Plasma Copolymerization of Allyl Alcohol/1,7-Octadiene: Surface Characterization and Attachment of Human Keratinocytes

Richard M. France, Robert D. Short,* Emmanuel Duval, and Frank R. Jones

Laboratory of Surface and Interface Analysis, Department of Engineering Materials, University of Sheffield, Mappin Street, Sheffield, S1 3JD U.K.

Rebecca A. Dawson and Sheila MacNeil

Department of Medicine, Clinical Sciences Centre, University of Sheffield, Northern General Hospital, Sheffield, S5 7AU U.K.

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Plasma copolymers (PCPs) of allyl alcohol/1,7-octadiene were prepared and characterized using X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and contact angle measurements. The use of a hydrocarbon diluent in the monomer feed allowed the deposition of films with controlled concentrations of hydroxyl groups. ToF-SIMS data have shown these PCPs to be rich in unsaturation. Dimeric allyl alcohol species were observed in the ToF-SIMS spectra, which reduced in intensity with the proportion of hydrocarbon diluent in the monomer feed. Contact angle measurements have shown the nondispersive component of surface energy to increase with hydroxyl group concentration, while the dispersive component remained approximately constant. Human keratinocytes were cultured on these PCP surfaces and collagen I. The level of keratinocyte attachment over 24 h was measured. Keratinocyte attachment increased with hydroxyl group concentration (the nondispersive component of surface energy). PCP surfaces containing high concentrations of hydroxyl groups (25%) were found to promote levels of keratinocyte attachment similar to that on collagen I, a well-established substratum for keratinocyte attachment.

Introduction

Plasma polymers (PPs) are thin polymeric pinhole-free films that can be produced from continuous-wave radio frequency induced nonequilibrium plasmas of volatile organic compounds. The technique is attractive, as deposition is possible onto almost all solid materials, irrespective of geometry, with minimal or no pretreatment required. It allows exact control over film thickness, and polymerization takes place in a clean environment. Films deposited by plasma polymerization are free of remnants of initiator or solvents. These materials have received much attention in the literature due to their application in a wide range of technologies.[1−3]

Tailoring of surface functionality requires judicious choice of monomer and careful control of plasma conditions. In a plasma, there is considerable fragmentation of the original compound and a wide range of functional groups are incorporated into the deposit. In this laboratory, we have examined the relationship between the plasma deposition parameters (plasma power, P, and monomer flow rate, F) and functional group retention in the deposit. We have shown that by employing low P/F it is possible, in many cases, to fabricate films with a high degree of functional group retention. This has been demonstrated for various methacrylates,[4] allyl alcohol,[5,6] and acrylic acid.[7]

Recently, plasma copolymerization has been used in this laboratory to control the concentration of specific functional groups present in the plasma polymer surface. The introduction of a hydrocarbon diluent into the monomer feed has been used to prepare plasma copolymers (PCPs) with carboxylic acid and nitrogen-containing functional groups.[8] The technique of plasma copolymerization also allows the production of films which are insoluble in aqueous media.

Surface (substratum) chemistry and/or energy have been implicated in many aspects of cell physiology, including adhesion, proliferation, and differentiation. Knowledge arising of how surface chemistry/energy influences cell−surface interactions may be applied in the development of agents to enhance or inhibit such interactions for tissue regeneration or biomaterials

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integration. Surface wettability is known to affect cellular adhesion;[9] enhanced cell adhesion has been observed at intermediate wettability for many cell types and material substrata, but there is considerable variation in the data from different laboratories. For fibroblasts, it has been shown that cell spreading is better on higher surface energy solids.[9] Surface chemistry, or specifically the presence of certain functional groups, has been implicated in cellular attachment. In particular, surface hydroxyl,[10] carbonyl,[11] and carboxylate[12] have been shown to affect cellular attachment. However, the relative effects of surface chemistry vs surface energy are still the subject of much debate in the literature. As yet, no general principles have been established which would allow prediction of cellular behavior on different surfaces. The study of cellular response to specific surface functional groups requires surfaces of well-defined chemistry. Cell−surface interactions have been investigated on ion-exchange materials,[13] self-assembled monolayers (SAMs),[12] and plasma polymers.[14]

The response of human keratinocytes to natural or synthetic surfaces is of importance in wound care and healing. The influence of extracellular matrix proteins on human keratinocyte attachment, proliferation, and transfer to a dermal wound bed model has been studied.[15] Matrigel, collagen I, and collagen IV were found to enhance initial attachment; RGD, vitronectin, fibronectin, and irradiated 3T3 fibroblasts did not.

In a previous study, we examined the attachment of human keratinocytes to plasma copolymers of acrylic acid/1,7-octadiene and allylamine/1,7-octadiene.[16] Enhanced attachment was observed at low carboxylic acid group concentrations and high nitrogen-containing functional group concentrations. The current study involves the characterization of PCPs of allyl alcohol/1,7-octadiene using time-of-flight secondary ion mass spectroscopy (ToF-SIMS) and contact angle measurements in addition to X-ray photoelectron spectroscopy (XPS). The level of keratinocyte attachment to hydroxyl functionalities, over a range of functional group concentrations, was studied and attachment correlated with both surface chemistry and surface energy. Collagen I and a hydrocarbon plasma polymer were employed as reference surfaces.

Experimental Section

Plasma Copolymerization. Allyl alcohol (>99%) and 1,7-octadiene (>99%) were obtained from Aldrich Chemical Co. (U.K.). All monomers were used as received, after several freeze−pump−thaw cycles. Polymerization was carried out in a cylindrical reactor vessel (8 cm diameter and 50 cm in length), evacuated by a two-stage rotary pump. The plasma was sustained by a radio frequency (13.56 MHz) signal generator and amplifier inductively coupled to the reactor vessel. The base pressure in the reactor was 3×10^{-3} mbar.

