Surface Reduction of Poly(aryl ether ether ketone) Film: UV Spectrophotometric, ^{3}H Radiochemical, and X-ray Photoelectron Spectroscopic Assays of the Hydroxyl Functions

Olivier Noiset,[†,‡] Catherine Henneuse,[†] Yves-Jacques Schneider,[‡] and Jacqueline Marchand-Brynaert*[,†]

Laboratoire de Chimie Organique de Synthèse and Laboratoire de Biochimie Cellulaire, Département de Chimie, Bâtiment Lavoisier, Université Catholique de Louvain, Place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium

Received March 11, 1996; Revised Manuscript Received October 16, 1996[⊗]

ABSTRACT: The surface reduction of amorphous poly(aryl ether ether ketone) (PEEK) film was successfully achieved by wet chemistry using a solution of $NaBH_4$ in DMSO at 120 °C for 3 h. The resulting PEEK-OH film was fully characterized by MIR, UV−visible, and ^{1}H NMR spectroscopies; all the data were consistent with those of the references, 4-(4-methoxyphenoxy)benzhydrol and bulk-reduced PEEK ("PEEK-OH"). The surface of PEEK-OH film was analyzed by X-ray photoelectron spectroscopy (XPS). From the fine structures of the C1s and O1s peaks, we could determine a ratio of reduction reaching 75−85% of the monomer units contained in the 10 outermost atomic layers. The surface reactivity of the hydroxyl groups was assayed by derivatization with [^{3}H]acetic anhydride followed by liquid scintillation counting (LSC) of the sample-associated radioactivity. The PEEK-OH film was reacted with *p*-nitrophenyl chloroformate to furnish an activated surface (PEEK-OCO_2PNP), the basic hydrolysis of which allowed the indirect spectrophotometric assay of the reactive OH groups. The PEEK-OCO_2PNP film was further used to covalently fix amine derivatives via a carbamate linkage. Using [^{3}H]lysine and trifluoroethylamine, we were able to assay the surface reactivity by LSC and XPS respectively. The ratios of surface derivatization were within 5−30%. The PEEK-OH film was used as substrate for the cultivation of CaCo2 epithelial cells; the presence of surface hydroxyl functions moderately improves the polymer biocompatibility.

Introduction

The use of synthetic materials for biomedical devices and biotechnological applications has increased considerably in recent years. Particularly, polymers possessing the appropriate mechanical and physical bulk properties make up the broadest and most diverse class of biomaterials,[1] *i.e.* materials designed and manufactured in order to be placed in contact with living tissues, cells, blood, or biological fluids. The biological interactions with synthetic polymers are strongly influenced, and even directed, by the chemical nature of the surface of the materials.[2] Therefore, the polymer surfaces are often purposely modified in order to improve their properties, or to achieve new properties.[3] However, predictive relationships that can be used in surface engineering to elicite the desired biological responses have yet not been established.[4]

Our research group is interested in the development of new substrates[5] for *in vitro* mammalian cell cultivation.[6] In this context, we decided to systematically investigate the role played by selected chemical motifs, exposed on the substrate surfaces, on the adhesion, proliferation, and differentiation of mammalian cells. Poly(aryl ether ether ketone) (PEEK), a highly resistant thermoplastic,[7] was chosen as reference substrate[8] in our study. The native PEEK film has been previously shown by one of us to be a poor substrate, extremely reluctant to allow cellular adhesion.[9] Therefore, the biological responses associated with each designed surface modification would be easily detected. Moreover, the absence of toxicity[10] as well as biological inertness of this polymer have been recently reported: in the field of medical engineering, carbon-fiber-reinforced PEEK gave rise to composites exhibiting excellent biomechanical compatibility with bone tissues.[11]

The subject of the present report is the controlled preparation, from the native PEEK film, of a new substrate for cell cultivation, the surface of which displays a high density of hydroxyl functions (substrate called PEEK-OH). This PEEK modification was designed in order (a) to increase the hydrophilicity of the substrate surface and, therefore, its capacity for adsorbing/desorbing proteins which mediate the cellular responses,[12] and (b) to introduce reactive anchorage points (the hydroxyl functions) for the covalent coupling of biologically active molecules susceptible to improving biocompatibility by interacting with specific cell receptors.[13] For this latter purpose, the usual spectroscopic techniques of surface analysis are only partially relevant to characterize the PEEK-OH samples. Thus we developed a series of complementary analyses, *i.e.* original surface reactivity assays[14] based on the derivatization with a spetrophotometric label, a ^{3}H-radioactive marker, or a fluorine-containing tag. We used the wet-chemistry technique (organic reactions carried out at the solid−liquid interface) for performing these covalent surface functionalizations under very mild conditions that mimick the situations likely to be encountered in the coupling of sensitive bioactive molecules. Accordingly, the analytical work presented in this paper, which characterizes the PEEK-OH surface in terms of usable chemical reactivity, establishes quantitative bases for the further development of biocompatibilization strategies.[15]

Experimental Section

Reagents and Solvents. Organic reagents (99+% purity) were purchased from Acros Chimica (Beerse, Belgium) or

* To whom correspondence should be addressed. Telephone: +32 10 47 27 46 or +32 10 47 27 40. Fax: +32 10 47 41 68.
† Laboratoire de Chimie Organique de Synthèse.
‡ Laboratoire de Biochimie Cellulaire.
⊗ Abstract published in *Advance ACS Abstracts,* January 15, 1997.

S0024-9297(96)00368-3 CCC: $14.00

Aldrich (Bornem, Belgium) and used as received. Inorganic reagents (analytical grade) were obtained from UCB (Braine-l'Alleud, Belgium) or Merck (Darmstadt, Germany) and used as received. The radiolabeled reagents were provided by Amersham (Little Chalfont, UK): the specific radioactivity of L-[4,5-^{3}H]lysine monohydrate in aqueous solution was 82 Ci/mmol; the activity of [^{3}H]acetic anhydride in toluene was 500 Ci/mmol. Dimethyl sulfoxide (DMSO), pyridine, and acetic anhydride were distilled before use. Acetone was dried over $CaCl_2$ and distilled. Methanol, ethanol, and isopropyl alcohol were dried over CaO and distilled. Benzene and toluene were dried over CaH_2 and distilled. Water (HPLC grade) was obtained with a Milli-Q system (Millipore, Bedford, MA). The phosphate buffered saline (PBS, pH 7.3) was prepared from NaCl (40 g), KCl (1 g), KH_2PO_4 (1 g), and $Na_2HPO_4 \cdot 2H_2O$ (7.1 g) dissolved in water (5 L).

Methods. Merck silica gel 60 (70−230 mesh ASTM) was used for column chromatographies; the R_f values were determined on Merck silica gel 60 TLC plates (F254, 0.2 mm of thickness) using I_2 vapor and UV lamp to make visible the spots. Melting points (Leitz microscope) are uncorrected. The IR spectra were taken with a Perkin-Elmer 681 instrument and calibrated with polystyrene (1601 cm^{-1}). The NMR spectra were recorded on Varian Gemini 200 and Bruker AM 500 spectrometers with tetramethylsilane as internal standard. The mass spectra were obtained with a Finnigan MAT-TSQ 70 spectrometer (EI mode, 70 eV). The UV−visible spectra were recorded with a Gilford Response S-200 instrument and printed with OKI Microline 192 Elite. The microanalyses were performed at the University College of London, Chemistry Department; the results indicated by the symbol of the elements were within ±0.3% of the theoretical values.

