Tailored Functionalization of Low-Density Polyethylene Surfaces

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ABSTRACT: Surface-functionalization chemistries were optimized to tailor the surface chemistry of polyethylene, and this made covalent attachment of bioactive molecules possible. This concept has relevance in biomaterials, biosensors, textiles, and active food-packaging applications. Clean polyethylene films were subjected to chromic acid oxidation to introduce carboxylic acids. A range of functional groups, including amine, aldehyde, thiol, and hydroxyl, were then introduced to the surface of the oxidized films with functionalized crosslinking agents and covalent bioconjugation chemistries. The quantity of functional groups was further increased by subsequent grafting of polyfunctional agents such as polyethylenimine and poly(acrylic acid). The number and type of functional groups were quantified by contact-angle, dye-assay, attenuated total reflectance/Fourier transform infrared, and X-ray photoelectron spectroscopy analyses. We optimized chemistries to introduce a variety of functional groups to the surface of low-density polyethylene in numbers ranging from several picomoles per centimeter squared to tens of nanomoles per centimeter squared. A range of bioactive compounds, including antimicrobials, antibodies, oligonucleotides, cell precursors, drugs, peptides, enzymes, and synthetic biomimetic agents, can be covalently bound to these functional groups in the development of nonmigratory biofunctionalized polymers. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 2940–2949, 2008

Key words: biological applications of polymers; biomaterials; functionalization of polymers; polyethylene (PE); surfaces

INTRODUCTION

There is a growing interest in the covalent immobilization of bioactive compounds onto polymer surfaces for applications in the biomedical,[1–10] textile,[11–19] bioanalytical,[20–28] bioprocessing,[29–31] and food-packaging[32–35] industries. In practice, a polymer substrate is chosen for its bulk properties (elasticity, strength, origin, clarity, degradability, etc.). The polymer surface is then functionalized to possess the desired type and quantity of functional groups. Finally, a bioactive compound of interest is covalently bound to the functionalized polymer surface. Although noncovalent adsorption may be useful in some applications, covalent immobilizations provide the most stable bond between the compound and the functionalized polymer surface. This approach offers advantages in biomedical applications, such as preventing metabolism of chemotherapeutic molecules that have antitumor activity when used locally but may be toxic if metabolized or allowing continued bioactivity of indwelling devices such as shunts, catheters, and vascular prostheses.[36,37] In nonmigratory active food-packaging applications, a covalent linkage may offer the regulatory advantage of not requiring approval as a food additive.[38–40]

There are a number of challenges to modifying the polymer surface so that the biomolecule can be covalently bound and retain bioactivity. Techniques in polymer surface modification have focused on increasing surface polarity to improve adhesion, wetting, and printability, with little focus on functional group specificity. Surface-functionalization techniques must therefore be adapted to introduce specific functional groups. The specific functionality imparted to the inert polymer surface must be compatible with the reactive sites on the biomolecule to be covalently attached to that surface. When a large number of surface-functional groups is required, branched or dendritic tether molecules can be used.[3,4,9,41–44] In addition, the activity of a bioactive compound often changes when it is covalently bound to a polymer substrate, and this may be a result of surface-induced hydrophobic denaturation, a change in local pH due to the presence of ionizable groups, or reduced biomolecule mobility.[45–47] It would therefore be useful to be able to control the chemistry of a polymer surface in terms of the type and quantity of functional group present as well as the length of the crosslinkers used to immobilize the biomolecule.

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The objective was to optimize the introduction of a range of functional groups useful in bioconjugation chemistries to the surface of low-density polyethylene (PE) films. PE was chosen as a model substrate because of its prevalence in packaging,[34,48] biomedicine,[49–53] and other applications. Parameters such as the reactant concentration, reaction time, and pH were investigated to characterize the effect of reaction conditions on the resulting number of available surface-functional groups. The long-term goal of this research is to develop surface-functionalization chemistries that can be used to produce a known type and quantity of functional groups on a polymer surface, to which a bioactive compound of interest can be covalently bound.

EXPERIMENTAL

Materials

Additive-free, low-density PE (640I; 100 μm) was kindly donated by Dow Chemical Co. (Midland, MI). Poly(acrylic acid) (weight-average molecular weight = 450,000) was purchased from Scientific Polymer Products (Ontario, NY). Chromium trioxide (anhydrous), ethylenediamine tetraacetic acid (EDTA), and toluidine blue O were purchased from Fisher Scientific (Fair Lawn, NJ). Acid orange 7, 5, 5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), ethylenediamine dihydrochloride (98%), glutaraldehyde (50 wt %), *N*-hydroxysuccinimide (NHS), polyethylenimine (PEI; weight-average molecular weight = 25,000), Schiff's reagent, and sodium cyanoborohydride coupling buffer (3 g/L) were purchased from Sigma–Aldrich (St. Louis, MO). L-Cysteine hydrochloride monohydrate (cysteine) was purchased from MP Biomedicals (Aurora, OH). 2-Iminothiolane·HCl (Traut's reagent) was purchased from Pierce Biotechnology, Inc. (Rockford, IL). All other reagents were obtained from commercial laboratory supply stores and were reagent-grade or better.