Monomers were copolymerized at a plasma power of 2.5 W and a total flow rate of 2.0 sccm. Plasma copolymers were deposited onto clean aluminum foil (for XPS and ToF-SIMS analysis), clean glass slides (for contact angle measurements), and tissue culture wells. The pressure during copolymerization was typically 4.0×10^{-2} mbar.

For all copolymerizations, a deposition time of 20 min was used. The monomer mixtures were allowed to flow for a further 20 min after the plasma was switched off. This was done in an attempt to minimize the uptake of atmospheric oxygen by the deposits on exposure to the laboratory atmosphere.

X-ray Photoelectron (XP) Spectroscopy. XP spectra were obtained on a VG CLAM 2 photoelectron spectrometer employing Mg Kα X-rays. Survey scan spectra (0−1100 eV) and narrow spectra were acquired for each sample using analyzer pass energies of 50 and 20 eV, respectively. Spectra were acquired using Spectra 6.0 software (R. Unwin Software, Cheshire, U.K.). Subsequent processing was carried out with Scienta data processing software (Scienta Instruments, Uppsala, Sweden). The spectrometer was calibrated using the Au 4f 7/2 peak position at 84.00 eV and the separation between the C 1s and F 1s peak positions in a sample of polytetrafluoroethylene measured at 397.2 eV, which compares well with the value of 397.19 eV reported by Beamson and Briggs.[17]

Time-of-Flight Secondary Ion Mass Spectrometry. ToF-SIMS analyses were carried out on a modified VGS IX23LS ToF-SIMS instrument. The vacuum in the spectrometer was typically better than 2×10^{-9} mbar. The original ion source was substituted by a pulsed liquid monoisotopic Ga ion source supplied by FEI (USA). This gave a primary beam energy of 25 keV and a beam current of approximately 1 nA, with a pulse width of 20 ns. Mass analysis was achieved using a Poschenreider type time-of-flight mass analyzer. Secondary ions were extracted from the sample through an aperture to the flight tube by the application of a ±5 kV bias to the sample stage, depending on the acquisition of positive or negative ions. The acquisition was controlled by VGX 7000T software. Charge compensation was achieved using a low-energy (<15 eV) electron flood gun, pulsed between the primary beam pulses. Spectra were acquired from 5 to 1000 amu with a channel width of 0.25 amu. Ion doses were $<10^{12}$ ions cm^{-2} for all samples.

Contact Angle Measurements. Contact angle measurements were performed by placing a 4 μL drop of liquid upon a PCP-coated glass slide. Distilled water and diiodomethane were used, with three measurements for each liquid being taken. Dispersive and nondispersive components of surface energy were approximated from the geometric mean method, given in eq 1.[18,19]

$$(1 + \cos\theta)\gamma_l = 2(\gamma_s^{d}\gamma_l^{d})^{1/2} + 2(\gamma_s^{nd}\gamma_l^{nd})^{1/2} \qquad (1)$$

In eq 1, θ is the contact angle, γ_l is the surface energy of the pure liquid, and γ_s is the surface energy of the solid. The superscripts "d" and "nd" correspond to the dispersive and nondispersive components of the surface energy, respectively. For water and diiodomethane, values of γ_l (72.8 and 50.8 mJ m^{-2}, respectively), γ_l^{d} (21.8 and 50.8 mJ m^{-2}, respectively) and γ_l^{nd} (51.0 and ≈0 mJ m^{-2}, respectively) were used.[20]

Cell Culture. Normal human dermal keratinocytes (obtained from breast reductions and adominoplasties) were

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Table 1. Summary of XPS Results for PCPs Prepared from Allyl Alcohol and 1,7-Octadiene[a]

		percentage of functionality in the C 1s core level			
F_{aa}/F_{tot}	O/C ratio	*C*−C, *C*−H	*C*−OH/R	*C*═O	*C*OOH/R
0	0.01	97.5	2.1	0.1	
0.54	0.07	90.7	9.1	0.5	0
0.60	0.09	87.8	11.9	0.7	0
0.76	0.13	85.3	13.6	1.6	0
0.90	0.21	76.5	19.5	4.0	0.1
1.00	0.30	68.7	25.3	5.5	0.6

[a] Note, a β shift (at +0.7 eV from the hydrocarbon) of equal magnitude to the carboxylate has been added to the peak fit where necessary. Conditions for polymerization were F_{tot} = 2.0 sccm, power = 2.5 W, deposition time = 20 min.

isolated from the dermal/epidermal junction as previously described.[21] Cells were cultured in complete Green's media, which included cholera toxin (0.1 nM), hydrocortisone (0.4 μg/mL), epidermal growth factor (10 ng/mL), adenine (1.8×10^{-4} M), triiodo-L-thyronine (2×10^{-7} M), insulin (5 mg/mL), transferrin (5 μg/mL), glutamine (2×10^{-3} M), fungizone (0.625 μg/mL), penicillin (1000 IU/mL), streptomycin (1000 μg/mL), and 10% fetal calf serum. Cells were cultured at 37 °C, in a 5% CO_2 atmosphere.

Experiments were carried out using freshly isolated cells. Tissue culture polystyrene (TCPS) was obtained from Corning Glass Co. (USA). Collagen-coated tissue culture plates were prepared by air-drying a solution of collagen I (32 μg/cm^2) in 0.1 M acetic acid (200 μg/mL) in a laminar flow cabinet overnight.

