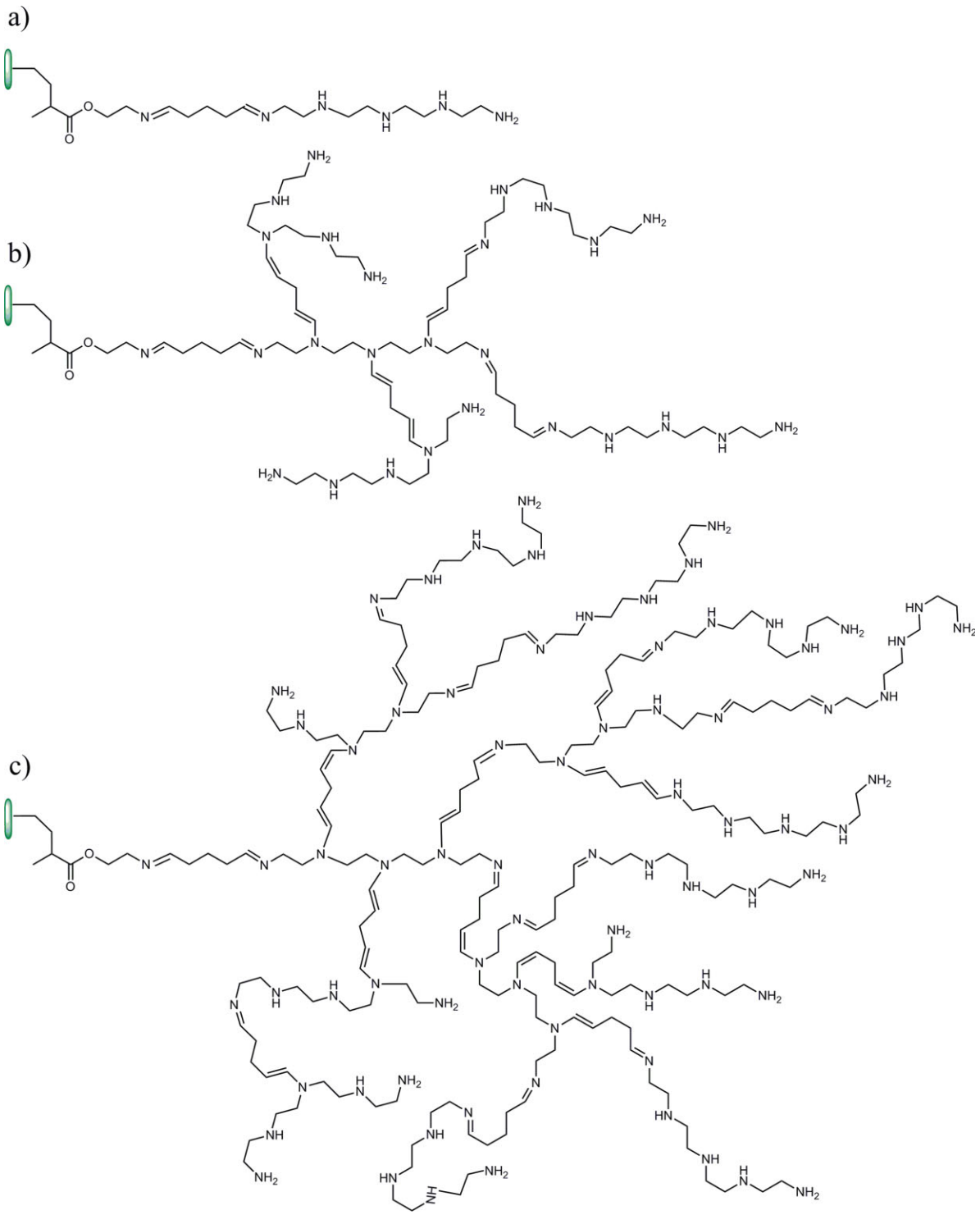

Figure 3 Proposed structures of a) **GII 1**; b) **GII.2** and c) **GII.3** built at the AEMA-functionalized PS surface. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

contrast to that, the concentration of BSA on nonplasma exposed plates did not exceed 5 μg mL^{-1}. We also note that the BSA concentration at the first generation **GII.1** was similar to that of its corresponding nonplasma treated system, indicating that for **GII.1** nitrogen plasma modification did not noticeably improve the extent of protein immobilization. Nevertheless, in the case of nonplasma treated plates, the BSA was only immobilized via (unspecific) adsorption, whereas chemical bonding between BSA and the support was achieved with plasma-treated, functionalized PS.