Surface functionalization

PE films (2 × 2 cm^2) were sonicated for 10 min in three aliquots of dichloromethane (99.9%), then acetone (99.7%), and finally deionized water. Cleaned films were then oxidized for 2 min in 70°C chromic acid [29 : 42 : 29 weight ratio of chromium trioxide, deionized water, and sulfuric acid (96%)], and this was followed by 15 min of soaking in 50°C nitric acid (70%).[34,54] Oxidized films [carboxyl-functionalized polyethylene (PE–COOH)] were subjected to various aqueous surface-functionalization treatments, followed by rinsing in copious volumes of distilled water, to change the quantity or type of the surface-functional groups. In all cases, *molar excess* is defined as the ratio of the moles of the bioconjugation reagent to the estimated moles of available surface-functional groups. Functionalized PE films that were not used immediately were stored in deionized water to inhibit the migration of polar surface-functional groups into the polymer bulk. Figures 1 and 2 illustrate the overall reaction scheme and identify abbreviations used to describe the resulting films.

PE–polyNH$_2$ films were prepared as previously reported by the shaking of PE–COOH films for 2 h at room temperature in a 30 mg/mL solution of PEI in

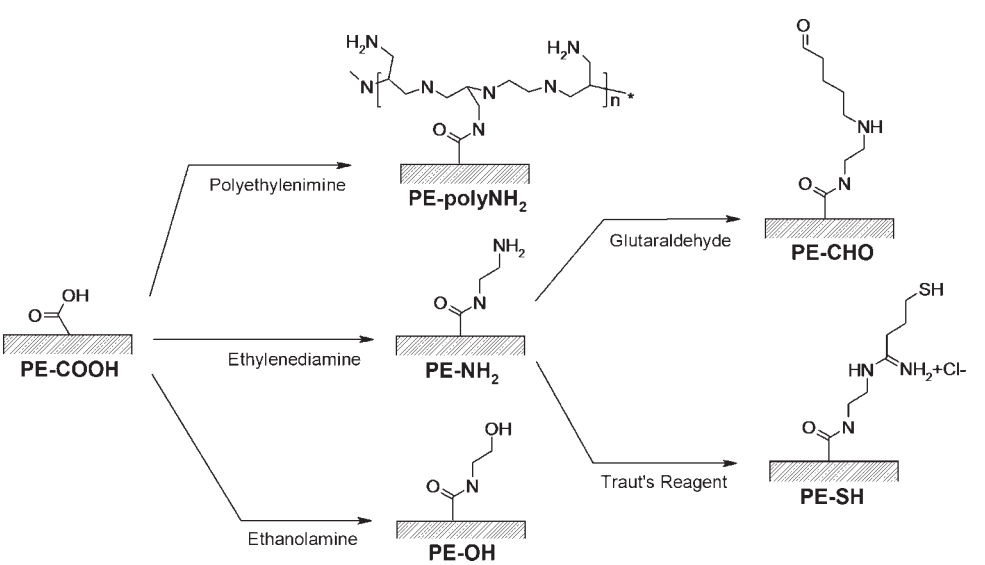

Figure 1 Reaction scheme for the synthesis of polyamine-functionalized polyethylene (PE–polyNH$_2$), amine-functionalized polyethylene (PE–NH$_2$), aldehyde-functionalized polyethylene (PE–CHO), thiol-functionalized polyethylene (PE–SH), and hydroxyl-functionalized polyethylene (PE–OH) surfaces.

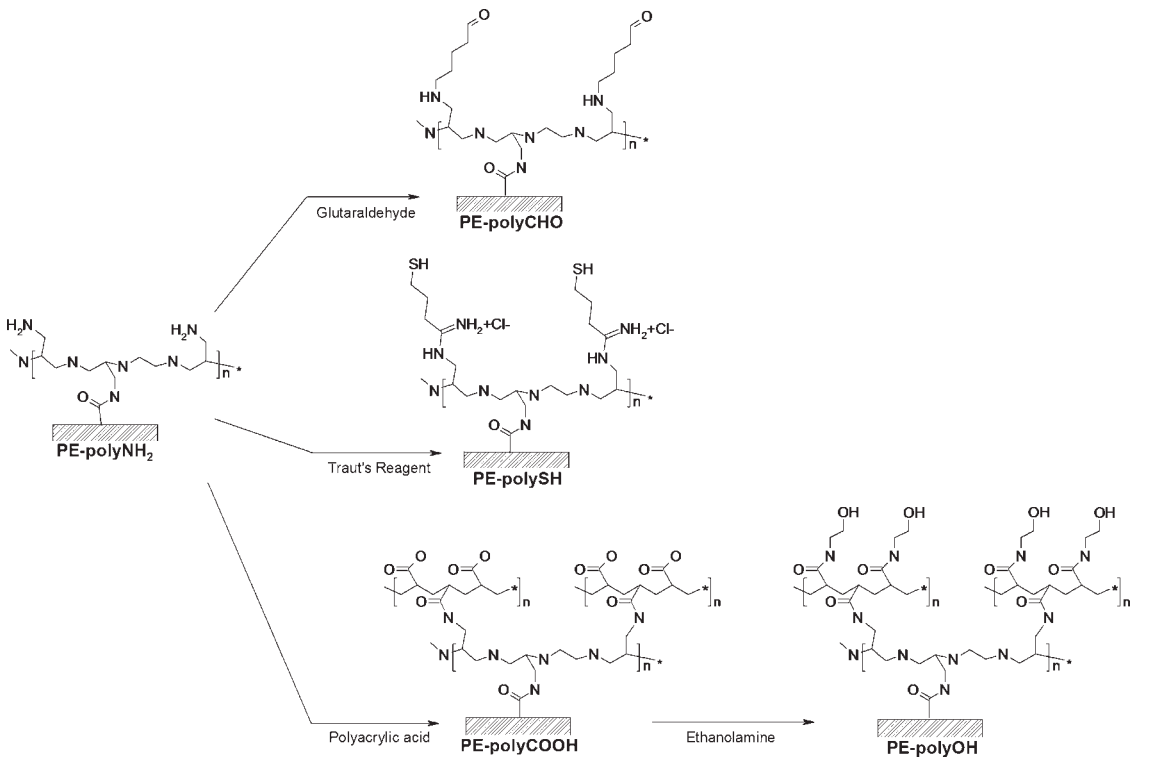

Figure 2 Reaction scheme for the synthesis of polyaldehyde-functionalized polyethylene (PE–polyCHO), polythiol-functionalized polyethylene (PE–polySH), polycarboxyl-functionalized polyethylene (PE–polyCOOH), and polyhydroxyl-functionalized polyethylene (PE–polyOH) surfaces.

