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ORIGINAL PAPER

Surface functionalization and biomolecule immobilization using plasma-generated free radicals on polypropylene

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Abstract In this study are presented evidences for the functionalization of polypropylene surfaces accomplished in a sequential process: argon- or oxygen-plasma enhanced generation of free radical sites on polypropylene surfaces was followed by "in situ" gas phase derivatization in the absence of plasma using ethylene diamine, or propylene diamine; and an "in situ", gas phase derivatization using oxallyl chloride or "ex situ" derivatization in the presence of glutaraldehyde. The free radicals' presence on the plasma-exposed polypropylene surfaces was confirmed using "in situ" sulfur dioxide or nitric oxide labeling techniques. It was shown that the free radical sites readily react under "in situ" conditions with the stable chain-precursor components and generate the desired spacer-chain molecules revealed by ESCA analysis. Functionalized polypropylene substrates were used for immobilization of α-chymotrypsin in the presence of spacer-chain molecules. The activity of the immobilized α-chymotrypsin was found to be comparable to the activity of the free enzyme when the spacer molecules have been used.

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Introduction

The most widely used synthetic polymer surfaces are usually characterized by low surface energy values, and some of the thermoplastics, including polyethylene and polypropylene (PP) for instance, are chemically inert. Characteristics like adhesion, wettability, dyebility, and reactivity require the presence of specific functionalities [1–3] on polymer substrate surfaces. New application areas such as sensors [4, 5], optical and electrical components, catalysts [6–8], etc., require specific nature and densities of functionalities to control the device performances.

Traditionally, PP has been bulk functionalized using reactive processing [9] with compounds such as diallylamino-triazine [10], sulfonyl azide [11], maleic anhydride [12–14], esters of itaconic acid [15], etc. in order to improve the compatibility with various materials, including polymers, for composites or block copolymer-advanced applications.

Recent studies report surface functionalization of PP by plasma treatments; grafting [16], implanting functional groups (OH, amine, and carboxyl, etc.) [17–20] or just modifying the roughness [21] were used to improve adhesion to various materials [22], immobilize biomolecules [23, 24], ad biocidal finishes to non-woven fabrics [25], etc.

Functionalities such as primary amine are between the most desirable due to their hydrophilic character, reactivity, and stability in comparison to secondary amines. Ammonia discharge environments were used for plasma-enhanced implantation of primary amine functionalities. A variety of amines or hydrogen and nitrogen mixtures were used as alternative primary amine group precursors [26–36] due to the extensive fragmentation of NH_3 in non-equilibrium plasmas; hydrazine plasma can be a better choice for the implantation of primary amine functionalities onto polymeric surfaces [37–39].

Reactive plasma functionalization using precursor molecules of the desired functionalities (e.g. ammonia, hydrazine, and amines, etc.) undergo fragmentation processes as a result of their interactions with the electrons and with the multitudes of precursor derived, nascent, charged and neutral species; in addition to the desired functionalities, other functional groups will be simultaneously implanted onto the surfaces, reducing the specificity of the functionalization process; free radical sites (reactive surface radicals and stable, "caged" free radicals) will initiate after plasma treatment, non-specific interactions with target molecules (e.g. biomolecules), which obviously will diminish significantly the molecular recognition capabilities (specificity) of the modified substrates.

Electron spin resonance (ESR) investigations, coupled with systematic computer simulation, show a variety of free radicals generated on inert polymer surfaces, such

as low and high density polyethylene (LDPE; HDPE), even under conditions when the substrates are exposed to inert-gas plasma environments [40]. Discrete, mid-chain alkyl, allylic, and large amounts of dangling-bond sites were identified both on plasma-exposed LDPE and HDPE surfaces as dominant radical species. It was demonstrated that crystalline and amorphous morphologies also influence significantly the densities and the nature of nascent free radicals. Thermally stable dangling-bonds were found to be the predominant radical species in the LDPE spectra, while the spectra of HDPE indicate the presence of the mid-chain alkyl radicals as the major entities. This was related to the differences in the intrinsic polymer morphologies (relative ratios of branched, amorphous regions vs. crystalline zones). It also has been shown that the plasma-treated substrates have an in situ and ex situ environmental-history. Both LDPE- and HDPE- origin dangling-bonds for instance, undergo an instant conversion into peroxy radicals under open laboratory conditions, while the mid-chain alkyl radicals have very low reactivity with oxygen.

The free radicals interact through combination, disproportionation, transfer, addition and decomposition reactions, and often involve inter and intra-macromolecular-chain processes [41]. These reactions usually alter the chemical structures of the nascent plasma-functionalized species. In addition, surface-active sites (ions and free radicals) interact with selected derivatizing agents including, aldehydes, carboxylic acids, ketones, alcohols, etc., under in situ environment, and generate stable compounds or new radicals with non-specific activities, often leading as a consequence, to erroneous analytical results.

In this contribution, the implantation of primary amine functionalities onto PP film surfaces will be presented, by taking advantage of the high reactivity of the plasma-generated free radical sites through introduction of stable primary amine precursor molecules under in situ environments. This approach uses a side process of plasma processing (free radicals generation) in a beneficial way by avoiding the development of undesired, plasma-mediated fragmentation reaction mechanisms which usually accompany reactive plasma functionalization increasing the chemical complexity of treated sample and by blocking the free radicals.

Materials and methods

Materials

Polypropylene film was provided by Cargill Dow LLC. PP samples were cut into square 110 $\times$ 110 mm^2 specimens. Acetone extraction of PP sample before plasma treatment was done in a beaker with agitation. Argon and oxygen used for the plasma-enhanced treatments and decontamination of the reactor were supplied by Airgas (Linde). Ethylene diamine (EA), propylene diamine (PA), oxalyl chloride (OC), glutaraldehyde (GL) (50% aqueous solution), sulfur dioxide, and nitric oxide were purchased from Sigma-Aldrich Co. All chemicals used for the enzyme immobilization reactions and assays have been described earlier [34, 38].

Surface modification of polypropylene

All plasma treatments were carried out in a cylindrical stainless steel, capacitively coupled (disc-shaped stainless steel electrodes; electrode diameter: 20 cm; gap: 3 cm), RF-plasma-reactor, equipped with 13.56 MHz power supplies [38, 39].