The contact angles of water were measured at room temperature using the sessile drop technique and an image analysis system (CCD camera of MXR 5010 type and contour processor PIO-12 with computer monitor 80 from Electronish Ontwerp Bureau De Boer, Holland). The values given in Table 2 are the average of 10 measurements. The surface IR spectra (MIR mode) were recorded on PE 580 and PE 1760 spectrometers using an optical deviation system from Perkin-Elmer and a thallium bromide−iodide crystal KRS-5 (incidence angle 45°); the instrument was coupled with a PE 3600 computer. The XPS spectra were obtained with a SSI X probe (SS-100/206) spectrometer from Fisons (Surface Science Laboratories, Mountain View, CA), equipped with an aluminum anode (10 kV, 17 mA) and a quartz monochromator. The direction of photoelectron collection made angles of 55° and 73° with the normal to the sample and the incident X-ray beam, respectively. The electron flood gun was set at 6 eV. The vacuum in the analysis chamber was 2.5×10^{-7} Pa. The binding energies of the peaks were determined by setting the C1s component due to carbon only bound to carbon and hydrogen at a value of 284.8 eV. The peak areas were determined with a nonlinear background subtraction. Intensity ratios were converted into atomic concentration ratios by using the SSI ESCA 8.3 D software package. The peaks were curve fitted using a nonlinear least-squares routine and assuming a Gaussian/Lorentzian (85/15) function. The amounts of radiolabeled molecules associated with the PEEK disks were measured by liquid scintillation counting (LSC). The disks were individually placed in 20 mL polyethylene vials (Milli-Q 20, Packard, San Diego, CA) and 5 mL of Aqualuma or Lipoluma cocktails (Lumac, Basel, Switzerland) were added in each vials. A Tri-Carb 1600 TR liquid scintillation analyzer (Packard) was used. The experimental counts per minute (cpm) were converted in disintegrations per minute (dpm) using the relationship dpm = cpm/counting efficiency.[5,14] The results are expressed in picomole per surface unit (cm^2); each value is the average of, at least, four independent measurements performed with four samples similarly treated. Since the PEEK samples are totally insoluble in the scintillation cocktails, surface quenching could probably occur, leading to some underevaluation of the counting; the corrective factor should be 2, as a maximum.

Model Chemistry. a. 4-(4-Methoxyphenoxy)benzophenone (1). A solution of 4-fluorobenzophenone (8 g, 0.04 mol) and potassium 4-methoxyphenoxide (8.4 g, 0.05 mol) in DMSO (50 mL) was refluxed for 3 h. After cooling, the mixture was poured into water (500 mL) under stirring. The precipitate was filtered off and washed twice with water, then twice with methanol. Drying under vacuum gave crude **1** (10.44 g, 84% yield) which was recrystallized from isopropyl alcohol: mp 106.3°C; R_f (SiO_2, benzene−MeOH 95:5) = 0.7; IR (KBr) ν 3058, 3051, 2988, 1655 (carbonyl), 1597, 1499, 1273 (ether), 1233, 845, 769 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.81 (s, 3H), 6.90−7.08 (m, 6H), 7.40−7.63 (m, 3H), 7.70−7.85 (m, 4H); ^{13}C NMR (125 MHz, CF_3SO_3D) δ 61.05, 117.33, 117.92, 121.68, 121.84, 128.76, 128.89, 132.90, 138.55, 140.18, 150.06, 151.00, 170.29, 201.35; UV (CF_3SO_3H) λ_{max} 380 nm (ϵ = 42 100 M^{-1} cm^{-1}); MS(EI) *m/e* 304. Anal. $C_{20}H_{16}O_3$ (C, H).

b. 4-(4-Methoxyphenoxy)benzhydrol (2). To a solution of **1** (0.5 g, 1.7 mmol) in ethanol (30 mL) was added sodium borohydride (0.07 g, 1.8 mmol). The mixture was refluxed under stirring for 1 h. After cooling, ammonium chloride (4 equiv) was added. After filtration, the solvent was removed under vacuum; the residue was dissolved in dichloromethane (30 mL) and washed with water. Drying over $MgSO_4$ and concentration gave crude alcohol **2** (442 mg, 85% yield) as an oil which was purified by column chromatography on silica gel: R_f (SiO_2, benzene−MeOH 95:5) = 0.6; IR (film) ν = 3400 (br, OH), 1010 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.95 (br s, OH), 3.80 (s, 3H), 5.80 (s, 1H), 6.8−7 (m, 6H), 7.2−7.4 (m, 7H); ^{1}H NMR (500 MHz, CF_3SO_3D) δ 8.68 (s, 1H, Ar*CH*$^+$Ar); ^{13}C NMR (125 MHz, CF_3SO_3D) δ 59.08, 124.99, 127.23, 132.69, 134.46, 135.84, 138.85, 143.24, 146.79, 147.88, 148.62, 159.07, 179.95, 190.52 (Ar*CH*$^+$Ar); UV (CF_3SO_3H) λ_{max} 472 nm (ϵ = 70 700 M^{-1} cm^{-1}); MS (EI) *m/e* 306. Anal. $C_{20}H_{18}O_3$ (C, H).

Polymer Sample. Amorphous PEEK film was received from ICI (UK); the sample was Stabar K200 (reference number K0310902; thickness of 25 μm). The contact angle of water was 71.2° (± 1.5°), and XPS analysis revealed an atomic O/C ratio of 0.20 (theoretical value for $C_{19}H_{12}O_3$: O/C = 0.158). This results from an industrial surface treatment by corona discharge. Native PEEK film was obtained as follows: (i) immersion of Stabar K200 in refluxing acetone for 48 h; (ii) rinsing twice with acetone; (iii) drying under vacuum (1 mmHg) for 3 h at 60 °C. After this treatment, the contact angle of water was 89° (±1°).[16] From XPS analysis we found an atomic O/C ratio of 0.148, close to the theoretical value.[17] The MIR spectrum showed a carbonyl stretching at 1650 cm^{-1}.[18] The ^{1}H NMR spectrum recorded in CF_3SO_3D solution at 500 MHz showed two sharp multiplets at 6.48 δ and 7.33 δ, in the ratio 2:1, corresponding to the protons in ortho position regarding the ether bonds and to the protons in ortho position regarding the carbonyl function, respectively. The λ_{max} observed in CF_3SO_3H solution was at 420 nm (ϵ = 57 500 M^{-1} cm^{-1}) and attributed to the protonated benzophenone moiety.

Surface Chemistry. The small film samples (disks of 1.2 or 2.4 cm in diameter) were put into a round-bottomed flask containing the reactive solution (5−10 mL per sample) and vigorously stirred with a magnetic barrel. The two faces of the samples are thus equally treated.

The large film samples (rectangles of 30 cm in length and 15 cm in width) were fixed on a homemade glass cylinder (height 16 cm; diameter 8.5 cm) and placed into a 1.5 L wide-neck (diameter 12 cm) reaction flask (Sovirel glassware) containing the reactive solution (1−1.2 L). The neck was fitted, with a large diameter flange joint, in a lid with a two-socket neck equipped with a reflux condenser and a drying tube. The two faces of the sample are not strictly similarly treated; the external side of the film was marked and considered for the surface analyses (MIR, XPS, contact angle with water). After chemical treatment and suitable rinsing, the film samples were dried under vacuum ($P \leq 1$ mmHg) at 60 °C. We used a Gallenkamp oven and an Edwards E2M5 pump, connected through two vapor condensers cooled with liquid air.

a. PEEK Film Reduction. DMSO (1.2 L) and sodium borohydride (2.4 g) were introduced in the reaction flask and heated at 120 °C under stirring (dissolution occurred). The film sample fixed on the glass cylinder was totally immersed into the reactive solution for 3 h at 120 °C, and then removed

from the support and rinsed, successively with methanol (15 min), water (10 min), 0.5 N HCl (10 min), water (10 min), and ethanol (10 min). After drying under vacuum (3 h, 60 °C), the sample (PEEK-OH) was stored, in the dark, in a polystyrene box (damp and dust proof).

b. PEEK-OH Film Activation. Toluene (50 mL) and *p*-nitrophenyl chloroformate (1 g) were introduced into a 100 mL flask, under argon atmosphere. The solution was heated at 50 °C, and then 5−8 disks cut from the previous PEEK-OH sample were immersed and stirred for 3 h at 50 °C. The disks were taken off with tweezers and rinsed, successively with toluene (20 min) and acetone (5 min). The samples (PEEK-OCO_2PNP) were directly used in the reactivity assays.