0.1*M* sodium carbonate buffer (pH 9.6) containing a 100 molar excess of NHS and a 1000 molar excess of EDC.[34] PE–NH_2 and PE–OH films were prepared by the shaking of PE–COOH films at room temperature in 0.067*M* phosphate buffer containing a 1000 molar excess of the crosslinker (ethylenediamine and ethanolamine, respectively), a 1000 molar excess of EDC, and a 100 molar excess of NHS. The buffer pH and reaction time were varied to determine which conditions provided maximum conjugation efficiency. PE–polyCHO films were prepared by the shaking of PE–polyNH$_2$ films at room temperature in 0.1*M* sodium carbonate buffer (pH 9.6) containing a 100 molar excess of sodium cyanoborohydride as a reducing agent. PE–polySH films were prepared by the conjugation of Traut's reagent to the amines present on PE–polyNH$_2$ films at room temperature in 0.067*M* phosphate buffer (pH 8.0) containing 5 m*M* EDTA.[55,56] To optimize surface functionalization of PE–polyCHO and PE–polySH films, the reaction time and concentration of the crosslinker were varied. PE–polyCOOH films were prepared by the shaking of PE–polyNH$_2$ films for 4 h at room temperature in 0.1*M* 2-(N-morpholino) ethane sulfonic acid buffer (pH 6.5) containing a 1 : 6 molar ratio of poly(acrylic acid) to available primary amines and equimolar quantities of both NHS and EDC to available primary amines. PE–polyOH, PE–CHO, and PE–SH films were prepared under optimum conditions identified in preparing PE–OH, PE–polyCHO, and PE–polySH films.

Surface analysis

After each step in the modification, polymer surfaces were analyzed for changes in hydrophilicity and surface chemistry. To measure surface hydrophilicity, water contact angles of control and functionalized PE films (n = 6, two droplets on each of three separate films) were measured on a Tantec (Schaumburg, IL) CAM-Plus goniometer with reagent-grade deionized water.[57,58]

The number of available carboxylic acids and/or primary amines was quantified with dyes that complex with specific functional groups in an equimolar ratio.[59] The number of carboxylic acids was determined with the toluidine blue O assay.[60] Control and modified PE films were shaken for 3 h at room temperature in 0.5 m*M* toluidine blue O in deionized water adjusted to pH 10 by NaOH and then rinsed with an NaOH solution at pH 10 to remove noncomplexed dye. Complexed dye was desorbed by the immersion of films in 50 wt % acetic acid, and absorbance of the acetic acid solution was read at 633 nm and compared to a standard curve made of dye in 50 wt % acetic acid. The number of available primary amines was similarly determined with an

TABLE I
Effect of Buffer pH on the Optimization of PE–NH_2 and PE–OH Conjugation Chemistry

Buffer pH	PE–NH_2 primary amine (nmol/cm^2)[a]	PE–OH carboxylic acid (nmol/cm^2)[b]
5.0	0.306 ± 0.03	2.13 ± 0.12
5.5	0.306 ± 0.16	2.22 ± 0.26
6.0	0.458 ± 0.04	2.26 ± 0.13
6.5	0.635 ± 0.18	2.20 ± 0.15
7.0	0.689 ± 0.12	**1.95 ± 0.20**
7.5	**0.846 ± 0.13**	2.30 ± 0.15
8.0	0.682 ± 0.11	2.48 ± 0.15
8.5	0.599 ± 0.10	2.54 ± 0.07
9.0	0.627 ± 0.11	2.49 ± 0.16
9.5	0.705 ± 0.26	2.65 ± 0.14

[a] Values are averages of at least three determinations plus or minus the standard deviation.
[b] Values are averages of at least six determinations plus or minus the standard deviation.

adaptation of the acid orange 7 method.[59] Films were shaken for 3 h at room temperature in 1 m*M* acid orange 7 in deionized water adjusted to pH 3 by HCl and then rinsed. Dye was desorbed in deionized water adjusted to pH 12 by NaOH, and absorbances were read at 460 nm. The number of surface thiol groups was determined with Ellman's reagent.[55,61] Films were immersed in 0.067*M* phosphate buffer (pH 8.0) containing 5 m*M* EDTA, to which 50 μL of a 4 mg/mL solution of Ellman's reagent in 0.067*M* phosphate buffer (pH 8.0) containing 5 m*M* EDTA was added. After 15 min of shaking at room temperature, absorbances were read at 412 nm and compared to a cysteine standard curve. The presence of surface aldehydes was qualitatively confirmed with Schiff's reagent, which turns pink when in contact with aldehydes.[62]

X-ray photoelectron spectroscopy (XPS) was conducted at the Penn State Materials Characterization Laboratory (State College, PA) on a Kratos Analytical Axis Ultra (Kratos Anaytical, Inc., Chestnut Ridge, NY) with a monochromatic Al Kα X-ray source operating at an X-ray power of 280 W. Spectra were collected with a 90° takeoff angle and a pass energy of 80 or 20 eV (for high-sensitivity and high-resolution scans, respectively) and were referenced to the C 1s binding energy at 285 eV. A charge neutralizer was used to compensate for charge buildup, and films were wrapped in aluminum foil, except for the spot analyzed, to minimize charging.