The presence of free radical sites on the polypropylene substrate surfaces, generated as a result of argon or oxygen plasma treatments, was demonstrated by reacting them in situ with SO_2 or NO. This was followed by vacuum-removal (the reactor having the modified samples was kept at base pressure level for 30 min) of the non-reacted SO_2 and NO, using the experimental conditions listed in Table 1, and analyzing the C1s, O1s, S2p and N1s regions by high resolution (HR) electron spectroscopy for chemical analysis (ESCA).

Functionalization and enzyme-immobilized reactions were carried out in a four-step process by the following scheme (Fig. 1): (i) Argon or oxygen plasma-assisted generation of surface free radicals. (ii) In situ, gas phase derivatization in the absence of plasma using, ethylene diamine, or propylene diamine. (iii) Second in situ, gas phase derivatization in the absence of plasma using oxalyl chloride or ex situ liquid-media derivatization using glutaraldehyde. (iv) Ex situ liquid-media immobilization of α-chymotrypsin (AC).

In a typical experiment, the reactor was first decontaminated (10 min consecutive oxygen- and argon plasma: RF-power: 300 W; pressure: 200 mT; flow-rate of oxygen or argon: 7 sccm) then the polypropylene substrate was placed on the lower electrode, and vacuumed to base pressure level. The pre-selected pressure was established in the reactor and the plasma was ignited for specific treatment conditions. At the end of the plasma-exposure of the substrates, the chamber was evacuated and ethylene diamine or propylene diamine was distilled into the installation until the pressure reached 1 Torr and was kept for 90 min. Then, the chamber was pumped down to base pressure for 20 min in order to remove the non-reacted amines.

Table 1 Atomic concentration of argon or oxygen plasma treated PP in situ reacted with sulfur dioxide and nitric oxide

Sample	Atomic concentration on surface (%)				Ratio of surface mers with free radicals
	Carbon	Oxygen	Nitrogen	Sulfur	
PP[AR]NO	75.9	17.5	6.6	–	0.261
PP[AR]SO	88.0	9.8	–	2.2	0.075
PP[OX]NO	83.5	16.5	No signal	–	–
PP[OX]SO	91.8	6.2	–	2.0	0.066

Plasma conditions: 100 W at 13.56 MHz; 1 min; 6 sccm of Ar or O_2

PP polypropylene, *AR* argon plasma-assisted surface activation of PP, *NO* in situ gas phase derivatization process of nitric oxide on PP activated surface, *SO* in situ gas phase derivatization process of sulfur dioxide on PP activated surface, *OX* oxygen plasma-assisted surface activation of PP

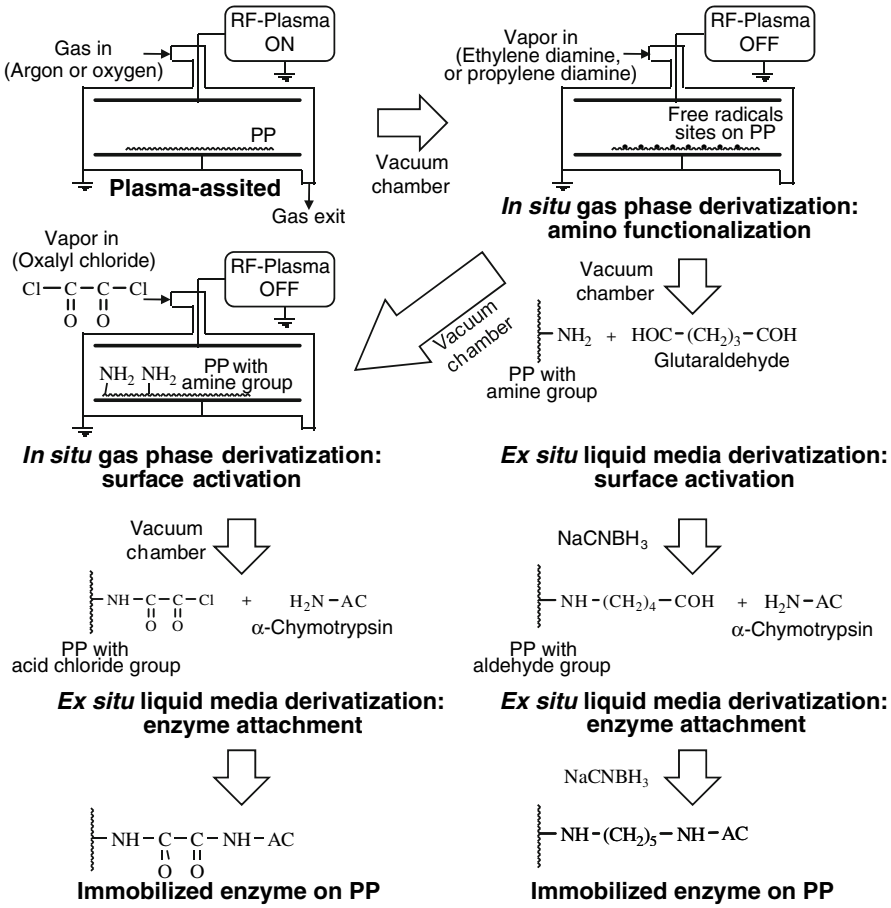

Fig. 1 Schematic diagram of surface functionalization and enzyme immobilization pathway

Immobilization of α-chymotrypsin on functionalized substrates

The oxalyl chloride activation was carried out by vacuum distilling into the reactor vapors and maintaining the vapor atmosphere under similar conditions to the EA and PA environments. The system was then evacuated and the PP bearing the acid chloride groups was removed and used immediately, consecutively for further analytical evaluations or enzyme immobilization, since the acid chloride is not very stable. α-chymotrypsin was immobilized according to the following procedure: the functionalized substrates were dipped into 50 mL of 0.02 M EPPS buffer solution. The pH of the buffer was corrected to 8.4 by adding 1 N NaOH, then 12 mg of α-chymotrypsin was added to each solution and the solutions were kept for 4 h. At the end of the procedure, the samples were washed with the buffer and then with distilled water (at least three times) and dried for further analysis. Immobilized α-chymotrypsin was quantified [42].