Cell Attachment Assay. Cells were seeded at a density of 2.5×10^5 cells/mL onto six-well (3.5 cm diameter) tissue culture plates. The wells were either PCP-coated or collagen I-coated. One milliliter of cell culture media was added to each well. Cells were seeded on all substrates within 48 h of preparation. Keratinocytes were allowed to attach for 24 h; unattached cells were removed by a gentle wash with 1 mL of phosphate buffer solution (PBS).

For all substrates, the level of cell attachment was determined by an estimation of total DNA content using Hoechst stain, as detailed in ref 22. Cells were incubated in a saline sodium citrate (SSC) + urea + sodium dodecyl sulfate (SDS) digestion buffer for 1 h. Following digestion, cells were stained with Hoechst stain (in an SSC buffer at 1 μg/mL). Fluorescence was measured using excitation and emission wavelengths of 355 and 460 nm, respectively. DNA content was then estimated from a standard curve of known DNA concentration.

To determine keratinocyte attachment and viability, an MTT-ESTA assay[23] was used. This estimates the viable cell number, values with this assay having previously been shown to parallel increases in cell number for human keratinocytes.[24] Cells were incubated with 0.5 mg/mL of MTT in PBS for 40 min. The stain was then eluted with acidified 2-propanol. An optical density measurement was then made at 540 nm with a protein reference wavelength of 630 nm which was subtracted.

Results

XPS Characterization. XP survey scan spectra of PCPs prepared from allyl alcohol and 1,7-octadiene revealed only carbon and oxygen in the deposits. The O/C ratios were measured and are shown in Table 1.

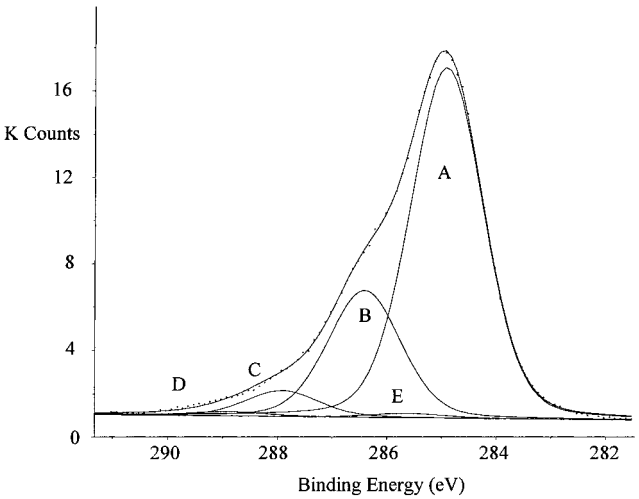

Figure 1. Peak fitted C 1s core level of PP of allyl alcohol. A: *C*−C, *C*−H; B: *C*−OH/R; C: *C*═O; D: *C*OOH/R; E: *C*−COOH/R.

The O/C ratio was observed to increase as the molar fraction of allyl alcohol in the monomer feed increased. If ideal gas behavior is assumed, then the molar fraction of allyl alcohol is equal to the flow rate ratio F_{aa}/F_{tot}, where $F_{tot} = F_{aa} + F_{oct}$. The measured O/C ratio was always lower than the O/C ratio of the monomer mixture, but the loss of oxygen (in any PCP) was no more than 10%. The C 1s core level spectra of the PCPs were peak fitted for various oxygen-containing functionalities.[17] First, spectra were corrected for sample charging by setting the hydrocarbon signal to 285 eV. The following functionalities were then fitted: alcohol/ether (*C*−OH/R) at a shift of +1.5 eV; carbonyl (*C*═O) at +3.0 eV; carboxylic acid/ester (*C*OOH/R) at +4.0 eV; and a β-shifted carbon bonded to carboxylate (*C*−COOH/R) at +0.7 eV. The results of peak fitting are shown in Table 1 and an example peak fit ($F_{aa}/F_{tot} = 1$) is shown in Figure 1. In the peak fits, the fwhm and Gaussian-to-Lorentzian ratio (*G*/*L*) of component peaks were kept equal and were in the range 1.4−1.6 eV and 0.8−0.9, respectively.

While XPS cannot distinguish between alcohol and ether groups, trifluoro acetic anhydride (TFAA) labeling of plasma-polymerized allyl alcohol has shown that, at the low powers employed in this study, the hydroxyl functionality of the monomer is retained in the deposit and the carbon singly bonded to oxygen peak in the XP spectra can be assigned to hydroxyl rather than ether.[6] A direct relationship was found between F_{aa}/F_{tot} and the concentration of hydroxyl groups in the PCPs. This can be seen in the data reported in Table 1. The percentage retention of hydroxyl functionality in the PP of allyl alcohol was 76%. This was calculated by dividing the percentage of carbons in hydroxyl functional groups in the PP (25.3%) by those in the monomer (33.3%). Other carbon−oxygen functionalities present in the PCPs (besides hydroxyl) included carbonyl and carboxylate. These arose as a result of fragmentation of the monomer in the plasma. Reaction between the deposit and water desorbed from the walls of the plasma vessel (during polymerization) and atmospheric oxygen and water (after polymerization) also contributed.