PEEK Bulk Reduction. DMSO (200 mL) and sodium borohydride (0.8 g) were introduced in a 500 mL round-bottomed flask equipped with a reflux condenser and a drying tube. The mixture was heated, under stirring, at 120 °C (dissolution occurred). PEEK film cut in small pieces (0.573 g) was added. The mixture was stirred for 3 days at 120 °C (dissolution occurred). After cooling, the crude solution (PEEK-ONa) was slowly poured into 0.05 N HCl (1.2 L); a white solid was formed. After filtration and washing with water (3 times), the solid "PEEK-OH" was dried under vacuum at 60 °C for 8 h, yield 0.429 g (75%).

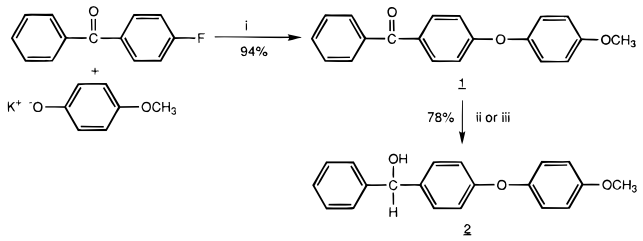
Reactivity Assays of Surface Hydroxyl Groups. a. Indirect Spectrophotometric Assay. Disk samples of 2.4 cm in diameter were cut from PEEK-OH film and activated with $ClCO_2PNB$ as described above. The samples (PEEK-OCO_2PNB) were individually hydrolyzed by immersion in 0.1 M NaOH (2.8 mL) for 24 h at 20 °C. The liquid phase was analyzed by UV−visible spectrophotometry between 350−600 nm (λ_{max} 405 nm; $\epsilon = 18\,000\ M^{-1}\ cm^{-1}$ for $PNBO^-$).

b. Radiochemical Assays. Direct Method. To a 10^{-2} M solution of acetic anhydride in toluene (30 mL) were added ^{3}H-labeled acetic anhydride (5 μL, specific activity 500 Ci/mmol) and pyridine (10 μL) as catalyst. PEEK-OH disks of 1.2 cm in diameter were individually treated by immersion in the previous solution distributed in small tubes equipped with tight stoppers (3 mL per tube). The samples were shaken with an Edmund Bühler stirrer (model KL-2) for 5 h at 20 °C, then taken off the reactive solution with tweezers, and rinsed as follows: successive treatment with toluene (3 mL per sample) under ultrasonication (15 min), acetone (3 mL per sample) under ultrasonication (15 min), and PBS buffer containing 0.1% of Triton X 100 (5 mL per sample) under ultrasonication (15 min). Sonication was effected in a Branson 5210 cleaning bath, at 50−60 Hz. The samples were then immediately placed in aqualuma (5 mL per tube) for LSC measurements. Blank samples were prepared from commercial PEEK samples treated as above; in this case, LSC assay gave the ratio of nonspecific adsorption of the radioactive label. This value has to be subtracted from the values found for the PEEK-OH samples.

Indirect Method. To a 10^{-3} M solution of lysine in PBS buffer (20 mL) was added ^{3}H-labeled lysine (50 μL, specific activity 83 Ci/mmol). PEEK-OCO_2PNB disks of 1.2 cm in diameter were individually treated by immersion in the previous solution distributed in small tubes (2.5 mL per tube). The samples were shaken (Edmund Bühler) for 3 h at 20 °C, then taken off the reactive solution with tweezers, and drained over filter paper. The samples were rinsed three times with PBS buffer containing 0.1% of Triton X 100 (2 mL per sample, 10 min shaking) and placed in aqualuma (5 mL per tube) for LSC measurements. Blank samples were prepared from unactivated PEEK-OH samples treated as above (measurements of the ratio of nonspecific lysine adsorption).

c. Indirect XPS Assay. A solution of 2,2,2-trifluoroethylamine (0.44 mL) in DMSO (49.5 mL) was placed in a 100 mL round-bottomed flask under argon atmosphere. Disks of PEEK-OCO_2PNB (3−5 samples of 1.2 cm in diameter) were immersed and magnetically stirred for 5 h at 20 °C. The samples were taken off with tweezers and rinsed as follows: treatment with DMSO (20 mL) for 10 min, and treatment with methanol (20 mL) for 10 min, three times. After drying under vacuum (3 h, 60 °C), the samples were analyzed by XPS. Blank samples were prepared by treatment of unactivated PEEK-OH samples as above. XPS analysis of PEEK-CF_3: 82.18% C (≈285 eV), 14.20% O (≈533 eV), 2.68% F (688.2 eV), 0.95% N (399.6 eV).

Scheme 1. Synthesis of the Model Compounds[a]



[a] Reagents and conditions: (i) DMSO, 100 °C, 72 h; (ii) $NaBH_4$, EtOH or DMSO, 60 °C, 1 h; then NH_4Cl; (iii) $LiAlH_4$, THF, reflux, 1 h; then NH_4Cl and chromatography.

Table 1. IR, ^{1}H NMR, and UV Spectroscopic Data

sample	IR (C=O str.) (KBr or film), cm^{-1}	^{1}H NMR (Ar*CH* + Ar) (CF_3SO_3D, 500 MHz), δ	UV−vis (CF_3SO_3H or H_2SO_4 95%) λ_{max}, nm	Δ, nm	ϵ, $M^{-1}\ cm^{-1}$
compound **1**	1655		380		42 100
compound **2**		8.66 (singlet)	470	+90	70 700
PEEK[a]	1650		420		57 500
"PEEK-OH" [b]		8.35 (singlet)	520	+100	78 500

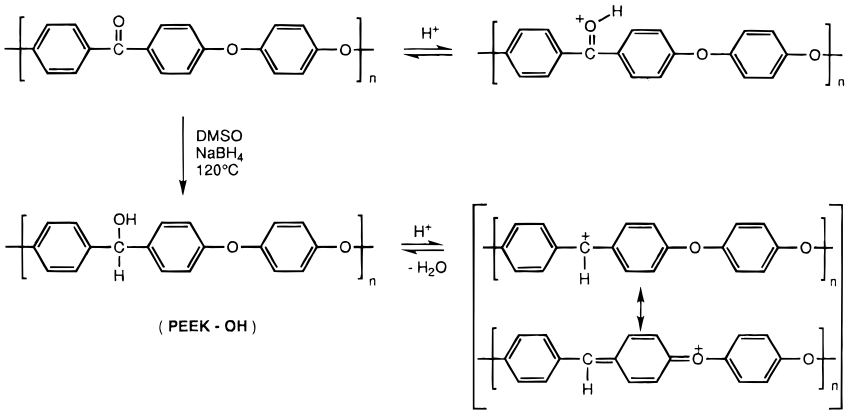
[a] Stabar K 200 film. [b] PEEK totally reduced ($NaBH_4$, DMSO, 120 °C, 3 days).

Cell Cultivation.[6a] CaCo2 cells (American type culture collection, Rockville, MD) have been previously adapted in synthetic, serum-free medium and are routinely maintained by repetitive subcultivation in flasks (Falcon, Becton Dickinson Labware, Lincoln Park, NJ). The synthetic medium is the basal defined medium (BDM) which is prepared on a customery basis by Gibco (Life Technologies Ltd, Paisley, UK). The CaCo2 cells were cultured in cell culture inserts (Falcon 3090, Becton Dickinson Labware) adapted for six-well microplates containing the sterilized polymer film (5 cm^2 of growth area) as the substrate. The tested substrates were native PEEK, PEEK-OH, TCPS (tissue culture polystyrene from Nunclon, Roskilde, Denmark), and PET-s (sulfonated membrane of poly(ethylene terephthalate) from Cyclopore, Louvain-la-Neuve, Belgium). Cells were inoculated with a density of 10^5 cells/cm^2 in 2.5 mL of BDM containing 1% of fetal calf serum (FCS), and incubated at 37 °C in a water-saturated air and 5% CO_2 atmosphere. Adhesion and cell growth were monitored as a function of incubation time by microscope examination and by determination of the cellular protein content. For the latter, the culture medium was removed and the inserts were washed three times with phosphate-buffered saline (PBS). The membranes of the adherent cells were solubilized by a detergent (2 × 1 mL of 1% sodium deoxycholate, adjusted to pH 11.3 with NaOH). The proteins were assayed by the Lowry method[19] with bovin serum albumin (BSA) as standard. The results of Figure 4 are expressed in micrograms of proteins per centimeter^2 of substrate. They are the average of three independent experiences.