The glutaraldehyde activation was carried out according to Hermanson et al. [43]. Immobilization of α-chymotrypsin was prepared using the following procedure: functionalized substrates were dipped into 12 mg of AC buffer solution for 3 h. Sodium cyanoborohydride (0.02 M) was then added and the functionalized substrates were suspended for 1 h, followed by washing as on OC procedure.

Assay of α-chymotrypsin activity

A solution of 2.4 mL ATEE (4 mM) and 0.15 M of 17.6 mL NaCl containing 0.5% (v/v) of Triton X-100 was used for the enzyme assay. The evaluation of pH changes was performed using a Virtual Instrument (LabView) serial connected to a pH-meter (Corning 443i). The pH data were recorded every 2 s in the interval of 0–20 min. Enzyme activity evaluations were performed on all substrates, involving at least three different and consecutive measurements, and assay/substrate-washing/assay cycles.

Electron spectroscopy for chemical analysis (ESCA)

The relative surface atomic concentrations and the C1s, O1s and N1s high resolution peaks corresponding to atoms in non-equivalent positions of plasma modified, derivatized, and enzyme attached samples were analyzed using a Perkin Elmer Physical Electronics 0 5400 small area ESCA system (Mg source; 15 kV; 300 W; pass energy: 89.45 eV; take off angle of 45 degrees). In order to correct surface-charge-origin binding energy (BE), shift calibrations were performed based on the well known C1s C–C (285 eV) peak.

Results and discussion

Identification of the presence of free radicals on argon and oxygen plasma-exposed polypropylene surfaces

Free radicals' high reactivity and presence of scavengers (e.g., oxygen) in typical laboratory environment requires special precautions (e.g., in situ investigations) and complex methods for their accurate evaluation. For gas phase measurements have been developed optical methods (including Laser-Induced Fluorescence) for free radical quantification and evaluation of their energies [44–61]. Biological and biochemical measurements of free radicals were investigated under liquid environment the compositions and, for medical applications, even in vivo [62–66]; consequently, scavengers or biochemical pathways' markers have been used.

Surface modification of polymers using plasma techniques raises the necessity for free radical quantification on the surface of treated polymers. Free radical scavengers (e.g., 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl [67]) have been used traditionally, but the concurrent reaction with other scavengers from the handling environment of the samples (atmosphere) are altering the quantitative evaluations. A different approach is the measurements in situ (e.g., ESR) by plasma treating the

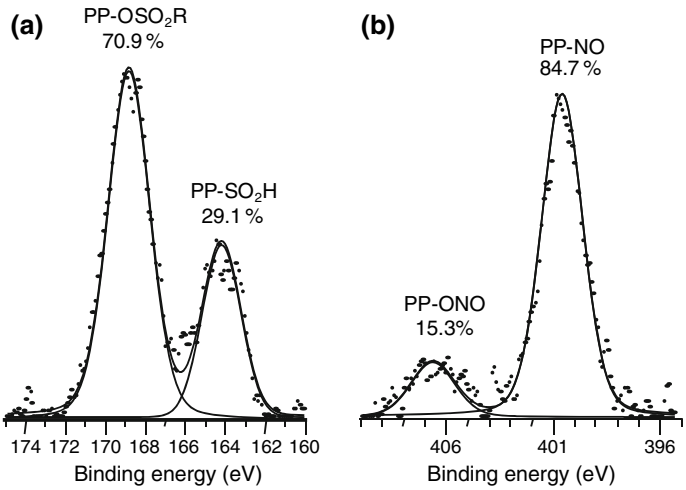

Fig. 2 High resolution S2p **(a)** and N1s **(b)** spectra of argon plasma treated PP in situ reacted with sulfur dioxide and nitric oxide

samples inside of analytical instrument, without exposing the samples to contaminants until the end of measurements; this method raises special design requirements and limits the plasma types/devices that can be studied. We used a simple free radical evaluation method [68] compatible with majority of vacuum-operated installations by using gas scavengers (vacuum manipulation simple) that avoid handling contamination of samples (in situ operation).

High resolution ESCA data resulting from argon and oxygen plasma-treated polypropylene surfaces subsequently in situ reacted with sulfur dioxide and nitric oxide clearly indicate that the radical scavengers have reacted with the plasma-exposed surfaces. The presence of sulfur and nitrogen in the surface-layer structures of modified substrates is shown in Table 1, and a typical HR S2p and N1s diagrams are presented in Fig. 2.

The presence of R′-OSO_2-R functionalities on PP[AR]SO surfaces, and R-ONO functionalities on PP[AR]NO indicates Ar-plasma-generated alkoxy radicals, while the presence of R-SO_2H and R-NO functionalities is indicative of the generation of carbon radicals [44, 45] according to the following reactions:

$$R^{\bullet} + SO_2 \rightarrow R\text{-}SO_2^{\bullet} \rightarrow R\text{-}SO_2H \quad (1)$$

$$R^{\bullet} + NO \rightarrow R\text{-}NO \quad (2)$$

$$R\text{-}O^{\bullet} + SO_2 \rightarrow R\text{-}OSO_2^{\bullet} \rightarrow R\text{-}OSO_2\text{-}R' \quad (3)$$

$$R\text{-}O^{\bullet} + NO \rightarrow R\text{-}ONO \quad (4)$$

The two binding energy peaks of the high resolution, bimodal S2p spectra (Fig. 2a) where assigned [46] to R′-OSO_2-R (168 eV) and R-SO_2H (165.5 eV) functionalities. On the other hand, high resolution N1s spectra (Fig. 2b) indicates the presence of R-ONO (405 eV) and R-NO (400 eV) groups. Accordingly, it can be suggested

that sulfur dioxide is more selective for reacting with alkoxy radicals and nitric oxide is more selective for reacting with carbon radicals. For sample PP[OX]SO, high resolution S2p spectrum showed a 168 eV energy binding peak, while the high resolution N1s spectrum of PP[OX]NO samples do not show the characteristic peaks. It can be concluded that argon plasma treatments generate alkoxy and carbon radicals on polypropylene surfaces, while oxygen plasmas predominantly produce alkoxy radicals.