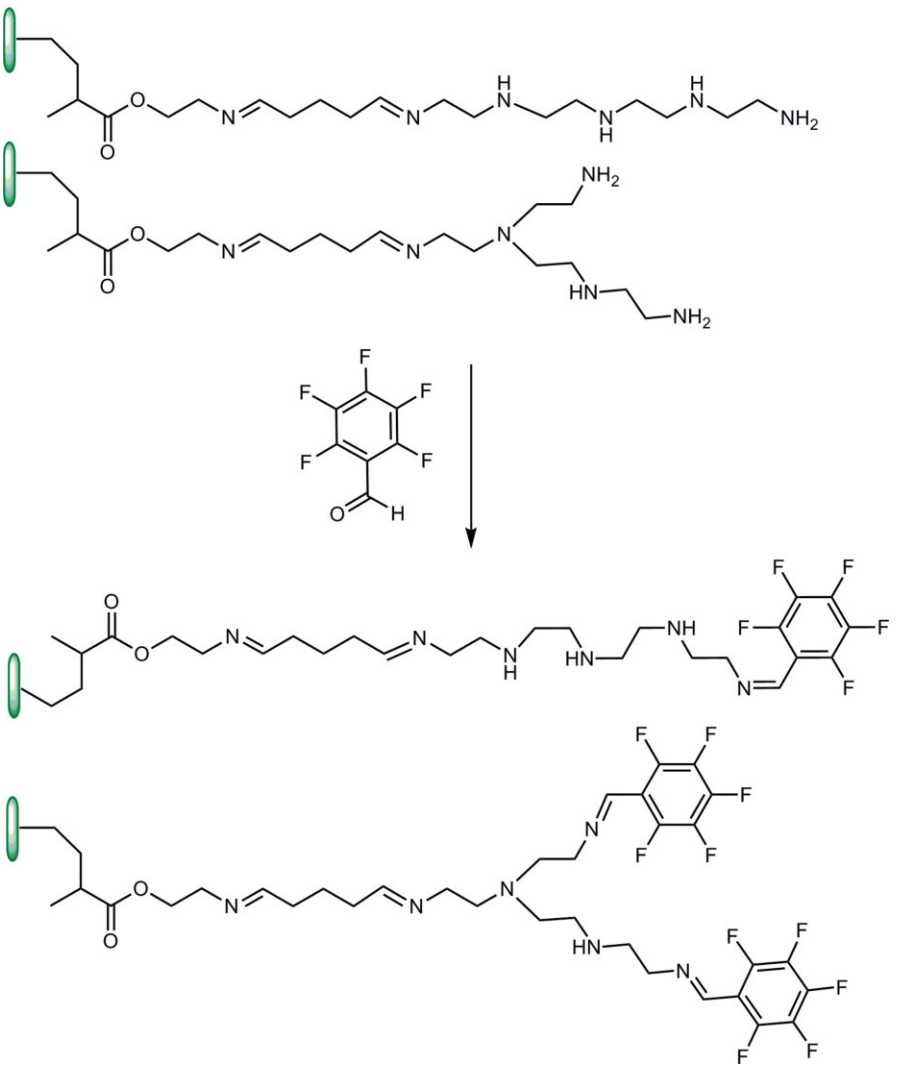

Figure 4 Derivatization of the amino groups of PS-AEMA-(GA-TEPA)$_1$ segments by PFBA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