Results and Discussion

Bulk functionalized PEEK samples have been previously obtained, either by using functionalized monomers in the polymer synthesis,[20−23] or by modifying the preformed polymer. However, this second method is practically limited to the sulfonation[24] due to the PEEK insolubility in usual organic solvents. Surface functionalized PEEK samples have been prepared by several plasma treatments[25] and by photooxidation.[26] The wet-chemistry technique has been illustrated by the work of McCarthy;[16] we selected this mild method for per-

Scheme 2. PEEK Reduction and Dissolution in Strong Acids: Direct Spectroscopic Assays (UV, ^{1}H NMR) of the Hydroxyl Functions (Total Sample Analysis)



forming specific chemical transformations leading to surfaces with well-defined chemical composition.

The surface hydroxylation of semicrystalline PEEK films has been performed by the reduction of some benzophenone motifs into the corresponding benzhydrol motifs, using sodium bis(2-methoxyethoxy)aluminum hydride as reducing agent.[16] As for us, starting from an amorphous PEEK film, we were able to reach very high levels of surface hydroxylation, by reduction with sodium borohydride under various conditions. The resulting PEEK-OH samples were characterized, after dissolution in strong acids, by UV and ^{1}H NMR spectroscopies; the recorded data were fully consistent with those of two references: the 4-(4-methoxyphenoxy)-benzhydrol and the bulk-reduced PEEK (called "PEEK-OH"). The surfaces of the PEEK-OH samples were further analyzed by contact angle measurements, and by the MIR and X-ray photoelectron spectroscopies. Finally, the reactivity of the hydroxylated surfaces was assayed by several direct and indirect methods.

Preparation of References. In order to record useful spectral references, a model study was carried out, with 4-(4-methoxyphenoxy)benzophenone (**1**) considered as the simplest small molecule to be representative of the PEEK monomer unit. This compound was easily obtained by aromatic nucleophilic substitution using potassium 4-methoxyphenoxide and 4-fluorobenzophenone as partners. Treatment of **1** with $NaBH_4$ in ethanol or DMSO at 60 °C, or with $LiAlH_4$ in refluxing THF led to the benzhydrol derivative **2** (Scheme 1). The IR spectrum of **2** showed a broad absorption band near 3400 cm^{-1} (O−H stretching) and the total disappearance of the carbonyl band of the precursor **1** at 1655 cm^{-1}. In the ^{1}H NMR spectrum, recorded in trifluoromethanesulfonic acid, the C−H benzhydryl proton gave a resonance line at 8.6 δ, typical of the carbocationic species Ar−*CH*$^+$−Ar.[27] The UV−visible spectrum, recorded in strong acids, showed an absorption at 470 nm (deep cherry-red coloring of the solution), confirming the formation of a highly stabilized cation[28] by dehydration of the protonated alcohol **2**. In the same conditions, the precursor **1** gave an absorption at 380 nm, corresponding to the protonated ketone species (yellow-orange coloring of the solution) (Table 1).

The PEEK film, cut into small pieces, was treated with a saturated solution of $NaBH_4$ in DMSO at 120 °C; complete dissolution of the polymer occurred after 3 days of reaction. Precipitation from diluted HCl furnished the bulk-reduced PEEK (called "PEEK-OH"). This new material was analyzed by IR, ^{1}H NMR, and UV−visible spectroscopies, and compared to the model compound **2** (Table 1). The PEEK carbonyl band at 1650 cm^{-1} was no more longer in the IR spectrum of "PEEK-OH"; new bands appeared, at 3300 (broad) and 1010 cm^{-1}, attributed to the O−H and C−O stretchings, respectively. The ^{1}H NMR spectrum showed the typical singlet at 8.35 δ due to the benzhydryl proton. Finally, the UV−visible spectrum gave a strong absorption at 520 nm corresponding to the stabilized benzhydryl cation (Scheme 2), while the native PEEK absorbed at 420 nm; a bathochromic effect of about 100 nm was observed, as in the case of the model compound **2**. The same UV−visible values were obtained when measuring the absorbance in CF_3SO_3H or H_2SO_4 95% solutions;[29] for economical reasons, this last solvent was routinely used for the analysis of PEEK samples.

Thus, having in hands practical spectroscopic tools (UV, NMR) that allow a rapid analysis of PEEK samples, we were ready to investigate various experimental conditions for selective surface reduction of the

Table 2. Advancing Contact Angles (Water) for Surface-Modified PEEK Films

	sample	θw
1	PEEK[a]	89° (± 1)
2	PEEK-corona[b]	71.2° (± 1.5)
3	PEEK-OH	76.5° (± 1.4)
4	PEEK-CF_3	87.4° (± 1.1)

[a] Commercial film washed in hot acetone = native PEEK. [b] Commercial film (Stabar K200) corona treated.

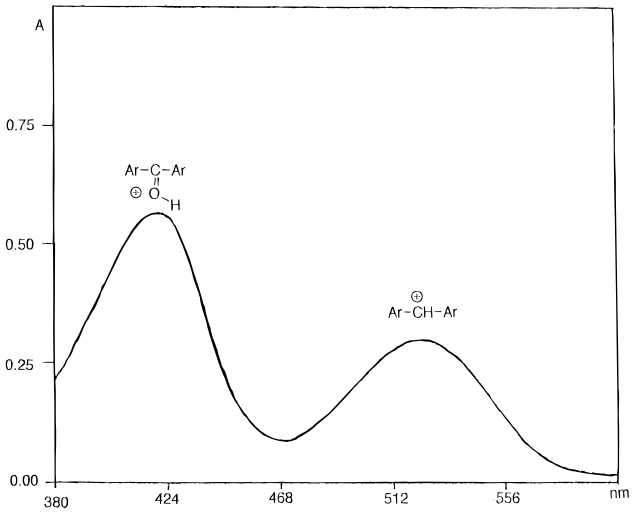


Figure 1. UV−visible spectrum of PEEK-OH film dissolved in H_2SO_4.

Table 3. PEEK-OH Assays

	method	analyzed sample (film)	experimental results	% of function-alization	depth of analysis
1	UV (direct method by dissolution in CF_3SO_3H)	PEEK-OH	λ_m: 520 and 420 nm	7%	total sample
		PEEK	λ_m: 420 nm		
2	^{1}H NMR (direct method by dissolution in CF_3SO_3D)	PEEK-OH	δ: 8.3 ppm (singlet)	8%	total sample
		PEEK	no aliphatic proton		
3	MIR (direct method)	PEEK-OH	ν 1650 cm^{-1}/ν 1490 cm^{-1}: 0.55	18%	1−10 μm
		PEEK	ν 1650 cm^{-1}/ν 1490 cm^{-1}: 0.67		
4	XPS (direct method)	PEEK-OH	*C*═O/*C*−O: 0.0545	74%	50−100 Å
		PEEK	*C*═O/*C*−O: 0.2381		
5	LSC (direct derivatization method by fixation of [^{3}H]Ac_2O)	PEEK-OH	[Ac]: ∼ 1050 pmol/cm^2	48%	∼100 Å
		PEEK	[Ac]: ∼ 25 pmol/cm^2		
6	UV (indirect method measuring [$PNPO^-$] in the hydrolysis solution)	PEEK-OCO_2PNP	[$PNPO^-$]: ∼ 645 pmol/cm^2	30%	∼100 Å
7	LSC (indirect derivatization method by fixation of [^{3}H]lysine)	PEEK-OCO_2PNP	[Lys]: ∼ 120 pmol/cm^2	5%	∼100 Å
		PEEK-OH	[Lys]: ∼ 10 pmol/cm^2		
8	XPS (indirect derivatization method by fixation of fluorinated tag $NH_2CH_2CF_3$)	PEEK-OCO_2PNP	F/C: 0.0326; N/C: 0.0115	22%	50−100 Å
		PEEK-OH	F/C: 0; N/C: 0		