ESCA analysis of modified PP surfaces

HR C1s ESCA diagrams of argon and oxygen plasma-modified PP samples subsequently, in situ, functionalized using EA or PA, in the absence of plasma, are presented in Fig. 3. The depth profile atomic concentration evaluations were performed at take off angles of 15, 45, and 75° (not presented here). The functionalized PP substrates have a significant relative surface nitrogen atomic concentration regardless of the nature of derivatization agents, and the depth-sampling measurements do not indicate significant atomic concentration changes. It is suggested that plasma-induced surface roughness changes might be responsible for the apparent constancy of the atomic compositions.

The presence of broad peaks is evident in all treated samples, covering a region that can be related to the existence of carbon, nitrogen, and oxygen (Table 2) containing linkages. The C1s peak fitted at 286 eV binding energy can be related to the presence of aliphatic amine primary groups (285.7 eV) or aliphatic amine

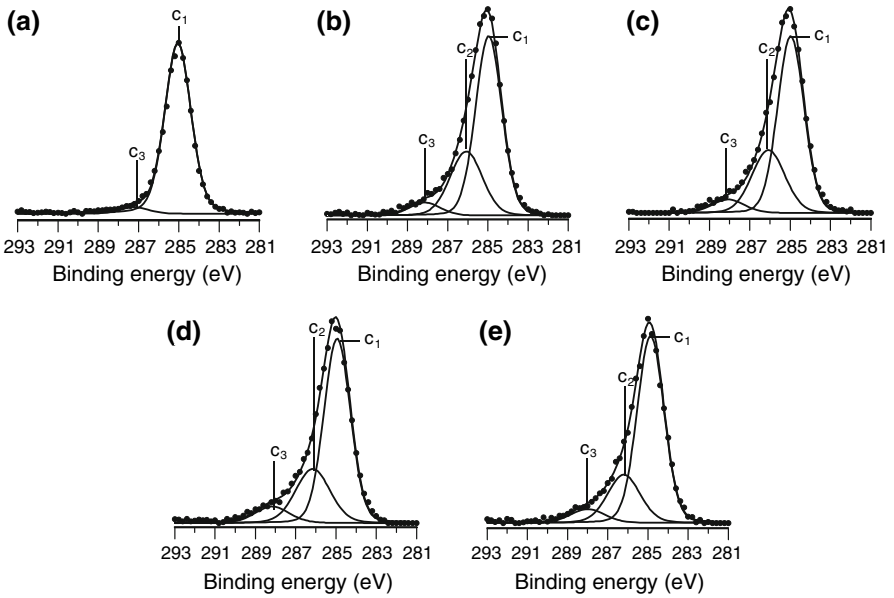

Fig. 3 High resolution C1s spectra of modified PP (Table 2): **a** PP; **b** PP[AR]EA; **c** PP[AR]PA; **d** PP[OX]EA; **e** PP[OX]PA

Table 2 Relative surface atomic composition and high resolution deconvoluted relative peak areas of virgin and modified PP

Sample	Atomic concentration (%)			Curve fit data of HR C1s spectra (%)		
	C	O	N	C_1 (285 eV)	C_2 (286 eV)	C_3 (288 eV)
PP	94.0	6.0	–	96.0	–	4.0
PP[AR]EA	81.8	12.3	5.9	65.8	26.8	5.6
PP[AR]PA	78.9	14.7	6.4	65.1	28.8	6.1
PP[OX]EA	80.2	13.9	6.1	66.1	25.8	8.1
PP[OX]PA	84.3	11.3	4.3	70.6	22.6	6.8

Plasma conditions: 100 watts; 1 min, 6 sccm of argon or oxygen; 13.56 MHz

EA in situ gas phase derivatization process of ethylene diamine on PP activated surface

PA in situ gas phase derivatization process of propylene diamine on PP activated surface

secondary groups (286.0 eV) [69] and is representative for a successful primary amine functionalization of the substrate. The slight increase of peak percentages fitted at 288 eV binding energy (carbonyl oxygen—288.0 eV—or amide groups—288.5 eV—or to a carbonyl and a non-carbonyl oxygen—289.4 eV) can be related to reduced polymer degradation during plasma treatments (higher on oxygen treatments, especially when is followed by EA functionalization) and post-plasma/functionalization reactions of reactive species created such as free radicals not quenched with amine during in situ reaction or imine functionalities.

The high resolution C1s region of PP and their derivatives generated as a result of functionalization reactions and AC attachment, and the binding energy values of carbon atoms suggested to be present in specific, non-equivalent positions are presented in Fig. 4. Once again, the very broad and complex nature of the C1s peaks make interpretations difficult. However, it is suggested that the range 287.2–289.4 eV of binding energy peak might be related to the presence of covalently attached AC on the substrate surfaces. Due to the broad nature of the diagrams, where the multi-modal character is more difficult to be defined, deconvolution of peaks and precise assignment were avoided. The ESCA atomic composition on the surface of samples (Table 3) shows the presence of nitrogen on all samples and chlorine only on the OC modified one.

Enzyme immobilization on modified substrates

Sensors, medical and biotech applications are demanding immobilization of biomolecules into a variety of substrates; physical, chemical, and nano methods [70–80] have been designed to control the attachment and activity of biomolecules. However, specific or non-specific attachment of biomolecules on surfaces is a difficult analytical problem [81]. Plasma techniques have been used to modify substrates [82–88] due to the efficacy even on most inert substrates.