The *C*-OH/R peak in the plasma polymer of 1,7-octadiene is thought to be predominantly ether. In this

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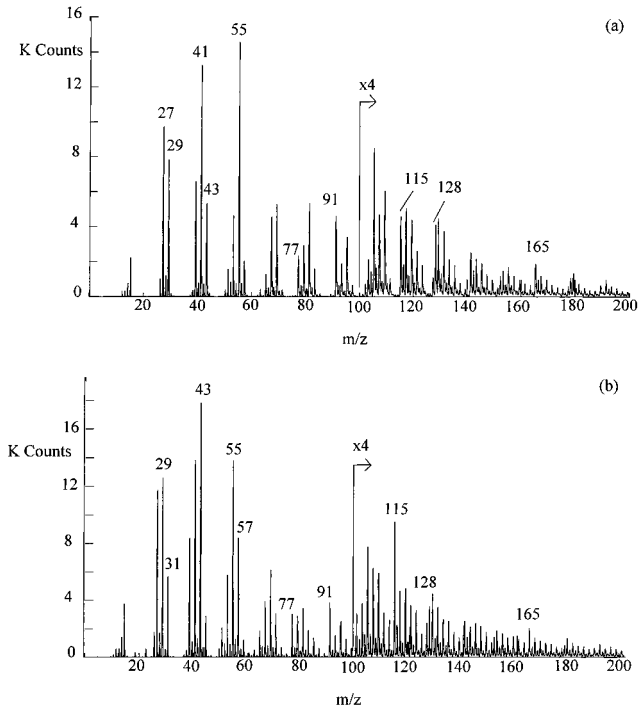

Figure 2. Positive ion ToF-SIMS spectra (m/z 0−200) for PPs of (a) 1,7-octadiene and (b) allyl alcohol.

pure hydrocarbon PP ($F_{aa}/F_{tot} = 0$), the O/C ratio determined directly from the C 1s and O 1s peak areas was 0.01. This is lower than the O/C calculated from the peak fit (0.02). In the calculation of the O/C ratio from the peak fit, the total number of carbons bonded to oxygen is divided by 100 carbon atoms to yield an O/C ratio (for $F_{aa}/F_{tot} = 0$, we calculate 2.1 oxygen atoms per 100 carbons). In the peak fit, the ether functionality will be counted twice, since two carbons experience the same shift brought about by one (shared) oxygen atom.

ToF-SIMS Characterization. In Figure 2a, the positive ion ToF-SIMS spectrum of plasma-polymerized 1,7-octadiene ($F_{aa}/F_{tot} = 0$) is shown. Evident in the spectrum are a number of ions typical of hydrocarbon materials. These correspond to $C_nH_{2n-1}^+$ and $C_nH_{2n+1}^+$ species, and appear at m/z 27, 29, 41, 43, 55, 57, etc.[25] Also present in the spectra are a number of ions typically associated with aromatic materials.[25] These ions appear at m/z 77, 91, 115, 128, and 165. They have been attributed to polycyclic aromatic ions (PCAs).[25] In Figure 2b, the positive ion ToF-SIMS spectrum of plasma-polymerized allyl alcohol ($F_{aa}/F_{tot} = 1$) is displayed. On initial inspection, the spectra of the two materials appear similar, however subtle differences exist: In the PP of allyl alcohol, the signals at m/z 31, 43, and 57 are of greater intensity, and the m/z 115 is dominant in the region m/z 100−200. These signals are assigned to CH_3OH^+, $C_2H_3O^+$, $C_3H_5O^+$, and $C_6H_{11}O_2^+$, respectively. (These assignments are discussed later.)

In Figure 3, the normalized peak intensities (NPIs) for the m/z 55 and 57 are plotted against F_{aa}/F_{tot}. In this normalization procedure, the counts from the ion of interest (summed between the minima of adjacent peaks) are divided by the counts obtained over a larger

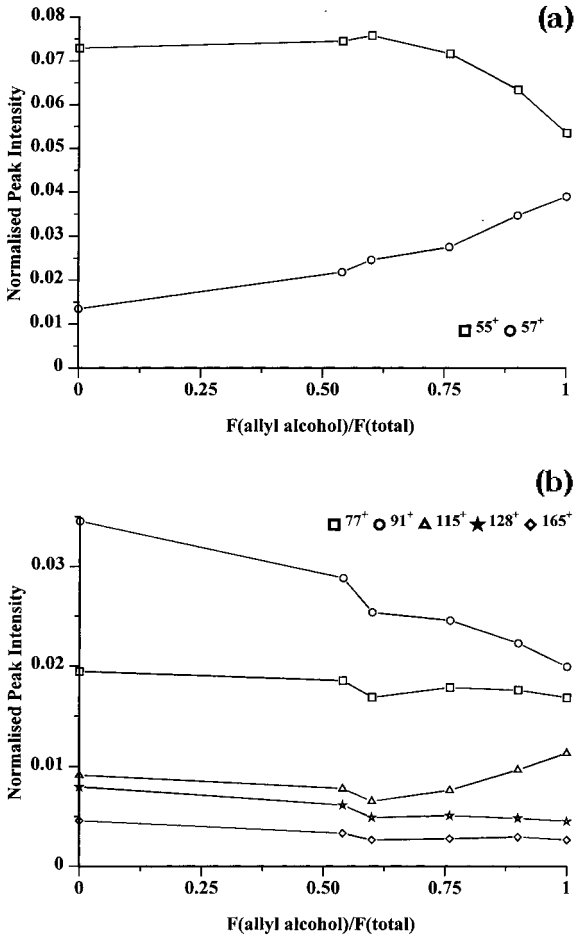

Figure 3. (a) Normalized peak intensities of positive ions m/z 55 and 57 vs F_{aa}/F_{tot} for PCPs prepared from allyl alcohol/1,7-octadiene. (b) Normalized peak intensities of positive ions m/z 77, 91, 115, 128, and 165 vs F_{aa}/F_{tot} for PCPs prepared from allyl alcohol/1,7-octadiene.