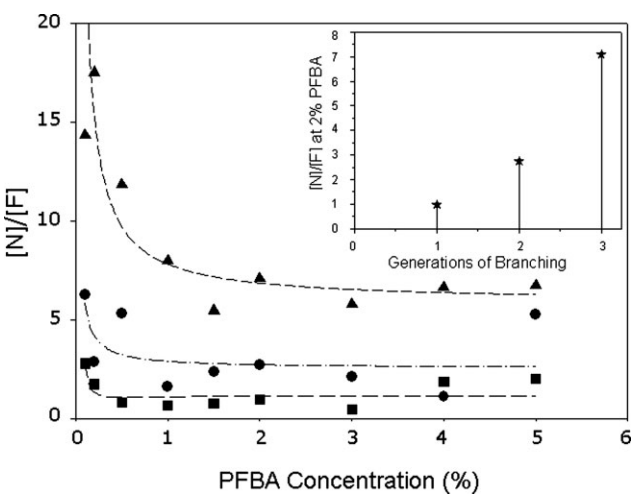

Figure 5 N/F atomic ratio versus PFBA concentration on **GII.1** (■), **GII.2** (●) and **GII.3** (▲) of PS-AEMA-(GA-TEPA)$_n$. Insert: variation of the N/F ratio versus number of generation, at a given concentration of 2% PFBA in ethanol.

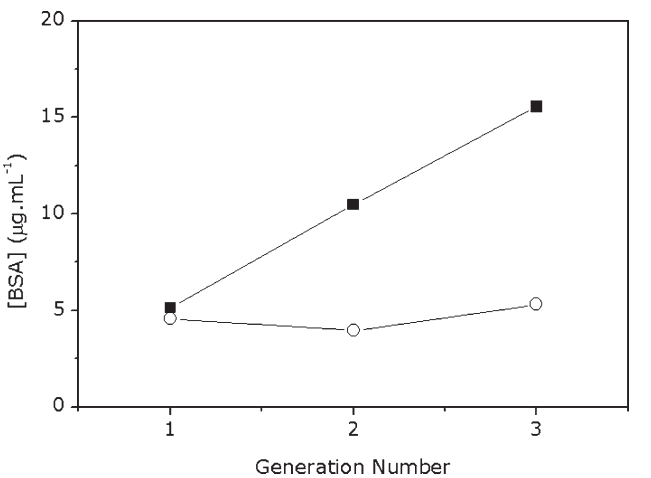

Figure 6 Concentration in BSA at the surface of the nitrogen plasma treated (■) and nonplasma treated (○) PS-AEMA-(GA-TEPA)$_n$ microtiter plates, determined by using the Pierce® BCA Protein Assay Kit. Each point represents the median value of twelve results.

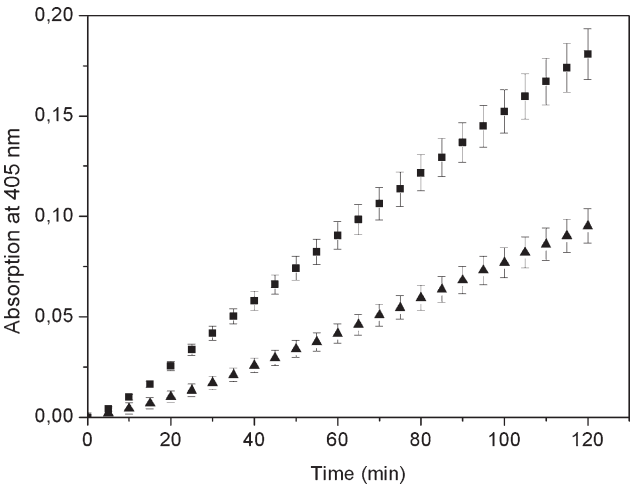

Figure 7 Activity of trypsin immobilized at the surface of nonplasma treated (▲) and nitrogen plasma treated (■) GII.3 PS-AEMA-(GA-TEPA)$_3$ microtiter plates, monitored by the release of *p*-nitroaniline measured at 405 nm as a function of time.