AC was immobilized on plasma-modified/derivatized PP substrates and the quantities have been evaluated from spectroscopic measurements (AC depletion from the solution by UV absorbtion at 274 nm [42]). Figure 5 exhibits the amounts

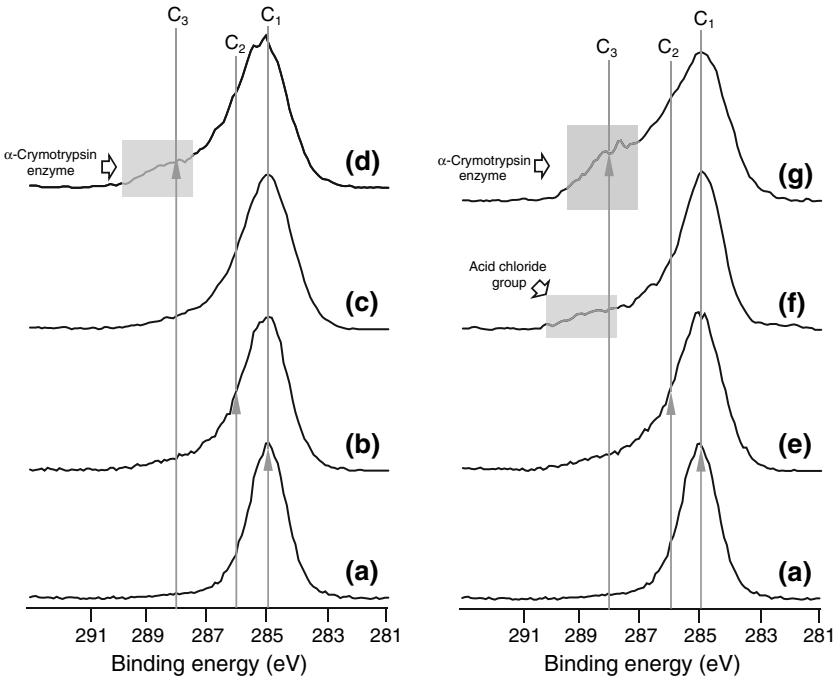

Fig. 4 High resolution C1s ESCA diagrams of PP surface during activation reaction sequence and immobilization of AC: **a** PP; **b** PP[AR]EA; **c** PP[AR]EAGL; **d** PP[AR]EAGLAC; **e** PP[OX]EA; **f** PP[OX]EAOC; **g** PP[OX]EAOCAC

Table 3 Relative surface atomic composition of surface activated and enzyme immobilized PP

Sample	Atomic concentration (%)			
	C	O	N	Cl
PP[AR]EAGL	83.5	11.8	4.7	–
PP[AR]EAGLAC	75.7	13.7	10.6	–
PP[OX]EAOC	72.1	16.0	9.3	2.7
PP[OX]EAOCAC	66.0	11.2	22.8	–

of AC attached to the modified PP substrates. It can be observed that the anchored-AC quantities are dependent on the specific surface functionalization technique. Slight dependence can be seen on plasma treatment technique (argon or oxygen). This might be related to the surface roughness characteristics.

Normalized comparative enzyme assay results of immobilized ACs are presented in Fig. 6. It can be noted that AC-immobilized both on argon and oxygen plasma-modified and post plasma-functionalized PP substrates, with terminal OC-based spacer chains, have higher activities in comparison to those of GL-bearing substrates. The highest activity of AC was achieved when anchored through EA/OC

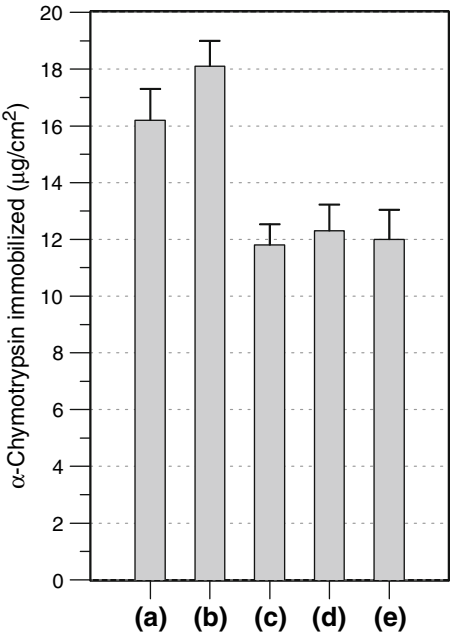

Fig. 5 Extent of α-crymotrypsin attachement on modified PP:
a PP[AR]EAOCAC;
b PP[OX]EAOCAC;
c PP[AR]EAGLAC;
d PP[OX]EAGLAC;
e PP[OX]PAGLAC

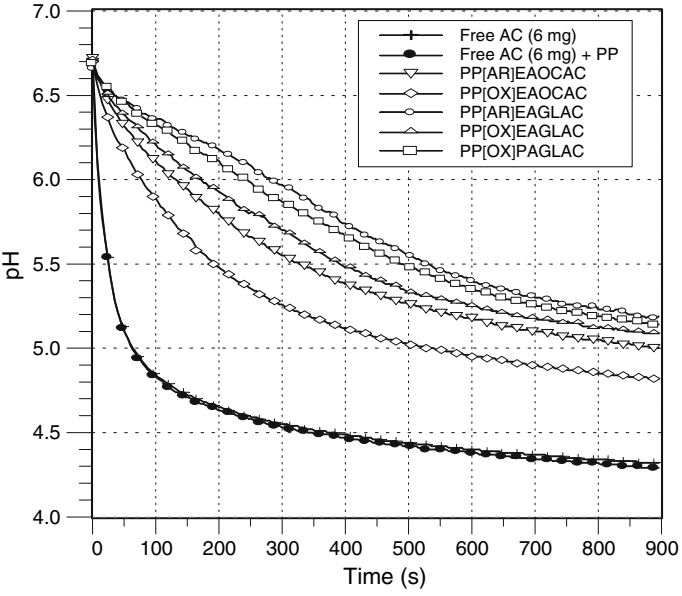

Fig. 6 Assay activity of α-crymotrypsin immobilized on modified PP

spacer-chain segments. However, the activities of the immobilized enzymes are significantly lower in comparison to the free enzyme or free enzyme in the presence of non-modified PP.

Conclusions

It was demonstrated that the oxygen and argon plasma species generate reactive free radical sites on the substrate surfaces that can interact with stable molecules under in situ conditions to produce amine functionalities.

α-Chymotrypsin was then immobilized on the oxygen- and argon-RF-plasma-exposed PP surfaces previously derivatized in the absence of plasma by attaching spacer-chain molecules between the substrates and AC. The spacer-chain molecules were sequentially built up from OC, EA, and GL molecules.