mass range,[26] in this case m/z 0−300. This has been shown to be a useful method to measure the contribution a particular ion makes to a spectrum and allows (qualitatively) changes in the concentration of specific surface moieties to be followed.[26] The errors associated with this method of quantitation of SIMS data have been well documented.[26,27] For a series of poly(methyl methacrylate) standards, the standard deviation in NPIs has been determined to be less than 13% in all key ions.[27] The data presented here show the NPI of the m/z 55 decreased as the molar fraction of allyl alcohol in the monomer feed was increased. The NPI of the m/z 57 increased. In Figure 3b, the NPIs of the m/z 77, 91, 115, 128, and 165 are plotted against F_{aa}/F_{tot}. The data show the NPIs of the m/z 77, 91, 128, and 165 decreased as the proportion of allyl alcohol in the monomer feed was increased. The NPI of the m/z 115 initially decreased over the range $F_{aa}/F_{tot} = 0-0.6$, but subsequently increased substantially (from $F_{aa}/F_{tot} = 0.6-1.0$). The monomer ratios were chosen to provide surfaces with a range of hydroxyl functional groups concentrations (see Table 1). No surfaces were prepared from monomer mixtures of $F_{aa}/F_{tot} < 0.5$.

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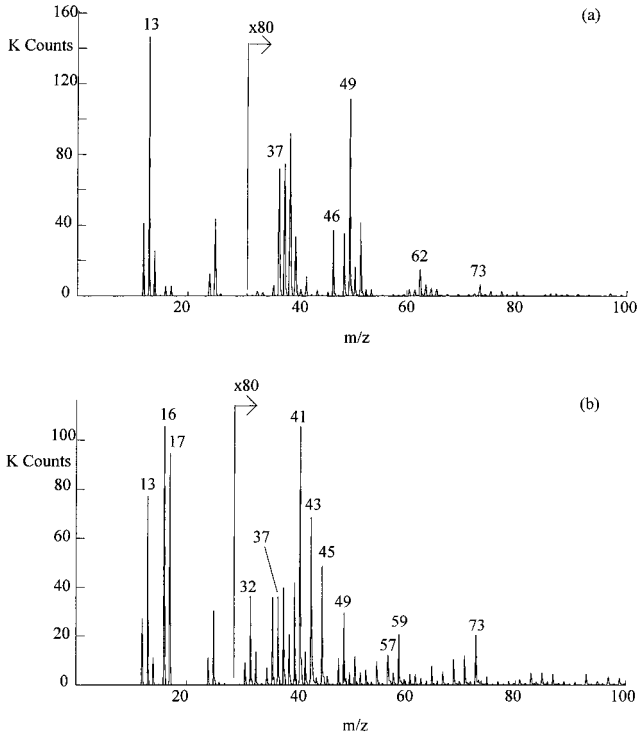

Figure 4. Negative ion ToF-SIMS spectra (m/z 0−100) for PPs of (a) 1,7-octadiene and (b) allyl alcohol.

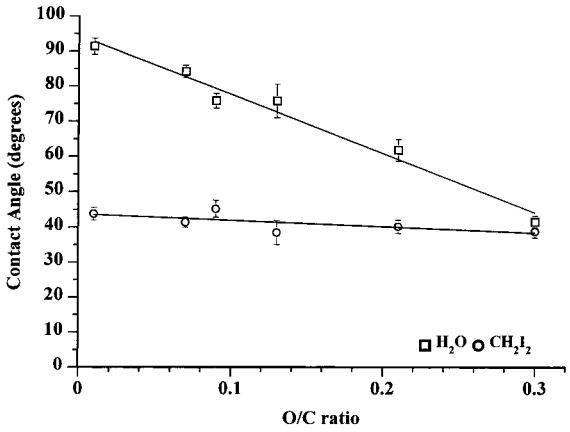

Figure 5. Contact angles of H_2O and CH_2I_2 vs O/C ratio for PCPs prepared from allyl alcohol/1,7-octadiene. Results shown are means ±standard deviation for triplicate 4 μL liquid drops.

Displayed in Figure 4a is the negative ion ToF-SIMS spectrum of the PP of 1,7-octadiene. The spectrum shows fragments typically associated with aromatics at m/z 37, 49, 62, and 73.[25] Low signals are observed from oxygen at m/z 16 (O^-) and 17 (OH^-). In Figure 4b, the negative ion ToF-SIMS spectrum for a PP of allyl alcohol is shown. Intense signals are observed at m/z 16 and 17. Oxygen-containing ions, absent in the spectra of plasma-polymerized 1,7-octadiene, are observed at m/z 41 (C_2HO^-), 43 ($C_2H_3O^-$), 45 ($C_2H_5O^-$), 57 ($C_3H_5O^-$), 59 ($C_2H_7O^-$, $CH_3O_2^-$). Although seen in a number of oxygen-containing polymers, they contain little structural information. The NPIs of m/z 16 and 17 increased almost linearly with O/C ratio.

Contact Angle Measurements. In Figure 5, the contact angles measured using H_2O and CH_2I_2 are plotted against O/C ratio for the PCP surfaces. The H_2O contact angle decreased from approximately 90° to 45° as the O/C ratio increased from 0.01 to 0.30. The contact angle of CH_2I_2 decreased slightly from 45° to 40°, as indicated by the negative slope. The dispersive and nondispersive components of the surface energy have been calculated using the geometric mean method (eq 1).[18,19] The data are shown in Figure 6. The dispersive component (γ^d) increased only very slightly as the oxygen content, as indicated by O/C ratio, increased. The nondispersive component (γ^{nd}) increased dramatically with O/C ratio. For all the PCP surfaces, the nondispersive component was less than the dispersive component.