To demonstrate that the technique elaborated here was also capable of immobilizing proteins in their bioactive form, trypsin, a proteolytic enzyme, was immobilized onto the PS-AEMA-(GA-TEPA)$_3$-GA surface. Trypsin is responsible for cleaving peptide bonds during digestion, and is specifically active on arginine and lysine segments.[46,47] This specificity of trypsin can be used to determine its activity on various substrates.[46,48–52] Here, this was realized by monitoring the hydrolytic cleavage of *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA) through the release of *p*-nitroaniline, by spectrophotometry at 405 nm. We observed (Fig. 7) only a low conversion rate on the unmodified surface. This most probably is a result of small amounts of trypsin nonspecifically adsorbed to the untreated PS surface, however, a significantly higher conversion rate of *p*-nitroaniline release was observed on plasma treated **GII.3** PS-AEMA-(GA-TEPA)$_3$ microtiter plates, indicating improved enzymatic activity.

CONCLUSIONS

Without affecting the bulk material properties, we have successfully attached tailored multifunctional branches to the surface of polystyrene via a method readily applicable to the modification of polystyrene microtiter plates used in biological arrays. Contact angle measurements indicate the hydrophilic character of the surface due to the presence of polar functional groups at the surface, which has also been demonstrated by the PFBA derivatization of the primary amine functional groups. These modified polymer surfaces turned out to be suitable for the covalent attachment of bioactive compounds, preserving their bioactivity.

References

1. Goddard, J. M.; Hotchkiss, J. H. Prog Polym Sci 2007, 32, 698.
2. Ghasemi, M.; Minier, M.; Tatoulian, M.; Arefi-Khonsari, F. Langmuir 2007, 23, 11554.
3. Jordan, R., Ed. Surface-Initiated Polymerization I; Springer: Berlin Heidelberg, 2006.
4. Jordan, R., Ed. Surface-Initiated Polymerization II; Springer: Berlin Heidelberg, 2006.
5. Hajji, C.; Haag, R. Top Organomet Chem 2006, 20, 149.
6. Stiriba, S. E.; Frey, H.; Haag, R. Angew Chem Int Ed Engl 2002, 41, 1329.
7. Sunder, A.; Heinemann, J.; Frey, H. Chem Eur J 2000, 6, 2499.
8. Voit, B. J Polym Sci Part A: Polym Chem 2000, 38, 2505.
9. Voit, B. J Polym Sci Part A: Polym Chem 2005, 43, 2679.
10. Zeng, F.; Zimmermann, S. C. Chem Rev 1997, 97, 1681.
11. Fischer, M.; Vögtle, F. Angew Chem 1999, 111, 934.
12. Bosman, A. W.; Janssen, H. M.; Meijer, E. W. Chem Rev 1999, 99, 1665.
13. Issberner, J.; Moors, R.; Vogtle, F. Angew Chem Int Ed Engl 1994, 33, 2413.
14. Graham, M. D. J Chem Technol Biotechnol 2001, 76, 903.
15. Trevisiol, E.; Berre-Anton, V. L.; Leclaire, J.; Pratviel, G.; Caminade, A.-M.; Majoral, J.-P.; Francois, J. M.; Meunier, B. New J Chem 2003, 27, 1713.
16. Beier, M.; Hoheisel, J. D. Nucleic Acids Res 1999, 27, 1970.
17. Kainthan, R. K.; Gnanamani, M.; Ganguli, M.; Ghosh, T.; Brooks, D. E.; Maiti, S.; Kizhakkedathu, J. N. Biomaterials 2006, 27, 5377.
18. Gillies, E. R.; Fréchet, J. M. J. Drug Discov Today 2005, 10, 35.
19. Petrat, F. M.; Wolany, D.; Schwede, B. C.; Wiedmann, L.; Benninghoven, A. Surf Interface Anal 1994, 21, 402.
20. Petrat, F. M.; Wolany, D.; Schwede, B. C.; Wiedmann, L.; Benninghoven, A. Surf Interface Anal 1994, 21, 274.
21. Harth, K.; Hibst, H. Surf Coat Technol 1993, 59, 350.
22. Leggett, G. J.; Ratner, B. D.; Vickerman, J. C. Surf Interface Anal 1995, 23, 22.
23. Ying, L.; Yin, C.; Zhuo, R. X.; Leong, K. W.; Mao, H. Q.; Kang, E. T.; Neoh, K. G. Biomacromolecules 2003, 4, 157.
24. Heldt, J. M.; Fischer-Durand, N.; Salmain, M.; Vessières, A.; Jaouen, G. Eur J Org Chem 2007, 5429.
25. Goddard, J. M.; Talbert, J. N.; Hotchkiss, J. H. J Food Sci 2007, 72, E036.
26. Walt, D. R.; Agayn, V. I. Trends Anal Chem 1994, 13, 425.
27. Richards, F. M.; Knowles, J. R. J Mol Biol 1968, 37, 231.
28. Ozan, E.; Ayhan, F.; Ayhan, H. J Bioact Compat Polym 2008, 23, 69.
29. Betancor, L.; Luckarift, H. R.; Seo, J. H.; Brand, O.; Spain, J. C. Biotechnol Bioeng 2008, 99, 261.
30. Longo, L.; Vasapollo, G.; Guascito, M. R.; Malitesta, C. Anal Bioanal Chem 2006, 385, 146.
31. Owens, D. K.; Wendt, R. C. J Appl Polym Sci 1969, 13, 1741.
32. Kaelble, D. H.; Uy, K. C. J Adhes 1970, 2, 50.
33. Rabel, W. Physikalische Blätter 1977, 33, 151.
34. Rulison, C. In Two-Component Surface Energy Characterization as a Predictor of Wettability and Dispersability; AN213/CR, KRUSS Application Note 213.
35. Smith, P. K.; Krohn, R. I.; Hermanson, G. T. Anal Biochem 1985, 150, 76.
36. Bullett, N. A.; Bullett, D. P.; Truica-Marasescu, F.-E.; Lerouge, S.; Mwale, F.; Wertheimer, M. R. Appl Surf Sci 2004, 235, 395.
37. Mizuta, T.; Sakaguchi, S.; Ishii, Y. J Org Chem 2005, 70, 2195.
38. Cestari, A. R.; Airoldi, C. Langmuir 1997, 13, 2681.