Table 4. XPS Analyses of PEEK Samples

		atomic concentration (%)					
		PEEK[a]		"PEEK-OH" [b]		PEEK-OH[c]	
binding energy (eV)	attribution	relative composition	% element	relative composition	% element	relative composition	% element
284.8	C1s **C**−C, **C**−H	70.5		67.8		67.7	
286.4	C1s **C**−O	16.8	87.13	23.7	84.81	22.0	85.16
287.1	C1s **C**═O	4.0		0.0		1.2	
291.5−291.7	C1s π	8.7		8.5		9.2	
531.3−531.7	O1s **O**═C	31.6		0.0		4.1	
532.4	O1s **O**−H	0.0	12.87	19.8	15.19	17.7	14.84
533.3−533.4	O1s **O**−C	68.4		80.2		72.7	
539.7−539.9	O1s π	0.0		0.0		5.8	

[a] Film of Stabar K 200 washed in hot acetone. [b] Pellet of PEEK totally reduced ($NaBH_4$, DMSO, 120 °C, 3 days). [c] Film of PEEK partially reduced on the surface ($NaBH_4$, DMSO, 120 °C, 3 h).

amorphous film. Indeed, the percentages of reduction with respect to the bulk could be determined from the ^{1}H NMR spectra in CF_3SO_3D, considering the benzhydryl proton integration (reduced units contribution) toward the aromatic protons integration (PEEK and PEEK-OH contributions). Similarly, from the UV−visible spectra in H_2SO_4 95%, using the Lambert−Beer equation, we could readily determine the ratio of reduced units (λ_{max} 520 nm; ϵ = 78 500 M^{-1} cm^{-1}) with regard to the unmodified units (λ_{max} 420 nm; ϵ = 57 500 M^{-1} cm^{-1}). Generally, both analyses gave very close results.

PEEK Film Reduction. Small disks of PEEK film (1.2 cm in diameter) were immersed and shaken into saturated solutions of inorganic hydrides. $LiAlH_4$ (0.5−1 M) in THF (20−70 °C, several hours) was rapidly abandoned because this reducing mixture caused the whitening and curling of the film samples. The reaction with $NaBH_4$ was examined in ethanol, isopropyl alcohol, and DMSO−ethanol (2:1), at 20 °C and 80 °C during 1−24 h, and in DMSO at 100 °C and 120 °C, during 1−5 h. The higher percentages of reduction (>5%; UV and ^{1}H NMR analyses) were obtained in hot DMSO: this nonprotic polar solvent offers good wetting properties for interacting with the PEEK film and allows the hydride dissolution (2.3 g/L at 120 °C). The selected conditions for large surface reduction (30 cm × 15 cm)[30] were the reaction with $NaBH_4$ in DMSO (2 g/L), at 120°C for 3 h; this was found to be the best compromise between efficient surface hydroxylation and modified interface dissolution. After this wet-chemistry treatment, the polymer film remained soft and smooth, and perfectly transparent;[31] this material was called PEEK-OH. According to our standard procedure, several samples of PEEK-OH film (30 cm × 15 cm) were prepared and analyzed as described in the following sections: all the results were reproducible within the limits of experimental errors.

PEEK-OH Film Analyses. The presence of surface hydroxyl groups significantly enhanced the PEEK-OH film wettability; its contact angle with water was 76.5° (Table 2, entry 3).

The ratio of reduced units with regard to the PEEK-OH total sample, determined by UV−visible (Figure 1) and ^{1}H NMR analyses, was around 7−8% (Table 3, entries 1 and 2); clearly, the modification has affected a relatively deep interface domain. This was confirmed by the MIR analysis which normally investigates a sample depth of about 5 μm. Two typical bands were considered in the MIR spectrum[18] of the native PEEK: the band at 1650 cm^{-1} due to the carbonyl function and the band at 1490 cm^{-1} due to the aromatic rings. Their relative intensity ratio was 0.67. After reduction, the PEEK-OH sample still showed the MIR band at 1490 cm^{-1} which is constant, but a diminution of the carbonyl band at 1650 cm^{-1} was clearly visible; the intensity ratio ν 1650 cm^{-1}/ν 1490 cm^{-1} became 0.55, corresponding to about 82% of remaining carbonyl groups in the explored domain. Accordingly, the ratio of reduction should be around 18% (Table 3, entry 3).

The surface of the PEEK-OH film was further analyzed by X-ray photoelectron spectroscopy (XPS), and compared to the native PEEK film[17] and the totally reduced material "PEEK-OH" (Table 4). The fine structure of the C1s peak (Figure 2) showed a significant diminution of the *C*═O component at 287.1 eV and a related increase of the *C*−O component at 286.4 eV. The experimental *C*═O/*C*−O atomic ratio was 0.0545; for the native PEEK, this ratio was 0.238 (theoretical value = 0.25). The "PEEK-OH" reference showed no *C*═O

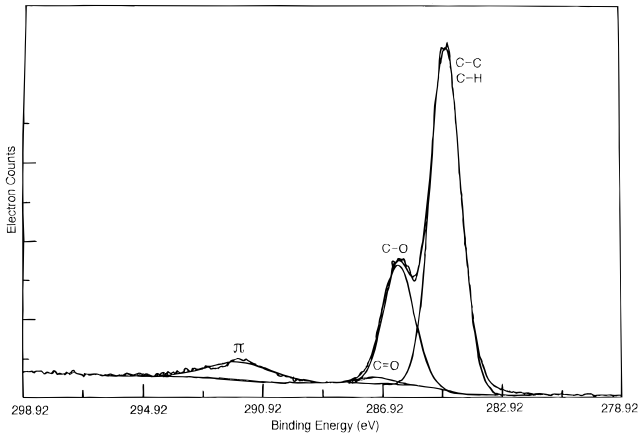


Figure 2. XPS analysis of PEEK-OH film: C1s peak.

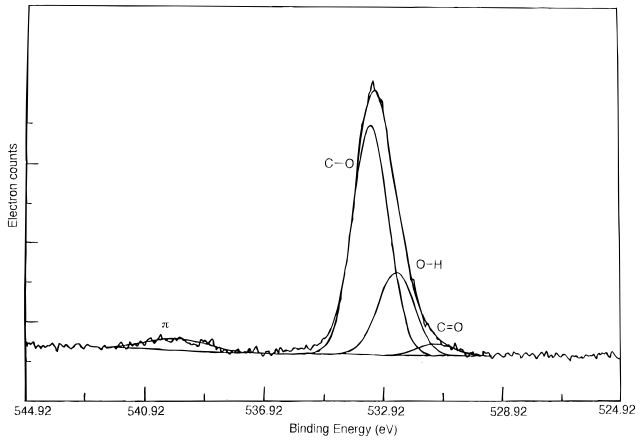


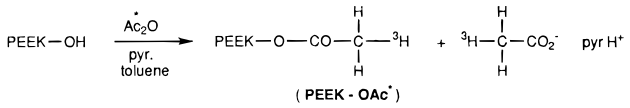
Figure 3. XPS analysis of PEEK-OH film: O1s peak.

component (*C*═O/*C*—O atomic ratio = 0), and the *C*—O contribution of both the ether and alcohol moieties appeared at 286.4 eV.[32] From the experimental *C*═O/*C*—O atomic ratio, we calculated[33] that 74% of the monomer units have been reduced in the explored domain, *i.e.* about 50−100 Å (corresponding to roughly 10 atomic layers). The fine structure of the O1s peak (Figure 3), in the PEEK-OH XPS spectrum (Table 4), revealed an important diminution of the *O*═C component at 531.5 eV, an increase of the *O*—C component at 533.3 eV, and the appearance of a new component at 532.4 eV attributed to the OH alcohol function.[32] This component was absent in the native PEEK spectrum, but clearly visible in the "PEEK-OH" reference (in which the *O*═C component was totally absent). The experimental atomic ratio *O*═C/(O—C + *O*—H) was 0.0453 for the PEEK-OH film. From that, we calculated[34] that 87% of the monomer units have been reduced, a value consistent with the previous one obtained by the C1s peak analysis. Considering the atomic ratio *O*═C/(*O*═C + *O*—H) = 0.1881, we concluded that the PEEK-OH sample contained about 19% of residual carbonyl functions. Thus, depending on the calculation method, we could established that the surface wet chemistry has affected 75−85% of the PEEK carbonyl functions contained in the 10 outermost atomic layers.