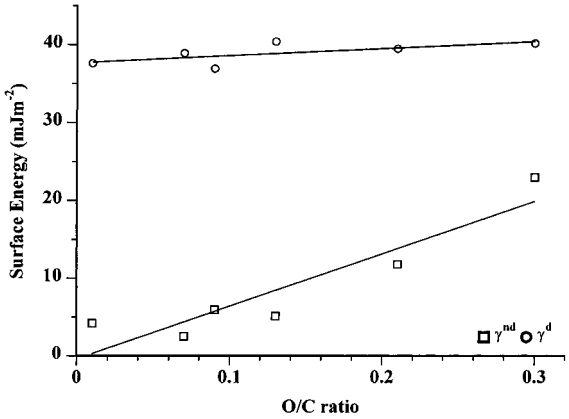

Figure 6. Dispersive (γ^d) and nondispersive (γ^{nd}) components of surface energy vs O/C ratio for PCPs prepared from allyl alcohol/1,7-octadiene.

Keratinocyte Culture. The effect of hydroxyl group concentration on keratinocyte attachment was investigated. Six-well tissue culture plates were coated with PCPs. Freshly isolated keratinocytes were seeded at 2.5×10^5 cells/mL on these PCP surfaces. Cell attachment 24 h later (after gentle removal of unattached cells) was estimated by both DNA and MTT-ESTA assays (three wells on the same tissue culture plate being used for each assay). The results are shown in parts a and b of Figure 7, respectively. In Figure 7a, the level of cell attachment is shown to have increased with the number of hydroxyl functionalities. The highest level of attachment occurred at high hydroxyl concentrations, where attachment approached that measured upon collagen I. The level of attachment as estimated by the MTT-ESTA assay, Figure 7b, shows a similar trend to the DNA assay. Enhanced cell attachment was observed at higher hydroxyl group concentrations. At the highest concentration, the level of attachment measured by this assay matches that achieved on collagen I.

Keratinocytes were examined by light microscopy prior to washing with PBS. Optical micrographs are shown in Figure 8. Cells on the hydrocarbon PCP, Figure 8a, showed poor attachment: the cells were rounded and light in appearance. The cells seeded upon PCPs containing hydroxyl functionalities, Figure 8b, also showed signs of poor attachment, although a greater number of keratinocytes were observed, consistent with the attachment assay results. The darker cells present on these micrographs are differentiated keratinocytes. Cells seeded upon collagen I, Figure 8c, displayed morphological features indicative of better attachment and spreading; the cells are darker in appearance and less rounded than those in parts a and b of Figure 8. In this micrograph, greater numbers of

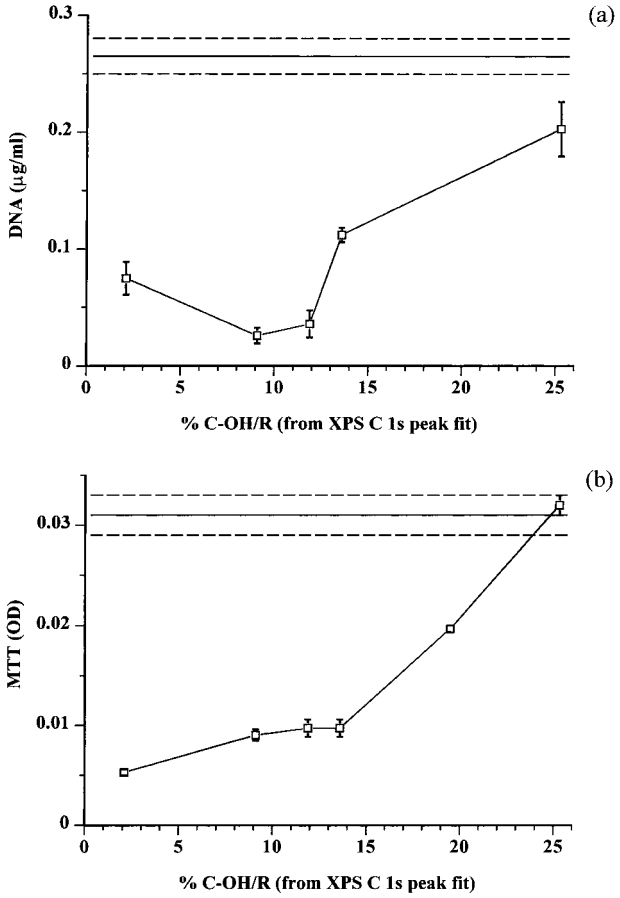

Figure 7. Attachment of keratinocytes to PCPs of allyl alcohol/1,7-octadiene and to collagen I. The level of attachment was estimated from (a) the total DNA content of cells and (b) the MTT-ESTA assay. All cells were freshly isolated (seeded at 2.5×10^5 cells/mL). Solid horizontal line indicates level of attachment to collagen I (dashed lines show error). Results shown are the means ±standard error of the mean of triplicate wells of cells.