Attenuated total reflectance (ATR)/Fourier transform infrared (FTIR) spectroscopy was conducted at the Cornell Nanobiotechnology Center (Ithaca, NY) on a Vertex 80v vacuum FTIR spectrometer (Bruker Optics, Inc., Ettlingen, Germany) with a liquid-nitrogen-cooled detector, evacuated optics, sample compartments, and a diamond ATR crystal. Each spectrum represents 1024 scans at an 8-cm^{-1} resolution taken against a reference spectrum of an empty ATR crystal. The resultant spectra were processed and analyzed with KnowItAll Informatics System 5.0 (Bio-Rad, Hercules, CA).

Statistical analysis

One-way analyses of variance (ANOVAs) and pairwise comparisons were conducted with Minitab release 14.1 from Statistical Software (State College, PA). Lines were fit to plotted data with nonlinear regression models with GraphPad PRISM (GraphPad Software, San Diego, CA), which was also used to generate graphs.

RESULTS AND DISCUSSION

Optimization of surface-functionalization chemistries

Conjugation of ethylenediamine to PE–COOH to form PE–NH_2 films was optimized by the variation of the pH and reaction time and quantification of the change in surface primary amines with the acid orange 7 assay.[59] One-way ANOVA ($P < 0.05$) followed by Fisher's pairwise comparison ($P < 0.10$) indicated that conjugation in 0.067*M* phosphate buffer (pH 7.5) generated the maximum number of primary amines (Table I). The gain in available primary amines as a function of increasing reaction time was fit to a one-phase exponential association model (Fig. 3), from which it was determined that 4 h was the optimum reaction time.

Synthesis of PE–OH films, via the immobilization of ethanolamine to PE–COOH films, was optimized by the variation of the pH and reaction time and

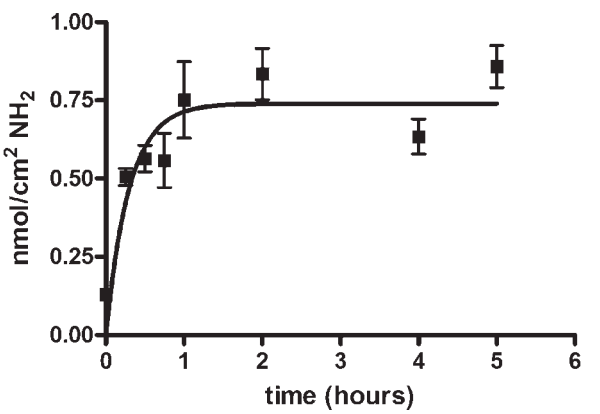

Figure 3 Effect of time on the optimization of PE–NH_2 conjugation chemistry. Values represent means ± standard error.

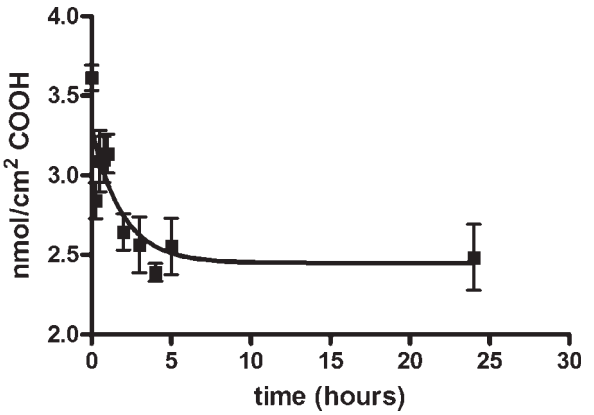

Figure 4 Effect of time on the optimization of PE–OH conjugation chemistry. Values represent means ± standard error.

quantification of the resulting loss in available carboxylic acids with the toluidine blue O assay.[60] One-way ANOVA ($P < 0.05$) followed by Fisher's pairwise comparison ($P < 0.05$) indicated that conjugation in 0.067*M* phosphate buffer (pH 7.0) resulted in the greatest loss of available carboxylic acids (Table I). The loss of available carboxylic acids as a function of increasing reaction time was fit to a one-phase exponential decay model (Fig. 4), from which it was determined that 4 h was the optimum reaction time.

Conjugation of glutaraldehyde to PE–polyNH$_2$, resulting in PE–polyCHO films, was optimized by the variation of the reaction time and molar excess of glutaraldehyde and quantification of the resulting loss in available primary amines with the acid orange 7 assay. The loss of available primary amines as a function of increasing conjugation time and the loss of available primary amines as a function of increasing molar excess of glutaraldehyde were fit to a one-phase exponential decay model [Fig. 5(a,b)]. As discussed later, glutaraldehyde forms several cyclic hemiacetal and linear polymers under basic and/or high concentration conditions; this polymerization results in an increase in UV absorbance.[63–65] There is an interest in maintaining homogeneous tether molecule length and chemistry, as these factors impact biomolecule denaturation, mobility, and microenvironment, which in turn affect activity.[47] In addition, because glutaraldehyde immobilization was quantified by a reduction in amines, not an increase in available aldehydes, there is concern that polymerized glutaraldehyde structures may crosslink the surface amines. For these reasons, the optimum conditions for glutaraldehyde immobilization (24-h conjugation time and a molar excess factor of 3000) were selected to be those which had the greatest reduction in available amines while minimizing glutaraldehyde polymerization, as determined by UV spectroscopy (data not shown). Nevertheless, it was observed that the PE–polyCHO films had increased opacity, which indicated that some polymerized forms of glutaraldehyde were present.