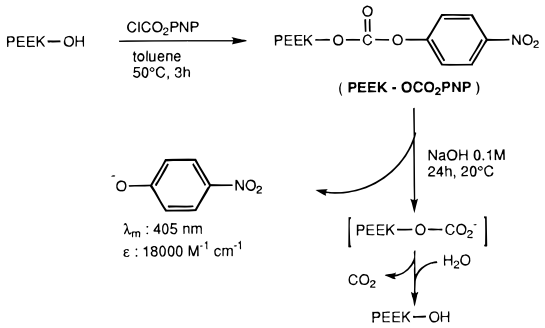
Reactivity Assays. Chemical derivatizations of PEEK-OH, based on acylation reactions, were undertaken using labeled reagents or spectroscopic tags in order to quantify the surface reactivity.

The PEEK-OH (disks of 1.2 cm in diameter) was treated by immersion and shaking into a 10^{-2} M solution of [^{3}H]acetic anhydride and pyridine in toluene

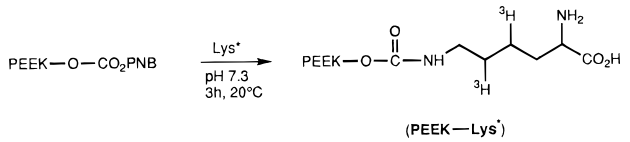
Scheme 3. PEEK-OH Acylation with [^{3}H]Acetic Anhydride for Direct Radiochemical Assay of the Reactive Hydroxyl Functions Displayed on the Film Surface



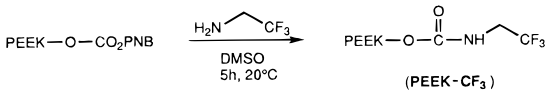
Scheme 4. PEEK-OH Activation and Indirect Spectrophotometric Assay of the Reactive Hydroxyl Functions Displayed on the Film Surface



Scheme 5. Coupling to L-[4,5-^{3}H]Lysine for Indirect Radiochemical Assay of the Reactive Hydroxyl Functions Displayed on the Film Surface



Scheme 6. Coupling to Trifluoroethylamine for Indirect XPS Assay of the Reactive Hydroxyl Functions Displayed on the Film Surface



for 5 h at 20 °C (Scheme 3). Liquid scintillation counting (LSC) of the radioactivity associated with the samples directly gave the ratio of reacted hydroxyl functions: we found a value of 1050 (±100) pmol/cm^2 (Table 3, entry 5). Under the same experimental conditions, the native PEEK film was practically unreactive (25 (±5) pmol/cm^2, corresponding to nonspecific adsorption of the label). Assuming, from crystallographic data,[35] that one monomer unit should occupy a volume of 6.24×10^{-22} cm^3, we calculated[36] that the interface domain of 10 atomic layers (~80 Å depth) covered by 1 cm^2 of surface contains 1.28×10^{15} monomer units, or 2123 pmol of $C_{19}H_{12}O_3$. Thus, a reactivity value of 1025 pmol/cm^2 (corrected value obtained by subtracting the nonspecific adsorption value) corresponded to 48% of fixed label, *i.e.* a surface chemistry yield of 60% (considering PEEK-OH with an average of 80% of hydroxyl functions).

We further examined the possibility to activate the surface OH groups by reaction with *p*-nitrophenyl chloroformate ($ClCO_2PNP$);[37] the resulting carbonate could react with various nucleophilic amines to give stable carbamates by *p*-nitrophenoxide displacement. Moreover, basic hydrolysis of PEEK-OCO_2PNP (Scheme 4) could provide an indirect assay of the reactive hydroxyl groups. Indeed, the concentration of *p*-nitrophenoxide released in the aqueous phase was readily determined by spectrophotometry. This original method was used for controlling the optimalization of the

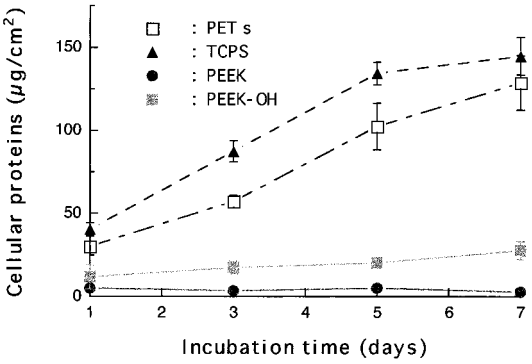


Figure 4. Cultivation of CaCo2 cells on the PEEK-OH substrate.

activation procedure. After examination of various parameters, (i) reagent concentration 1−10% of $ClCO_2$-PNP in toluene, (ii) temperature 20−100 °C, and (iii) duration 1−6 h, we selected the following conditions: 2% of $ClCO_2PNP$ in toluene, at 50 °C for 3 h. Thus, treatment of PEEK-OH as above (disks of 2.4 cm in diameter), followed by appropriate rinsing, gave the activated surface PEEK-OCO_2PNP, the basic hydrolysis of which in 0.1 M NaOH (24 h, 20 °C) liberated 645 (±15) pmol/cm^2 of $PNPO^-$ (Table 3, entry 6), corresponding to 30% of derivatized hydroxyl groups (surface chemistry yield 37%).

The PEEK-OCO_2PNP film (disks of 1.2 cm in diameter) was reacted with [^{3}H]lysine in aqueous solution (10^{-3} M in PBS buffer) for 3 h at 20 °C (Scheme 5). After appropriate rinsing, the LSC measurement gave a value of 120 (±10) pmol/cm^2 of fixed label (Table 3, entry 7). The nonspecific lysine adsorption (~10 pmol/cm^2) was determined by similarly treating the PEEK-OH. The corrected value of 110 pmol/cm^2 corresponded to 5% of derivatization into PEEK-Lys*, *i.e.* a yield of 17% from PEEK-OCO_2PNP. We could conclude that about 80% of the carbonate functions were hydrolyzed instead of giving the substitution with lysine.

Using a fluorine tag as nucleophile, we could assay the surface reactivity of PEEK-OCO_2PNP by XPS analysis. Thus, the activated PEEK-OH film (disks of 1.2 cm in diameter) was immersed into a solution of trifluoroethylamine (1%) in DMSO, for 5 h at 20 °C (Scheme 6) and then adequately rinsed. The blank sample was prepared by similarly treating the native PEEK-OH. The XPS spectrum of PEEK-CF_3 showed well the presence of new elements, fluorine (688.2 eV) and nitrogen (399.6 eV) atoms in the 3/1 ratio, while the blank sample contained only the carbon and oxygen atoms of the polymer backbone. The experimental F/C and N/C atomic ratios were 0.0326 and 0.0115, respectively. From these values, we calculated[38] that about 22% of the monomer units have been derivatized by the fluorine tag (surface chemistry yield of 73% from PEEK-OCO_2PNP). As compared to the previous assay (LSC method), in the present case, hydrolysis of PEEK-OCO_2PNP was not a competitive reaction; the functionalization yield was therefore significantly higher (Table 3, entry 8). Coupling of the CF_3 motif on the PEEK-OH surface restored the hydrophobic character of the polymer; the contact angle with water was 87.4° (Table 2, entry 4).