The activities of the immobilized enzymes were lower in comparison to the activity of the free enzyme; the best activity was recorded on PP-modified substrates using oxygen plasma treatment, EA functionalization, and OC derivatization before AC immobilization. The immobilization of enzyme molecules in a three-dimensional complex matrix (roughness after plasma treatment) reduces the freedom of mobility of the biomolecules and alters their supramolecular morphology as a result of multi-point interactions.

References

1. Ratner BD, Chilkoti A, Lopez GP (1990) Plasma deposition and treatment for biomaterial applications. In: d'Agostino R (ed) Plasma deposition, treatment, and etching of polymers. Academic Press Inc., London, pp 464–511
2. Shi FF (1996) Recent advances in polymer thin films prepared by plasma polymerization synthesis, structural characterization, properties and applications. Surf Coat Technol 82(1–2):1
3. Denes F, Manolache S (2004) Macromolecular plasma-chemistry: an emerging field of polymer science. Prog Polym Sci 29(8):815
4. Cavallini M, Biscarini F, Matacotta FC, Massaccesi G (2008) Device and method for protection and activation of sensors. PCT Int. Appl.; WO 2008110503 A2
5. Glasel JA, Freeman CJ (2004) Food pathogen sensor using molecularly imprinted polymers. PCT Int. Appl.; WO 2004020655 A2
6. Rappe AK, Polyakov OG, Bormann-Rochotte LM (2008) Catalyst structure and polymer tacticity: the theory behind site control in propylene polymerization. In: Baugh LS, Canish JAM (eds) Stereoselective polymerization with single-site catalysts. CRC Press, Boca Raton, FL, pp 169–200
7. Colacot TJ (2008) Palladium based FibreCat and SMOPEX as supported homogenous catalyst systems for simple to challenging carbon-carbon coupling reactions. Top Catal 48(1–4):91
8. Guo C, Fan H, Li BG, Zhu S (2006) Branched-PE/i-PP reactor blends prepared through ethylene gas-phase polymerization catalyzed by α-diimine nickel supported on iPP particles. Studies in surface science and catalysis. Prog Olefin Polym Catal Polyolefin Mater 161:245
9. Bash TF, Karian HG (1999) Functionalization and compounding of polypropylene using twin-screw extruders. In: Handbook of polypropylene and polypropylene composites, vol 51. Marcel Dekker Inc., New York, pp 335–365
10. Badrossamay MR, Sun G (2008) Preparation of rechargeable biocidal polypropylene by reactive extrusion with diallylamino triazine. Eur Polym J 44(3):733
11. Li Q, Tzoganakis C (2007) Functionalization of PP with sulfonyl azide through reactive processing. Int Polym Proc 22(3):311
12. Nachtigall SMB, Baumhardt NR, Mauler RS (1999) A factorial design applied to polypropylene functionalization with maleic anhydride. Polym Eng Sci 39(4):630
13. Oromehie AR, Hashemi SA, Meldrum IG, Waters DN (1997) Functionalization of polypropylene with maleic anhydride and acrylic acid for compatibilizing blends of polypropylene with poly(ethylene terephthalate). Polym Int 42(1):117

14. Borsig E (1999) Polypropylene derivatives. J Macromol Sci A Pure Appl Chem A36(11):1699
15. Yazdani-Pedram M, Vega H, Quijada R (2001) Melt functionalization of polypropylene with methyl esters of itaconic acid. Polymer 42(10):4751
16. Gupta B, Saxena S, Ray A (2008) Plasma induced graft polymerization of acrylic acid onto polypropylene monofilament. J Appl Polym Sci 107(1):324
17. Hoffmann K, Mix R, Friedrich JF, Buschmann HJ, Resch-Genger U (2009) Anchoring of fluorophores to plasma-chemically modified polymer surfaces and the effect of Cucurbit[6]uril on dye emission. J Fluoresc 19(2):229
18. Joshi RS, Friedrich JF, Wagner MH (2009) Study of carboxylic functionalization of polypropylene surface using the underwater plasma technique. Eur Phys J D At Mol Opt Plasma Phys 54(2):249
19. Friedrich JF, Mix R, Schulze RD, Meyer-Plath A, Joshi R, Wettmarshausen S (2008) New plasma techniques for polymer surface modification with monotype functional groups. Plasma Proc Polym 5(5):407
20. Lee KT, Goddard JM, Hotchkiss JH (2009) Plasma modification of polyolefin surfaces. Packag Technol Sci 22(3):139
21. Bhoj AN, Kushner MJ (2008) Repetitively pulsed atmospheric pressure discharge treatment of rough polymer surfaces: II. Treatment of micro-beads in $He/NH_3/H_2O$ and $He/O_2/H_2O$ mixtures. Plasma Sources Sci Technol 17(3):035025/1
22. Meyer-Plath A, Mix R, Friedrich J (2007) Aspects of amino functionalization of polypropylene surfaces for adhesion promotion of thermally evaporated copper films. In: Mittal KL (ed) Adhesion aspects of thin films. VSP, Utrecht, pp 177–198
23. Tang J, He Q, Chen H, He N (2005) Synthesis and hybridization studies of DNA on functionalized polypropylene surfaces. J Nanosci Nanotechnol 5(8):1225
24. Bratskaya S, Marinin D, Nitschke M, Pleul D, Schwarz S, Simon F (2004) Polypropylene surface functionalization with chitosan. J Adhes Sci Technol 18(10):1173
25. Wafa DM, Breidt F, Gawish SM, Matthews SR, Donohue KV, Roe RM, Bourham MA (2007) Atmospheric plasma-aided biocidal finishes for nonwoven polypropylene fabrics. II. Functionality of synthesized fabrics. J Appl Polym Sci 103(3):1911
26. Hollahan JR, Wydeven T (1973) Synthesis of reverse osmosis membranes by plasma polymerization of allylamine. Science 179:500
27. Yasuda H (1985) Plasma polymerization. Academic Press Inc., New York, pp 4–10
28. Gombotz WR, Guanghui W, Hoffman AS (1989) Immobilization of poly(ethylene oxide) on poly(ethylene terephthalate) using a plasma polymerization process. J Appl Polym Sci 37(1):91
29. Lub J, van Vroonhoven FCBM, Bruninx E, Benninghoven A (1989) Interaction of nitrogen and ammonia plasmas with polystyrene and polycarbonate studied by X-ray photoelectron spectroscopy, neutron activation analysis and static secondary-ion mass spectrometry. Polymer 30(1):40
30. Holmes S, Schwartz P (1990) Amination of ultrahigh-strength polyethylene using ammonia plasma. Compos Sci Technol 38(1):1
31. Chinn JA, Ratner BD, Horbett TA (1992) Adsorption of baboon fibrinogen and the adhesion of platelets to a thin film polymer deposited by radio-frequency glow discharge of allylamine. Biomaterials 13(5):322
32. Terlingen JG, Brenneisen LM, Super HT, Pijpers AP, Hoffman AS, Feijen J (1993) Introduction of amine groups on poly(ethylene) by plasma immobilization of a preadsorbed layer of decylamine hydrochloride. J Biomater Sci Polym Ed 4(3):165
33. Favia P, Stendardo MV, d'Agostino R (1996) Selective grafting of amine groups on polyethylene by means of NH_3-H_2 RF glow discharges. Plasmas Polym 1(2):91
34. Ganapathy R, Wang X, Denes F, Sarmadi M (1996) Primary amine implantation onto polypropylene surfaces from melamine and urea RF plasmas. J Photopolym Sci Technol 9(2):181
35. Peric D, Bell AT, Shen M (1997) Reverse osmosis characteristics of composite membranes prepared by plasma polymerization of allylamine. Effects of deposition conditions. J Appl Polym Sci 21(10):2661
36. Sarra-Bournet C, Ayotte G, Turgeon S, Massines F, Laroche G (2009) Effects of chemical composition and the addition of H_2 in a N_2 atmospheric pressure dielectric barrier discharge on polymer surface functionalization. Langmuir 25(16):9432
37. Denes F, Manolache S, Young RA (1999) Synthesis and surface functionalization under cold-plasma conditions. J Photopolym Sci Technol 12(1):27