Synthesis of PE–polySH films, via immobilization of Traut's reagent to PE–polyNH$_2$ films, was optimized by the variation of the reaction time and molar excess of Traut's reagent and quantification of the resulting gain in available thiols with Ellman's reagent.[55,61] One-way ANOVA ($P < 0.05$) followed by Fisher's pairwise comparison ($P < 0.10$) suggested that a 4-h conjugation resulted in the maximum number of available thiol groups [Fig. 6(a)]. Although more stable against hydrolysis than many other thiolating reagents,[39,48,54,66] over time the ring structure of Traut's reagent will hydrolyze to methyl 4-mercaptobutyrimidate, which possesses a free terminal thiol. Therefore, it is expected that at some point, the rate of hydrolysis of Traut's reagent and subsequent formation of disulfide bonds to the surface thiols will be greater than the rate of introduction of thiols to the surface amines. This may explain the observed decrease in available thiols, as measured by Ellman's reagent, after 4 h of conjugation. The gain in available thiol groups as a function of increasing molar excess of Traut's reagent was fit to

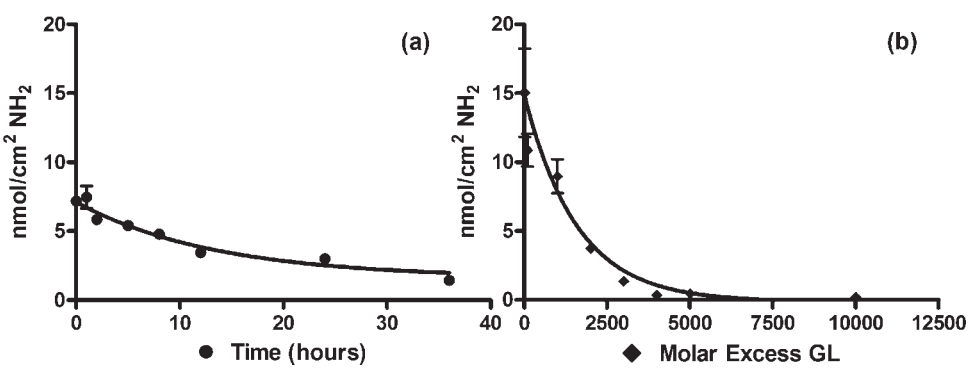

Figure 5 Effects of (a) time and (b) a molar excess of glutaraldehyde on the optimization of PE–polyCHO conjugation chemistry. Values represent means ± standard error.

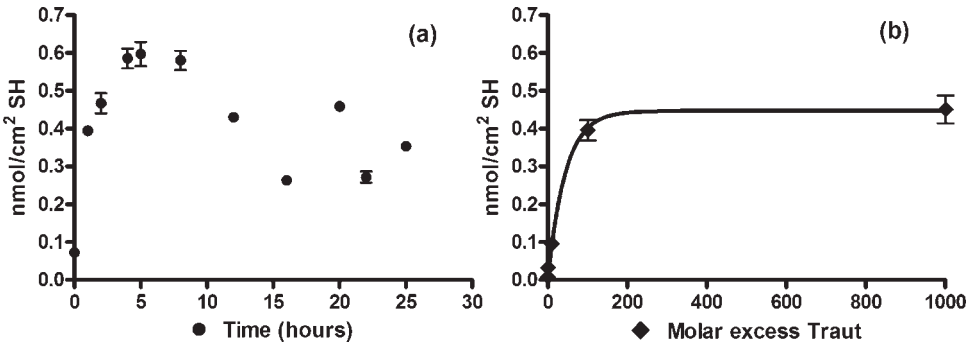

Figure 6 Effects of (a) time and (b) a molar excess of Traut's reagent on the optimization of PE–polySH conjugation chemistry. Values represent means ± standard error.

a one-phase exponential association model [Fig. 6(b)]. Optimum conditions for the preparation of PE–polySH films were found to be a 4-h reaction time and a molar excess factor of 100.

Surface chemistry and hydrophilicity

Changes in surface functionality and hydrophilicity resulting from the conjugations illustrated in Figures 1 and 2 are summarized in Table II. Oxidation by chromic acid introduced 6.12 nmol/cm^2 carboxylic acids. Conjugation of PEI to PE–COOH reduced the available carboxylic acids, indicating a covalent linkage, and increased the number of primary amines to 18.2 nmol/cm^2. Conjugation of ethylenediamine to PE–COOH reduced the number of available carboxylic acids by 2.4 nmol/cm^2 and introduced 2.1 nmol/cm^2 primary amines. The observed discrepancy between the loss in carboxyls and the gain in amines may be a result of variations between dye assays, or possible intramolecular crosslinking of carboxylic acids within crevasses of the oxidized surface, as etching is reported to occur during chromic acid oxidation.[55] Because ethanolamine is heterobifunctional, intramolecular crosslinking is unlikely, and it can be assumed that the loss in carboxylic acids indicates the presence of 3.2 nmol/cm^2 hydroxyls. Conjugation of glutaraldehyde to PE–poly NH_2 reduced the number of available primary amines by 17.8 nmol/cm^2. Although some crosslinking of glutaraldehyde within the polyaminated surface is expected, the presence of available aldehydes was confirmed by a positive response to Schiff's reagent. Conjugation of Traut's reagent to PE–polyNH_2 resulted in the introduction of 170 pmol/cm^2 available thiols. The increase in available amines may be a result of an interaction between the acid orange 7 dye and the imine side chain of Traut's reagent. Of all the functional groups investigated for PE surface functionalization, thiols are the most reactive and the most vulnerable to oxidation. EDTA was included in the conjugation buffer to minimize metal-catalyzed oxidation, but because surface thiols may form disulfide crosslinks, thiol-functionalized