Cell Cultivation. In previous studies,[39] it has been found that cell adhesion and growth strongly depends on the substrate chemistry, i.e. mainly water wettability and surface charge. However, the role played by surface hydroxyl functions appears to be still a matter of controversy.[40]

The biocompatibility of the PEEK-OH substrate (surface displaying about 80% of hydroxylated polymer units) was examined by culturing CaCo2 cells (human epithelial cell line from colon adenocarcinoma), in synthetic medium containing 1% of serum. The reference substrates were the native PEEK film, TCPS (commercially available tissue-culture-grade polystyrene), and PET-s [commercially available sulfonated membrane of poly(ethylene terephthalate)]. The substrates were not pretreated by coating with adhesive proteins (like collagen, fibronectin, or laminin), a treatment that would mask the real effect of the surface functionality on the adhesion and growth of anchorage-dependent cells.[12]

The cultures were visually examined over a period of 7 days. The native PEEK did not allow cellular adhesion at all; the PEEK-OH showed moderate adhesive properties in comparison with the reference substrates, but cell proliferation was not really detected.

After 1, 3, 5, and 7 days of incubation time, the protein content of the adherent cells was assayed by the Lowry method;[19] the protein densities could be correlated to the number of cells fixed on the substrates. The quantitative results collected in the Figure 4 confirmed our qualitative observations; the surface hydroxylation of the native PEEK moderately improves the performances of the substrate in the CaCo2 cell cultures.

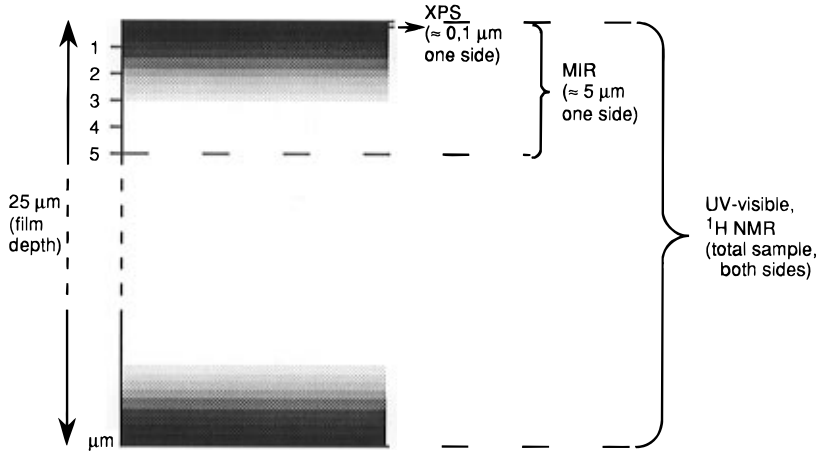


Figure 5. Modified interface of PEEK-OH film.

Conclusion

Surface hydroxylation of amorphous PEEK film was successfully achieved by selective reduction of the benzophenone motif with $NaBH_4$ in DMSO (120 °C, 3 h). This wet-chemistry procedure affected 8% (UV−visible and ^1H NMR analyses) of the monomer units with regard to the total sample (film thickness of 25 μm). It means that about 1 μm of interface (on both sides of the film) has been totally modified, or most likely, that a gradient of modification has affected a few micrometers of the interface domain. At 120 °C, solvent and reagent diffusion into the polymer film could occur, leading to such modification in depth. Accordingly, the MIR analysis showed the presence of 20% of reduced units in an interface domain of about 5 μm (Figure 5). Finally, from the XPS analyses, we confirmed that the 10 outermost atomic layers mainly consisted of hydroxylated units (75−85%). Since sulfur and boron atoms were not detected in the spectrum, we concluded that the rinsing procedure was efficacious: molecules of solvent and/or reagent were not entrapped in the modified domain explored by XPS.

The use of original derivatization techniques in conjunction with LSC and XPS analyses allowed for a quantitative estimation of the PEEK-OH surface reactivity under various experimental conditions. The hydroxyl groups were reacted using the mild conditions likely to be encountered in the covalent coupling of biologically active molecules (peptides, proteins). For instance, activation of PEEK-OH with $ClCO_2PNP$ followed by substitution with a water-soluble amine provided at least 100 pmol/cm^2 of fixed molecules of interest. This level of derivatization appears to be high enough for developing active biocompatibilization strategies.[6c]

We have achieved the cultivation of epithelial cells on the PEEK-OH film; the presence of surface hydroxyl functions moderately improves the polymer biocompatibility.

Acknowledgment. The authors thank Professors R. Legras and J. Devaux for stimulating discussions. The XPS analyses were realized in the laboratory of Professor P. Rouxhet. This work was supported by the Fonds National de la Recherche Scientifique (Belgium), l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (fellowships to O.N. and C.H.), and the Fonds du Développement Scientifique (UCL, Louvain-La-Neuve).

References and Notes

(1) (a) Williams, D. *Concise Encyclopedia of Medical and Dental Materials*; Pergamon Press: Oxford, 1990. (b) Szycher, M. *High Performance Biomaterials: a Comprehensive guide to Medical and Pharmaceutical Applications*; Technomic Publishing Co.: Lancaster-Basel, 1991.

(2) (a) Silver, F.; Doillon, C. Polymers. In *Biocompatibility, Interactions of Biological and Implantable Materials*; VCH Publishers, Inc.: New-York, 1989; Vol. 1. (b) Silver, F. H. *Biomaterials, Medical Devices and Tissue Engineering, an Integrated Approach*; Chapman and Hall: London, 1994.

(3) (a) Engers, G. H.; Feijen, J. *Int. J. Artif. Organs* **1991**, *14*, 199. (b) Ikada, Y. *Biomaterials* **1994**, *15*, 725. (c) Hubbell, J. A. *Trends Polym. Sci.* **1994**, *2*, 20.

(4) (a) Feast, W. J.; Munro, H. S. *Polymer Surfaces and Interfaces*; John Wiley and Sons: New York, 1987. (b) Peppas, N. A.; Langer, R. *Science* **1994**, *263*, 1715.

(5) (a) Marchand-Brynaert, J.; Deldime, M.; Dupont, I.; Dewez, J.-L.; Schneider, Y.-J. *J. Colloid Interface Sci.* **1995**, *173*, 236. (b) Mougenot, P.; Koch, M.; Dupont, I.; Schneider, Y.-J.; Marchand-Brynaert, J. *J. Colloid Interface Sci.* **1996**, *177*, 162.

(6) (a) Sergent-Engelen, T.; Delistrie, V.; Schneider, Y.-J. *Biochem. Pharmacol.* **1993**, *46*, 1393. (b) Halleux, C.; Schneider, Y.-J. *J. Cell. Physiol.* **1994**, *158*, 17. (c) Jaumotte-Thelen, S.; Dozot-Dupont, I.; Marchand-Brynaert, J.; Schneider, Y.-J. *J. Biomed. Mater. Res.* **1996**, in press.

(7) Kroschwitz, J., Ed. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Wiley-Interscience Publication: New York, 1988; Vol. 12, p 313.

(8) Dewez, J.-L.; Doren, A.; Schneider, Y.-J.; Legras, R.; Rouxhet, P. In *Interfaces in New Materials*; Grange, P., Delmon, B., Eds.; Elsevier Applied Science: London, 1991; p 84.

(9) Schneider, Y.-J.; Sergent-Engelen, Th. Unpublished results.

(10) Morrison, C.; Macnair, R.; MacDonald, C.; Wykman, A.; Goldie, I.; Grant, M.H. *Biomaterials* **1995**, *16*, 987.

(11) (a) Williams, D. F.; McNamara, A.; Turner, R. M. *J. Mater. Sci. Lett.* **1987**, *6*, 188. (b) Brown, S. A.; Hastings, R. S.; Mason, J. J.; Moet, A. *Biomaterials* **1990**, *11*, 541. (c) Petillo, O.; Peluso, G.; Ambrosio, L.; Nicolais, L.; Kao, W. J.; Anderson, J. M. *J. Biomed. Mater. Res.* **1994**, *28*, 635.

(12) (a) Lee, J. H.; Lee, H. B. *J. Biomater. Sci. Polym. Ed.* **1993**, *4*, 467. (b) Tamada, Y.; Ikada, Y. *J. Colloid Interface Sci.* **1993**, *155*, 334.