38. Martinez AJ, Manolache S, Gonzalez V, Young RA, Denes F (2000) Immobilized biomolecules on plasma functionalized cellophane. I. Covalently attached α-chymotrypsin. J Biomater Sci Polym Ed 11(4):415
39. Martínez-Gómez AJ, Manolache SO, Gónzalez-Álvarez V, Young RA, Denes FS (2009) Surface functionalization via *in situ* interaction of plasma-generated free radicals with stable precursor-molecules on cellulose. Cellulose 16(3):501–517
40. Kuzuya M, Yamashiro T, Kondo S-I, Sugito M, Mouri M (1998) Plasma-induced surface radicals of low-density polyethylene studied by electron spin resonance. Macromolecules 31(10):3225
41. Kerr JA (1973) Rate processes in the gas phase. In: Kochi JK (ed) Free radicals, vol 1. Wiley, London, pp 1–36
42. Ganapathy R, Manolache S, Sarmadi M, Simonsick WJ Jr, Denes F (2000) Immobilization of active α-chymotrypsin on RF-plasma-functionalized polymer surfaces. J Appl Polym Sci 78(10):1783
43. Hermanson GT, Mallia AK, Smith PK (1992) Immobilized affinity ligand techniques. Academic Press, New York, pp 77
44. Daltrini AM, Moshkalev SA, Morgan TJ, Piejak RB, Graham WG (2008) Plasma diagnostics in an inductively coupled plasma – gaseous electronics conference reference cell. ECS Trans 14(1): 345–373
45. Stillahn JM, Trevino KJ, Fisher ER (2008) Plasma diagnostics for unraveling process chemistry. Annu Rev Anal Chem 1:261–291
46. Sasaki K (2007) Plasma and spectroscopic analysis. V. Plasma diagnostics by spectroscopic methods. Bunko Kenkyu 56(5):224–236
47. Donne AJH, Barth CJ (2006) Laser-aided plasma diagnostics. Fusion Sci Technol 49(2T):375–386
48. Keesee AM, Scime EE, Boivin RF (2004) Laser-induced fluorescence measurements of three plasma species with a tunable diode laser. Rev Sci Instrum 75(12):4091–4093
49. Kim JH, Yoon THo, Chung KH, Yoo YS (2004) Study of the correlation between CF_2 radicals and the plasma parameters measured by using laser-induced fluorescence and a cutoff probe. Sae Mulli 48(5):478–484
50. Muraoka K, Uchino K, Yamagata Y (2004) Plasma diagnostics using lasers. Oyo Butsuri 73(2): 220–223
51. Kranzusch S, Peth C, Doering S, Mann K (2004) Plasma generation and characterization in the extreme ultraviolet spectral range. In: Laser-generated and other laboratory X-ray and EUV sources, optics, and applications. Proc SPIE Int Soc Opt Eng 5196:71–85
52. Sung YM, Honda C (2002) Studies on the plasma localization of a magnetic neutral loop discharge using normalized radio frequency electric field. J Vac Sci Technol B 20(4):1457–1464
53. Hargus WA Jr, Cappelli MA (2002) Interior and exterior laser-induced fluorescence and plasma measurements within a hall thruster. J Propuls Power 18(1):159–168
54. Oshikane Y, Kawashima S, Endo K, Kataoka T, Inoue H, Mori Y (2001) CF_x radicals and electric field measurement in capacitive VHF plasma at atmosphere by laser induced fluorescence and optical emission spectroscopy. In: Goto T (ed) Proceedings of 25th ICPIG, International conference on phenomena in ionized gases, Nagoya, Japan, July 17–22, vol 1. pp 181–182
55. Aramaki M, Sakawa Y, Shoji T, Hayasaka K (2001) Laser-induced fluorescence measurement of buffer-gas-cooled one-component plasma in toroidal RF ion trap. Purazuma Kaku Yugo Gakkaishi 77(5):476–483
56. Causse E, Malatray P, Calaf R, Charpiot P, Candito M, Bayle C, Valdiguie P, Salvayre R, Couderc F (2000) Plasma total homocysteine and other thiols analyzed by capillary electrophoresis/laser-induced fluorescence detection: comparison with two other methods. Electrophoresis 21(10):2074–2079
57. Brockhaus A, Georg A, Wingsch V, Engemann J (2000) Plasma erosion of a magnesium radio-frequency cathode measured by laser-induced fluorescence. J Vac Sci Technol A 18(3):927–932
58. Gracin D, Denkelmann R, Maurmann S, Andreic Z (2000) LIF spectroscopy of a cylindrical magnetron discharge. Contrib Plasma Phys 40(1–2):120–125
59. Goncalves BMS, Varandas CAF, Malaquias A, Mendonca JT, Cabral JAC (1999) Plasma potential profile determination by a new diagnostic based on neutral particle beam injection and laser induced fluorescence. Rev Sci Instrum 70(1, Pt. 2):908–911
60. Freegarde TGM, Hancock G (1997) A guide to laser-induced fluorescence diagnostics in plasmas. J Phys 4:7. (C4, XXIIIrd International Conference on Phenomena in Ionized Gases, 1997): C4/15-C4/29
61. Nakagawa H (1997) Plasma measurement using LIF. Optronics 190:139–144