TABLE II
Surface Chemistry of Control and Modified PE Films

Film sample	Carboxylic acid (nmol/cm^2)[a]	Primary amine (nmol/cm^2)[b]	Aldehyde[c]	Thiol (nmol/cm^2)[a]	Water contact angle[d]
PE	0.36 ± 0.1	0.07 ± 0.1	−	0.008 ± 0.046	100.5 ± 1.4
PE–COOH	6.12 ± 2.0	0.07 ± 0.1	−	−0.031 ± 0.008	66.8 ± 0.9
PE–polyNH$_2$	0.52 ± 0.0	18.20 ± 3.5	−	−0.039 ± 0.023	58.7 ± 1.2
PE–NH$_2$	3.70 ± 0.6	2.17 ± 0.3	−	0.039 ± 0.034	62.8 ± 0.4
PE–OH	2.94 ± 0.6		−		69.0 ± 1.1
PE–polyCHO		0.41 ± 0.2	+		43.8 ± 0.8
PE–polySH		26.27 ± 6.5	−	0.169 ± 0.069	60.8 ± 0.5
PE–polyCOOH	9.53 ± 0.3	11.33 ± 0.8	−		20.2 ± 0.5
PE–polyOH	5.55 ± 1.0		−		10.7 ± 0.6
PE–CHO		1.89 ± 0.2	+		60.5 ± 1.5
PE–SH		2.01 ± 0.6	−	0.251 ± 0.008	69.8 ± 3.9

[a] Values are averages of three determinations plus or minus the standard deviation.
[b] Values are averages of four determinations plus or minus the standard deviation.
[c] Indicates a negative (−) or positive (+) response to Schiff's reagent.
[d] Values are averages of six determinations (two droplets on each of three separate films) plus or minus the standard deviation.

polymer surfaces should be used shortly after preparation or protected with a reversible blocking agent.[55]

Conjugation of poly(acrylic acid) to PE–polyNH$_2$ reduced the number of primary amines by 6.9 nmol/cm^2 and resulted in 9.5 nmol/cm^2 available carboxylic acids. Longer conjugation times resulted in a greater reduction in the number of available primary amines but a decrease in the number of available carboxylic acids (data not shown), a consequence of the reactive polyfunctional crosslinker. To prevent carboxylic acids from continuing to react with the PEI linker layer, resulting in a net decrease in surface functionality, films were rinsed after conjugation to remove EDC/NHS.

Conjugation of ethanolamine to the resulting PE–polyCOOH films bound 4.0 nmol/cm^2 carboxylic acids, likely introducing as many hydroxyls. Conjugation of glutaraldehyde to PE–NH$_2$ films bound 0.28 nmol/cm^2 primary amines; introduction of aldehyde functionality was confirmed by a positive response to Schiff's reagent. Finally, Traut's reagent was bound to PE–NH$_2$ films, generating 0.25 nmol/cm^2 thiols.

Cleaned, unmodified PE films possessed a hydrophobic surface, with a water contact angle of 100.5° (Table II). Oxidation by chromic acid created a hydrophilic surface and reduced the water contact angle to 66.8°. Subsequent functionalized surfaces remained hydrophilic, with increased hydrophilicity for PE–polyCHO surfaces (43.8°) and full wettability for PE–polyCOOH (20.2°) and PE–polyOH (10.7°) surfaces. It is interesting to note the differences in contact angles between the pairs of surfaces (PE–OH/PE–polyOH, PE–SH/PE–polySH, etc.). In each case, the polyfunctional version has a lower contact angle because of the molecular flexibility and increased wettability provided by the long-chain polymers PEI and poly(acrylic acid).

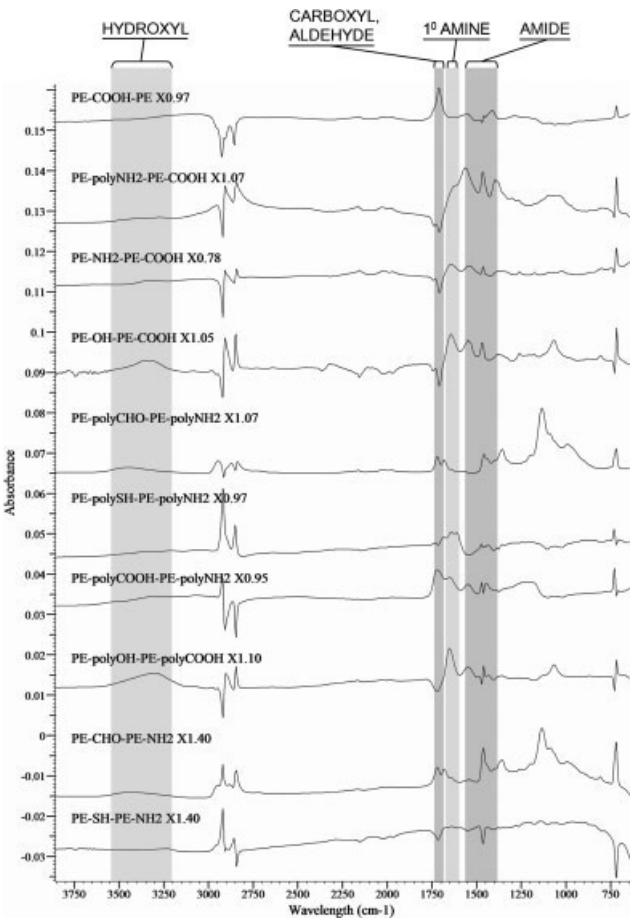

Figure 7 ATR–FTIR difference spectra of modified PE films. The values (0.78–1.40) represent scaling factors used in spectral subtractions. Gray bands highlight functional group absorptions.