(13) Kobayashi, H.; Ikada, Y. *Biomaterials* **1991**, *12*, 747.

(14) (a) Deldime, M.; Dewez, J.-L.; Schneider, Marchand-Brynaert, J. *Appl. Surf. Sci.* **1995**, *90*, 1. (b) Mougenot, P.; Marchand-Brynaert, J. *Macromolecules* **1996**, *29*, 3552. (c) Boxus, T.; Deldime-Rubbens, M.; Mougenot, P. Schneider, Y.-J.; Marchand-Brynaert, J. *Polym. Adv. Technol.* **1996**, *7*, 589.

(15) Marchand-Brynaert, J.; Pantano, G.; Noiset, O. *Polymer* **1996**, in print.

(16) (a) Franchina, N. L.; McCarthy, T. J. *Macromolecules* **1991**, *24*, 3045. (b) Franchina, N. L.; McCarthy, T. J. In *Chemically Modified Surfaces*; Mottola, H. A., Steinmetz, J. R., Eds.; Elsevier Science Publishers: London, 1992; p 173.

(17) Payne, R. S.; Beamson, G. *Polymer* **1993**, *34*, 1637.

(18) (a) Cole, K. C.; Casella, I. G. *Polymer* **1993**, *34*, 740. (b) Voice, A. M.; Bower, D. I.; Ward, I. M. *Polymer* **1993**, *34*, 1164.

(19) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem* **1951**, *193*, 265.

(20) (a) Mohanty, D. K.; Wu, S. D.; McGrath, J. E. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, *29*, 352. (b) Lindfors, B. E.; Mani, R. S.; McGrath, J. E.; Mohanty, D. K. *Makromol. Chem., Rapid Commun.* **1991**, *12*, 337. (c) Brink, A. E.; Gutzeit, S.; Lin, T.; Marand, H.; Lyon, K.; McGrath, J. E.; Riffle, J. S.; *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1992**, *33*, 402.

(21) (a) Wang, F.; Roovers, J.; Toporowski, P. M. *Macromolecules* **1993**, *26*, 3826. (b) Wang, F.; Roovers, J. *Macromolecules* **1993**, *26*, 5295. (c) Wang, F.; Roovers, J. *Polym. Mater. Sci. Eng.* **1993**, *68*, 44.

(22) Clark, J. H.; Denness, J. E. *Polymer* **1994**, *35*, 5124.

(23) Koch, T.; Ritter, H. *Macromolecules* **1995**, *28*, 4806.

(24) (a) Bailly, C.; Williams, D. J.; Karasz, F. E.; McKnight, W. J. *Polymer* **1987**, *28*, 1009. (b) Shibuya, N.; Porter, R. S. *Macromolecules* **1992**, *25*, 6495. (c) Daoust, D.; Godard, P.; Devaux, J.; Legras, R.; Strazielle, C. *Polymer* **1994**, *35*, 5491.

(25) (a) Brennan, W. J.; Feast, W. J.; Munro, H. S.; Walker, S. A. *Polymer* **1991**, *32*, 1527. (b) Matienzo, L. J. *Polymer* **1991**, *32*, 3057. (c) Jama, C.; Dessaux, O.; Goudmand, P.; Gengembre, L.; Grimblot, J. *Surf. Interface Anal.* **1992**, *18*, 751. (d) Baalmann, A.; Vissing, K. D.; Born, E.; Gross, A. *J. Adhes.* **1994**, *46*, 57. (e) Hopkins, J.; Badyal, J. P. S. *J. Phys. Chem.* **1995**, *99*, 4261.

(26) (a) Munro, H. S.; Clark, D. T.; Recca, A. *Polym. Degrad. Stabil.* **1987**, *19*, 353. (b) Shard, A. G.; Badyal, J. P. S. *Macromolecules* **1992**, *25*, 2053. (c) Mathieson, I.; Bradley, R. H. *J. Mater. Chem.* **1994**, *4*, 1157.

(27) Volz, H.; Mayer, W. D. *Liebigs Ann. Chem.* **1975**, 835.

(28) DMS. *UV Atlas of Organic Compounds*; Verlag Chemie: Weinheim, 1966.

(29) In concentrated sulfuric acid, PEEK sulfonation rapidly occurred. Addition of a small quantity of water dramatically slowed down the sulfonation reaction.

(30) The preparation of substrates for cell cultivation required large samples of surface-modified PEEK films.

(31) The transparency of the substrate was an interesting advantage for the observation of cell culture by microscopy.

(32) Lopez, G. P.; Castner, D. G.; Ratner, B. D. *Surf. Interface Anal.* **1991**, *17*, 267.

(33) We considered a theoretical monomer unit consisting of $[(PEEK\text{-}OH)_x + (PEEK)_y]$, where $(x + y) = 1$. For $x = 0.74$ and $y = 0.26$, we calculated the $C{=}O/C{-}O$ atomic ratio as follows: $0.26/(0.74 \times 5 + 0.26 \times 4) = 0.0548$.

(34) For $x = 0.87$ and $y = 0.13$, we calculated the $O{=}C/(O{-}C + O{-}H)$ atomic ratio as follows: $0.13/(0.87 \times 3 + 0.13 \times 2) = 0.0453$.

(35) (a) Brandrup, J., Immergut, E. H., Eds. *Polymer Handbook*, 3rd ed.; John Wiley: New York, 1989. (b) Fratini, A. V.; Cross, E. M.; Whitaker, R. B.; Adams, W. W. *Polymer* **1986**, *27*, 861. (c) Deslandes, Y.; Alva Rosa, E. *Polym. Commun.* **1990**, *31*, 269. (d) Blundell, D. J.; Newton, A. B. *Polymer* **1991**, *32*, 308.

(36) Volume of one monomer unit: 6 Å × 13 Å × 8 Å = 624 Å^3 = 6.24×10^{22} cm^3. Volume of 10 atomic layers (80 Å) covered by 1 cm^2 (XPS domain) = 8.10^{-7} cm^3; this volume contains 8×10^{-7} cm^3/6.24×10^{22} cm^3 = 1.28×10^{15} monomer units, corresponding to $1.28 \times 10^{15}/6.03 \times 10^{23} = 0.2123 \times 10^{-8}$ mol of $C_{19}H_{12}O_3$, or 2123 pmol.

(37) (a) Henneuse, C.; Gillard, K.; Noiset, O.; Marchand-Brynaert, J. *Bull. Soc. Chim. Fr.* **1995**, *132*, 333. (b) Wilchek, M.; Miron, T. *Biochem. Internat.* **1982**, *4*, 629.

(38) We considered a theoretical monomer unit consisting of $[(\text{PEEK-OH})_x + (\text{PEEK-CF}_3)_y]$, *i.e.* $[(C_{19}H_{14}O_3)_x + (C_{22}H_{16}O_4\text{-}NF_3)_y]$, where $(x + y) = 1$. For $x = 0.79$ and $y = 0.21$, the calculated the F/C atomic ratio was: $3 \times 0.21/(0.79 \times 19 + 0.21 \times 22) = 0.0321$. For $x = 0.77$ and $y = 0.23$, the calculated N/C atomic ratio was: $1 \times 0.23/(0.77 \times 19 + 0.23 \times 22) = 0.0117$.

(39) (a) Anderson, J. M.; Kotke-Marchant, K. *CRC Crit. Rev. Biocompat.* **1985**, *1*, 111. (b) Tamada, Y.; Ikada, Y. In *Polymer in Medicine II*; Chellini, E., Giusti, P., Migliaresl, C., Nicolais, L., Eds.; Plenum Press: New York, 1986; p 101.

(40) (a) Curtis, A. S. G.; Forrester, J. V.; Clark, P. *J. Cell. Sci.* **1986**, *86*, 9. (b) Lee, J. H.; Park, J. W. Lee, H. B. *Biomaterials* **1991**, *12*, 443. (c) Lee, J. H.; Jung, H. W.; Kang, I.-J.; Lee, H. B. *Biomaterials* **1994**, *15*, 705.

MA960368+