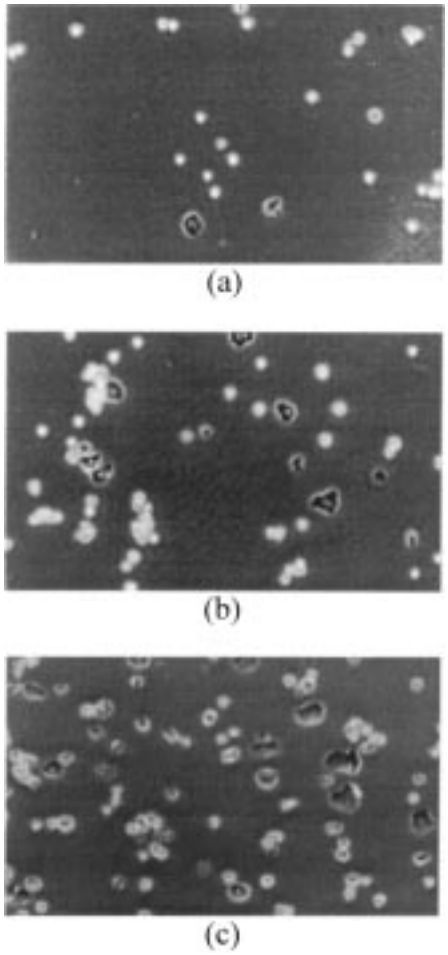

Figure 8. Freshly isolated keratinocytes on PCPs prepared from allyl alcohol/1,7-octadiene and collagen I. Micrographs were taken 24 h post cell addition, prior to washing with phosphate buffer solution. (a) Hydrocarbon PCP (no hydroxyl groups). (b) PCP surface containing 25.3% hydroxyl groups. (c) Collagen I.

keratinocytes are observed, cf. PCPs, again consistent with the cell attachment assay results.

Discussion

Plasma copolymerization of allyl alcohol and 1,7-octadiene has afforded the production of a range of PCP surfaces with controlled concentrations of hydroxyl functionalities. The XPS results showed a nonlinear relation between the O/C ratio and the molar fraction of allyl alcohol in the monomer feed. The nonlinearity of the relation stems from the differing number of carbons in each monomer (three in allyl alcohol and eight in 1,7-octadiene). A different relation between O/C and monomer feed was observed with hexane as the hydrocarbon diluent.[8] In plasma polymerization, loss of oxygen from monomer to product is always observed.

The ToF-SIMS spectra of plasma homopolymers of both 1,7-octadiene and allyl alcohol contained signals diagnostic of PCAs. The detection of these ions does not necessarily indicate that aromatic structures were present in these PPs, but rather the structures which were predisposed to form these ions in the SIMS process, most probably moieties that contained unsaturation. In the spectrum of plasma-polymerized allyl alcohol, ions were observed which were seen previously in the gas-phase plasma mass spectrometry (MS) of this compound. These appeared at *m/z* 31, 43, 57, and 115.[5] In plasma mass spectrometry the first two ions have been assigned to CH_3OH^+ and $C_2H_3O^+$.[5] These ions result from fragmentation of the monomer within the plasma. The latter two have been assigned to $(M-H)^+$ and $(2M-H)^+$, where M is the monomer unit.[5] Oligomerization reactions in the plasma gas phase have been proposed, proceeding though the addition of M to $(M-H)^+$ to produce the dimer $(2M-H)^+$.[5] A simple normalization procedure was used to follow the relative contribution that these ions made to the ToF-SIMS spectra over the monomer composition range. In Figure 3a it can be seen that the NPI of *m/z* 57 $[(M-H)^+]$ increased with F_{aa}/F_{tot}. In an earlier study *m/z* 55 was assigned to $(M-3H)^+$.[6] However this ion did not appear in abundance in the gas-phase plasma MS of allyl alcohol. This, coupled with the trend in NPI, suggests that this ion can be assigned to $C_4H_7^+$. In Figure 3b the NPI of *m/z* 115 is plotted against F_{aa}/F_{tot}. The NPI of *m/z* 115 initially reduced in intensity with increased F_{aa}/F_{tot}, up to 0.6. Below this monomer ratio, the chance of the molecular ion $(M-H)^+$ reacting with M in the gas phase is low due to the presence of the octadiene in the plasma. As F_{aa}/F_{tot} increased, the ability of $(M-H)^+$ to react with M increased and *m/z* 115 (assigned at least in part to

$(2M-H)^+$) was observed in increased amounts at the surface. The one complicating factor is that the *m*/*z* 115 can also be assigned to a PCA. However, the plots in Figure 3b suggest that similar ions (e.g. *m*/*z* 77, 91, 128, and 165) reduced in intensity with increased F_{aa}/F_{tot}.

The negative ion ToF-SIMS spectrum of plasma-polymerized 1,7-octadiene (Figure 4a) contained signals diagnostic of PCAs, which most likely arose from unsaturation at the surface. Some of these ions were observed in the ToF-SIMS spectrum of plasma-polymerized allyl alcohol (Figure 4b), although this negative ion spectrum was more dominated by oxygen-containing ions. Normalization was used to assess the relative contribution these ions made as a function of F_{aa}/F_{tot}. The NPIs of the *m*/*z* 16 and 17 were approximately linear with increasing F_{aa}/F_{tot}, reflecting the presence of one functionality upon the surface, namely hydroxyls.

In Figure 5, the H_2O contact angle is seen to decrease markedly with increasing O/C ratio in the PCP surfaces. However, there is only a slight reduction in the CH_2I_2 contact angle with O/C ratio. Using the geometric mean method it is shown that the component of surface energy that underwent the greatest change with increasing O/C ratio was the nondispersive component (Figure 6). This observation is consistent with the introduction of polar functionalities (predominantly hydroxyl) at the surface. The method used to calculate surface energy contains an approximation.[18,19] The nondispersive component of the surface energy of CH_2I_2 is approximated to zero, and other components to the surface energy of the liquids are ignored. Therefore, the values calculated are not exact measurements. However, given the distinct change observed, this method is suited for the material under investigation. The slight changes in dispersive forces may indicate subtle structural differences between the PCPs as also observed in the SIMS of these materials.