39. Kawahara, J.-I.; Ohmori, T.; Ohkubo, T.; Hattori, S.; Kawamura, M. Anal Biochem 1992, 201, 94.
40. Hopwood, D. Histochem J 1972, 4, 267.
41. Cass, T.; Ligler, F. S. Immobilized Biomolecules in Analysis: A Practical Approach; Oxford University Press: New York, 1998.
42. Okuda, K.; Urabe, I.; Yamada, Y.; Okada, H. J Ferment Bioeng 1991, 71, 100.
43. Luzinov, I.; Minko, S.; Tsukruk, V. V. Prog Polym Sci 2004, 29, 635.
44. Suginome, M.; Tanaka, Y.; Hasui, T. Synlett 2006, 1047.
45. Bayramoglu, G.; Yilmaz, M.; Senel, A. Ü.; Arica, M. Y. Biochem Eng J 2008, 40, 262.
46. Elsner, C.; Grahn, S.; Bauer, S.; Ullmann, D.; Kurth, T.; Jakubke, H. D. J Mol Catal B: Enzym 2000, 8, 193.
47. Migneault, I.; Dartiguenave, C.; Vinh, J.; Bertrand, M. J.; Waldron, K. C. J Liq Chromatogr Relat Technol 2008, 31, 789.
48. Ma, J.; Liang, Z.; Qiao, X.; Deng, Q.; Tao, D.; Zhang, L.; Zhang, Y. Anal Chem 2008, 80, 2949.
49. Dulay, M. T.; Baca, Q. J.; Zare, R. N. Anal Chem 2005, 77, 4604.
50. Xi, F.; Wu, J.; Jia, Z.; Lin, X. Process Biochem 2005, 40, 2833.
51. Nicoli, R.; Gaud, N.; Stella, C.; Rudaz, S.; Veuthey, J. L. J Pharm Biomed Anal 2008, 48, 398.