62. Wang CC, Rogers MS, Pang CP (2006) Measurements of total antioxidant activity. In: Panglossi HV (ed) New developments in antioxidants research. Nova Science Publishers, Inc., Hauppauge, NY, pp 153–175
63. Dusse LMSA, Morais S, Rivia M, Vieira LM, Carvalho MG (2005) Does plasma nitrite determination by the Griess reaction reflect nitric oxide synthesis? Clin Chim Acta 362(1-2):195–197
64. Wang ZY (2005) Impact of anthocyanin from Malva sylvestris on plasma lipids and free radical. J For Res 16(3):228–232
65. Oehlschlaeger S, Albrecht S, Hakenberg OW, Schrodter S, Froehner M, Manseck A, Wirth MP (2003) Clinical impact of the measurement of free radicals and nitric oxide to identify the reperfusion injury in human renal transplantation. Transpl Proc 35(2):843–844
66. Zarban A, Taheri F, Chahkandi T, Sharifzadeh G, Khorashadizadeh M (2009) Antioxidant and radical scavenging activity of human colostrum, transitional and mature milk. J Clin Biochem Nutr 45(2):150–154
67. Carley JF, Kitze P (1980) Corona-discharge treatment of polymeric films. II. Chemical studies. Polym Eng Sci 20(5):330
68. Hon DNS, Feist WC (1993) Interaction of sulfur dioxide and nitric oxide with photoirradiated wood surfaces. Wood Sci Technol 25(2):136
69. Beamson G, Briggs D (1992) High resolution XPS of organic polymers. The Scienta ESCA300 Database, Wiley, New York
70. Soloducho J, Cabaj J, Swist A (2009) Structure and sensor properties of thin ordered solid films. Sensors 9(10):7733–7752
71. Elaissari A, Fessi H (2009) Advances in the biomedical applications of reactive colloids. Braz J Phys 39(1A):146–149
72. Ishihara K, Takai MJR (2009) Bioinspired interface for nanobiodevices based on phospholipid polymer chemistry. Soc Interface 6(Suppl 3):S279–S291
73. Matsuno R, Ishihara K (2009) Molecular-integrated phospholipid polymer nanoparticles with highly biofunctionality. Polymers at frontiers of science and technology—MACRO 2008. Macromol Symposia 279:125–131
74. Vajda S, Kozakov D (2009) Convergence and combination of methods in protein-protein docking. Curr Opin Struc Biol 19(2):164–170
75. Prieto-Simon B, Campas M, Marty JL (2008) Biomolecule immobilization in biosensor development: tailored strategies based on affinity interactions. Protein Peptide Lett 15(8):757–763
76. Millner PA, Hays HCW, Vakurov A, Pchelintsev NA, Billah MM, Rodgers MA (2009) Nanostructured transducer surfaces for electrochemical biosensor construction-interfacing the sensing component with the electrode. Semin Cell Dev Biol 20(1):34–40
77. Petrou PS, Kakabakos SE, Misiakos K (2009) Silicon optocouplers for biosensing. Int J Nanotechnol 6(1/2):4–17
78. Jonkheijm P, Weinrich D, Schroeder H, Niemeyer CM, Waldmann H (2008) Chemical strategies for generating protein biochips. Angew Chem Int Edit 47(50):9618–9647
79. Teles FRR, Fonseca LP (2008) Applications of polymers for biomolecule immobilization in electrochemical biosensors. Mat Sci Eng C Bio S 28(8):1530–1543
80. Mendes PM (2008) Stimuli-responsive surfaces for bio-applications. Chem Soc Rev 37(11):2512–2529
81. McArthur SL, Fowler GJS, Mishra GJ (2008) Surface analysis of biomolecules: unravelling biointerfacial interactions. Surf Anal 14(4):370–375
82. Selim KM, Kamruzzaman SKM, Kang IK (2008) Immobilization of biomolecules on polymeric surfaces using plasma glow discharge and their interaction with biocomponents. In: Tanaka J (ed) Surface design and modification of biomaterials for clinical application. Transworld Research Network, Trivandrum, India, pp 61–93
83. Kuzuya M, Sasai Y, Yamauchi Y, Kondo S (2008) Pharmaceutical and biomedical engineering by plasma techniques. J Photopolym Sci Technol 21(6):785–798
84. Lopez LC, Gristina R, d'Agostino R, Favia P (2008) Chemical immobilization of biomolecules on plasma-modified substrates for biomedical applications. In: d'Agostino R (ed) Adv Plasma Technol. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 269–286
85. Siow KS, Britcher L, Kumar S, Griesser HJ (2006) Plasma methods for the generation of chemically reactive surfaces for biomolecule immobilization and cell colonization – a review. Plasma Process Polym 3(6/7):392–418

86. Foerch R, Zhang Z, Knoll W (2005) Soft plasma treated surfaces: tailoring of structure and properties for biomaterial applications. Plasma Process Polym 2(5):351–372
87. Meyer-Plath AA, Schroder K, Finke B, Ohl A (2003) Current trends in biomaterial surface functionalization-nitrogen-containing plasma assisted processes with enhanced selectivity. Vacuum 71(3):391–406
88. Hoffman AS (1984) Ionizing radiation and gas plasma (or glow) discharge treatments for preparation of novel polymeric biomaterials. Adv Polym Sci 57:141–157