Human keratinocytes have been successfully cultured on PCP surfaces containing hydroxyl groups. Low levels of attachment were observed with low concentrations of hydroxyl functionalities. Keratinocytes showed high levels of attachment to PCP surfaces containing high levels of hydroxyl functionalities (25%). The attachment increased with the number of hydroxyl functionalities, which in turn correlates with increased surface energy. The number of cells attached to these surfaces was comparable to the number of cells attaching to collagen I, a preferred substratum for the culturing of keratinocytes. Optical microscopy of cells cultured on these two surfaces revealed morphologies characteristic of attachment, but poor signs of spreading. Better attachment and evidence of spreading was observed upon collagen I.

In previous experiments we employed collagen I and TCPS as control surfaces.[16] However we noted that TCPS did not make for a good control surface, with considerable variation in cell attachment to TCPS from experiment to experiment in contrast to the reproducible attachment to collagen I and the hydrocarbon PP surface. This may be due to variability between different batches of TCPS. Therefore TCPS has not been employed as a control surface in this study. In previous experiments, there were approximately 20 times more cells attaching to the positive control collagen I than to the hydrocarbon PP surface,[16] which was used as the negative control, failing to promote attachment. We used the results on these two surfaces to "normalize" our data. In this study there were approximately 6 times more cells attaching to collagen I than to the hydrocarbon polymer (cf. 20:1). Keratinocytes from different donors do display considerable variation in culture as we have previously noted.[15] This variation is thought to account for the lower attachment to collagen I, cf. the hydrocarbon PP in this study.

Other methods have been used to prepare surfaces of well-defined chemistry, on which cellular attachment has then been measured. Recent papers describe the culturing of cells on self-assembled monolayers (SAMs) with a variety of different surface chemistries.[28−30] 3T3 murine fibroblasts have been successfully cultured on SAMs formed by the adsorption of carboxylic acid- and methyl-functionalized alkylthiols on gold surfaces.[12] A strong preference was exhibited for the COOH-terminated monolayer. Human osteoblasts[30] and endothelial cells[31] have also been demonstrated to have a preference for COOH-terminated surfaces. The preference exhibited here by keratinocytes for PCPs containing hydroxyl functional groups (over a hydrocarbon surfaces) confirms other findings with osteoblasts cultured upon hydroxyl-terminated SAMs.[30] However, a study of endothelial cell attachment to SAMs showed enhanced attachment on a hydrocarbon surface over hydroxyl functionalities.[31]

From the data presented here, it could be concluded that surface chemistry/energy both play a part in directing the attachment of keratinocytes to these surfaces; enhanced attachment was observed as both functional group concentration and surface energy increased. However, in our previous experiment looking at keratinocyte attachment to PCPs prepared from acrylic acid and 1,7-octadiene, we observed greater keratinocyte attachment to PCP surfaces with low amounts (2.3%) of carboxylic acid functionality, cf. high amounts (21%) of carboxylic acid functionality. Subsequently we have measured the components of surface energy on these two surfaces. Values of γ_s^d (45.4 and 38.8 mJ m^{-2} for the 2.3 and 21% *C*OOH/R PCP surfaces, respectively) and γ_s^{nd} (4.6 and 37.0 mJ m^{-2} for the 2.3 and 21% *C*OOH/R PCP surfaces, respectively) were calculated using the methodology described.[32] Thus, while in this study keratinocyte attachment correlated with both surface chemistry and energy, in the case of carboxylic acid-functionalized PCPs no such correlation was observed with surface energy; poorer attachment was seen on the surface with the highest surface energy. The best attachment was afforded upon PCP surfaces with low concentrations of carboxylic acids and lower surface energy. Hence, in these PCPs, surface chemistry appears to be the controlling factor at the surface.

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Thus PCPs can be used to help delineate the effects of surface chemistry vs surface energy on keratinocyte attachment.

The study of cell−surface interactions, whether using SAMs or PPs, is very much in its infancy. Yet, general trends are already emerging, particularly in the case of carboxylate vs hydrocarbon chemistries. However much work is still to be undertaken. The link between protein adsorption (to the substrate) and cellular attachment is extremely important, as surface functional groups influence the nature and conformation of the adsorbed protein layer and may only indirectly influence cellular attachment. Understanding of the nature of the adsorbed protein layer will be a critical in understanding cellular interactions with substrates and will form the basis of future work from this laboratory.

Conclusions

Plasma copolymerization of allyl alcohol/1,7-octadiene has been used to prepare surfaces with controlled amounts of hydroxyl functionalities. Surface characterization has been carried out using XPS, ToF-SIMS, and contact angle measurements. The amount of hydroxyl functionality at the surface is observed to increase as the proportion of allyl alcohol (F_{aa}/F_{tot}) in the monomer feed is increased. ToF-SIMS data have indicated the presence of oligomeric fragments (dimers) at the surface of plasma-polymerized allyl alcohol. These fragments decrease in intensity as the proportion of 1,7-octadiene is increased in the monomer feed. All plasma polymer surfaces display ions diagnostic of aromaticity/unsaturation. Contact angle analysis using the geometric mean method for two liquids has shown the nondispersive component of surface energy to undergo the greatest change with F_{aa}/F_{tot}. The dispersive component undergoes little change. Hence, we propose that plasma copolymerization may be used as a method to prepare surfaces on which to explore the effects of surface energy and surface chemistry.

Here, we demonstrate this method using PCP surfaces to examine the effect of hydroxyl group concentration on the level of keratinocyte attachment. Enhanced attachment was observed on surfaces containing high concentrations of hydroxyl functionalities. By comparison with earlier work, we show that the enhanced attachment most probably arises specifically from the different interactions of the hydroxyl and carboxylic acid groups and not changes in surface energy.

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