ATR–FTIR analysis

ATR–FTIR analyses were conducted to confirm changes in surface chemistry. Because surface modifications extend nanometers into the polymer surface and ATR–FTIR analysis probes to a depth nearing a micrometer, spectral subtraction was necessary for spectral peak identification (Fig. 7). Spectra of surface-functionalized films were subtracted from spectra of the next step in the functionalizations illustrated in Figures 1 and 2.

Clean, unmodified PE films had absorption bands at the expected frequencies for saturated aliphatic groups, including strong intensities at 2936–2916, 2863–2843, and 750–720 cm^{-1} for methylene vibrations, as well as a less intense absorption at 1380–1375 cm^{-1} indicating methyl groups. Chromic acid oxidized films presented a low-intensity absorption at 3100–2900 cm^{-1}, a strong, sharp absorption at 1725–1700 cm^{-1}, and a lower intensity absorption at 1440–1395 cm^{-1}, all indicating the presence of carboxylic acids. PE–polyNH$_2$ films had absorptions at 1650–1590 and 1650–1550 cm^{-1}, which were attributed to primary and secondary amines. The loss in intensity of the carboxylic acid absorption (1725–1700 cm^{-1}) as well as the presence of characteristic amide bands (1680–1630, 1570–1515, and 1490–1440 cm^{-1}) suggested a covalent linkage between PEI and PE–COOH. Similar to PE–polyNH$_2$, PE–NH$_2$ films showed characteristic amide and amine absorption bands and a corresponding loss in carboxylic acid intensity, indicating that ethylenediamine was covalently linked to the PE–COOH surface, but at lower intensity than the bands observed in the PE–polyNH$_2$ spectrum, as expected. For PE–OH films, the introduction of a broad hydroxyl group absorption (3400–3200 cm^{-1}) and amide bands at 1680–1630, 1570–1515, and 1490–1440 cm^{-1}, in addition to the loss of carboxylic acid band intensity

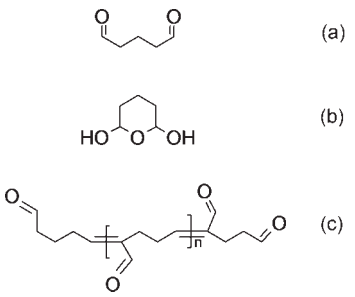

Figure 8 Molecular structures of native and polymerized glutaraldehyde.

at 1725–1700 cm^{-1}, indicated that ethanolamine was covalently linked to PE–COOH surfaces.

The ATR–FTIR spectrum of PE–polyCHO had a narrow absorption at 1740–1720 cm^{-1} and weaker bands at 2775–2695 and 1392–1388 cm^{-1}, all characteristic aldehyde vibrations. Also evident were secondary amine absorptions at 3500–3300, 1146–1132, 750–700, and 1650–1550 cm^{-1}, indicating reductive amination. The PE–polyCHO spectrum had a variety of other absorption bands as well, and this supports reports that glutaraldehyde polymerizes to several molecular structures in an aqueous solution, often as a result of basic conditions or a high glutaraldehyde concentration.[63–65] Figure 8(a–c) illustrates the native structures of glutaraldehyde and two representative molecular structures of polymerized glutaraldehyde, a cyclic hemiacetal structure and a linear polymer with ethylene double bonds. The presence of the cyclic hemiacetal form [Fig. 8(b)] was evident by alcohol absorption bands at 3400–3200, 1410–1310, and 1210–1100 cm^{-1}, as well as the slight six-ring ether asymmetric stretching vibration band at 1110–1090 cm^{-1}. Characteristic alkene bands (1692–1667 and 995–985 cm^{-1}) and higher intensity alkane bands (1485–1445 and 750–720 cm^{-1}) suggest the presence of the linear polymer form of glutaraldehyde [Fig. 8(c)].

Characteristic thiol stretching vibrations at 2590–2540 cm^{-1} were not present in either the PE–SH or PE–polySH films because of a combination of factors: the deep sampling depth of ATR–FTIR, which approaches 1 μm, and the low concentration (picomolar) of thiols on the polymer surface. The ATR–FTIR spectrum of PE–polyCOOH had sharp absorption bands at the characteristic stretching and bending vibrations of carboxylic acids (1725–1700 and 1440–1395 cm^{-1}, respectively) as in the PE–COOH spectrum. It also possessed a broad carboxyl absorption band at 3100–2900 cm^{-1}, but with greater intensity than in PE–COOH, as expected. An ester stretching vibration at 1300–1160 cm^{-1} was also present on the PE–polyCOOH, but not PE–COOH, spectrum, an attribute of the polyfunctional nature of poly(acrylic acid). As in the PE–OH spectrum, the PE–polyOH spectrum showed a broad band at the hydroxyl group stretching vibration (3400–3200 cm^{-1}), but with greater intensity, which supports the dye-assay indication that more ethanolamine conjugated to PE–polyCOOH films than to PE–COOH films (Table II). In addition, the characteristic amide band at 1680–1630 cm^{-1} was more pronounced than in PE–OH, representing the numerous amide bonds in the underlying poly(acrylic acid)/PEI linkage. Again, a loss in carboxyl band intensity and a gain in amide band intensity point to a covalent linkage between ethanolamine and PE–polyCOOH. The ATR–FTIR spectrum for PE–CHO films had aldehyde and secondary amine absorption bands similar to those found in the PE–polyCHO spectrum, as well as bands indicative of the presence of polymerized glutaraldehyde (hydroxyl, alkene, and ether), but at lower intensity, as expected.

XPS analysis

The high carbon and low oxygen contents of the unmodified PE suggest a clean surface (Table III). PE–COOH films saw a marked increase in the oxygen percentage, as expected from the oxidation. The increase in nitrogen and corresponding decrease in oxygen and carbon of PE–polyNH$_2$ films, in comparison with PE–COOH films, confirms the addition of PEI to the surface. Likewise, PE–NH$_2$ films had an increase in nitrogen and decrease in oxygen and carbon versus PE–COOH, but to a lesser extent, as expected. Conjugation of the ethanolamine to PE–COOH resulted in slight increases in nitrogen, from the introduction of the amide linkage, and carbon, from the aliphatic tether, and a corresponding decrease in oxygen. The marked increase in oxygen

TABLE III
Atomic Compositions of Control and Modified PE Films as Determined by XPS[a]

Film sample	O 1s	N 1s	C 1s	S 2p
PE	1.2	–	98.7	–
PE–COOH	7.6	0.3	91.4	0.1
PE–polyNH$_2$	6.0	5.7	87.6	0.1
PE–NH$_2$	6.2	1.6	91.1	0.2
PE–OH	6.2	1.5	92.0	–
PE–polyCHO	24.7	0.1	74.7	0.1
PE–polySH	6.4	6.8	84.8	1.4
PE–polyCOOH	16.4	5.9	77.3	0.1
PE–polyOH	15.8	8.7	74.9	0.1
PE–CHO	11.9	1.0	86.8	–
PE–SH	5.6	1.5	92.3	0.4

[a] Silicon, sodium, and chlorine were contaminants present on films at levels less than 1.0% and accounted for film sample atomic compositions totaling less than 100.0%. Low levels of sulfur (<0.2%) on nonthiolated films were also attributed to contamination.

after conjugation of glutaraldehyde to PE–polyNH$_2$ films, along with the corresponding decrease in nitrogen and carbon, confirms the findings from ATR–FTIR analysis, that the high concentration of glutaraldehyde used in the conjugation buffer fostered polymerization, which in turn resulted in a more complex crosslinker structure and therefore a thicker tether layer than the one indicated in Figure 2. After covalent immobilization of Traut's reagent to PE–polyNH$_2$ films, the atomic composition (%) of both sulfur and nitrogen increased, as predicted from the cyclic imidothioester structure of Traut's reagent. Conjugation of poly(acrylic acid) to PE–polyNH$_2$ films resulted in a marked increase in oxygen, as expected, as well as a slight decrease in carbon. As with the difference between PE–COOH and PE–OH films, PE–polyOH films had a slight decrease in oxygen and slight increase in nitrogen when compared to PE–polyCOOH films. Again, these changes confirm the immobilization of ethanolamine to the modified surface. Likewise, PE–CHO films had higher oxygen and lower nitrogen than PE–NH$_2$ films; this was similar to what was observed between PE–polyNH$_2$ and PE–polyCHO films, but on a smaller scale. Finally, the atomic composition of PE–SH films confirmed the introduction of Traut's reagent via the increase in sulfur, but to a lesser extent than that observed in PE–polySH films. This contrasts with the dye-assay result, which indicates more thiols on PE–SH than PE–polySH films. It is possible that the more flexible PEI linkage allows the formation of disulfide bonds in the PE–polySH films, thus reducing the available thiol groups.

CONCLUSIONS

We have demonstrated the ability to tailor the chemical functionality of the surface of low-density PE in both type and quantity of functional group, without affecting the desirable bulk material properties. Using a combination of spectral (XPS and ATR–FTIR) and chemical (dye assay and contact angle) surface analytical tools, we were able to characterize the conjugation chemistries as affected by pH, time, and molar excess factor and confirm the presence and quantity of the desired functional group at each step in the modification process. Reactive functional groups important to bioconjugation chemistry, including primary amine, carboxylic acid, hydroxyl, thiol, and aldehyde functionalities, were each introduced to the surface of PE film. The low deviation observed in dye-assay and contact-angle results suggests a uniform surface coverage of the desired functionalities. By varying reaction conditions, we were able to change the quantity of a given functional group, ranging from 10 pmol/cm^2 to 10–20 nmol/cm^2. The contact angles of the PE surfaces ranged from 10 to 100°, suggesting that in addition to generating specific functional groups, these surface-modification chemistries could be used to create surfaces with defined hydrophilicity. By taking such a systematic approach to polymer surface functionalization, we have developed surfaces to which a number of bioactive compounds can be covalently linked for applications in biomedicine, bioanalytical assays, antimicrobial surfaces, and active food packaging. Forthcoming research focuses on using dry chemical techniques such as plasma oxidation and ozone/UV irradiation for initial oxidation.

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