JOURNAL OF Materials CHEMISTRY

Attachment of human keratinocytes to plasma co-polymers of acrylic acid/octa-1,7-diene and allyl amine/octa-1,7-diene

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Plasma co-polymers (PCPs) of acrylic acid/octa-1,7-diene and allyl amine/octa-1,7-diene have been prepared and characterised using X-ray photoelectron spectroscopy (XPS). The use of a hydrocarbon diluent in the monomer feed allowed the deposition of films with controlled concentrations of carboxylic acid and nitrogen-containing functional groups. Human keratinocytes were cultured on these PCP surfaces, tissue culture poly(styrene) (TCPS) and collagen I. The level of keratinocyte attachment over 24 h was measured. PCP surfaces containing low concentrations of carboxylic acid groups (2.3%) were found to promote keratinocyte attachment. The performance of these PCPs was similar to collagen I, a well established substratum for attachment. Nitrogen-containing PCP surfaces were found to promote attachment at higher functional group concentrations, although the attachment did not attain the level achieved on the acid functionalised PCP surfaces.

Plasma polymers (PPs) are thin polymeric pinhole free films, that can be produced from continuous-wave radio-frequency induced non-equilibrium 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 pre-treatment required. It allows exact control over film thickness, and polymerisation takes place in a clean environment. Films deposited by plasma polymerisation are free of remnants of initiator or solvents. 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,[1] allyl alcohol[2,3] and acrylic acid.[4]

Recently, plasma co-polymerisation has been used in this laboratory to control the amount of functional groups present in the plasma polymer. The introduction of a hydrocarbon diluent into the monomer feed has been used to prepare plasma co-polymers (PCPs) with controlled amounts of carboxylic acid and nitrogen-containing functional groups.[5] The technique of plasma co-polymerisation also allows the production of films which are insoluble in aqueous media.

Cell–surface interactions influence or control many aspects of cell physiology, including adhesion, proliferation and differentiation. Knowledge arising from the study of cell–surface interactions may be applied in the development of agents to enhance or inhibit cell–substrate interactions for tissue regeneration or biomaterials integration. In general cell–surface interactions are poorly understood. This is due to the complexity of these interactions and the large number of parameters which control them. Surface chemistry is known to influence cellular attachment, either directly, or through the protein layer which rapidly absorbs to surfaces in contact with serum containing culture media. To examine cell–surface interactions, many simplified models have been used. Biologically active molecules have been coated or immobilised to various substrata to produce bioactive surfaces. Molecules that have been investigated include glycosaminogylcan matrix components and proteins such as fibronectin, laminin, vitronectin and collagen.[6] The tripeptide sequence Arg–Gly–Asp (RGD) has been identified in many cell adhesion proteins.[7] Synthetic RGD has been coated on surfaces[8] or covalently bound[9] to study the dependence of cell adhesion on RGD surface concentration.

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.[10] Matrigel, collagen I and IV were found to enhance initial attachment; RGD, vitronectin, fibronectin and irradiated 3T3 fibroblasts did not. Proliferation of cells was also found to be positively influenced (although to a lesser extent than initial attachment) on matrigel, collagen I and IV and irradiated 3T3 fibroblasts.

The response of cells to specific surface functional groups represents the most fundamental level of study. The basic requirement for this type of study is a surface of well defined chemistry. Cell–surface interactions have been investigated on ion exchange materials,[11] self assembled monolayers (SAMs)[12] and plasma polymers.[13]

This study investigates the attachment of human keratinocytes to PCP surfaces. The level of keratinocyte attachment to carboxylic acid and nitrogen functional groups, over a range of functional group concentrations has been studied. Tissue culture poly(styrene) (TCPS), a hydrocarbon plasma polymer and collagen I have been employed as reference surfaces. TCPS is generally acknowledged to be a poor substrate for the keratinocyte, while collagen I has been shown to be a preferred substrate for the keratinocyte.[10]

Experimental

Plasma co-polymerisation

Acrylic acid, allyl amine and octa-1,7-diene were obtained from Aldrich (UK). All monomers were used as received, after several freeze–pump–thaw cycles. Polymerisation was carried out in a cylindrical reactor vessel (of 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.

Acrylic acid and octa-1,7-diene were co-polymerised at a plasma power of 2 W and a total flow rate of 2.0 sccm [sscm = cm^3(STP) min^{-1}]. Allyl amine and octa-1,7-diene were co-polymerised at a plasma power of 3 W and a total flow rate of 2.0 sccm. Plasma co-polymers were deposited onto tissue culture wells and clean aluminium foil (for XPS analysis). The pressure during co-polymerisation was typically 4.0×10^{-2} mbar.

For all co-polymerisations, 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 minimise the uptake of atmospheric oxygen by the deposits on exposure to the laboratory atmosphere.

X-Ray photoelectron 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 analyser pass energies of 50 and 20 eV respectively. Spectra were acquired using Spectra 6.0 software (R. Unwin Software, Cheshire, UK). 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 PTFE measured at 397.2 eV, which compares well with the value of 397.19 eV reported by Beamson and Briggs.[14]

Cell culture

Normal human dermal keratinocytes (obtained from breast reductions and adominoplasties) were isolated from the dermal/epidermal junction as previously described.[15] Cells were cultured in complete Green's media, which included cholera toxin (0.1 nM), hydrocortisone (0.4 $\mu g\ ml^{-1}$), EGF (10 $ng\ ml^{-1}$), adenine (1.8×10^{-4} M), triiodo-L-thyronine (2×10^{-7} M), insulin (5 $mg\ ml^{-1}$), transferrin (5 $\mu g\ ml^{-1}$), glutamine (2×10^{-3} M), fungizone (0.625 $\mu g\ ml^{-1}$), penicillin (1000 IU ml^{-1}), streptomycin (1000 $\mu g\ ml^{-1}$) and 10% fetal calf serum. Cells were cultured at 37 °C, in a 5% CO_2 atmosphere.

Experiments were carried out using both freshly isolated and subcultured (passaged) cells. For the latter, cells were cultured (in collagen I coated flasks) for four days, being re-fed once with fresh media. The cells were then trypsinised (0.5% trypsin in EDTA), prior to seeding on the experimental substrates. TCPS was obtained from Corning Glass Co. (USA). Collagen coated tissue culture plates were prepared by air drying a solution of collagen I (32 $\mu g\ cm^{-2}$) in 0.1 M acetic acid (200 $\mu g\ ml^{-1}$) in a laminar flow cabinet overnight.

Cell attachment assay

Cells were seeded at densities of between 1.6 and 7.0×10^5 cells ml^{-1} onto six well (3.5 cm diameter) tissue culture plates. The wells were either uncoated, PCP coated or collagen I coated. 1 ml was added to each well. Cells were seeded on PCPs 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 the Hoechst stain, as detailed in ref. 16. 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 $\mu g\ ml^{-1}$). 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 concentrations.

To determine keratinocyte attachment and viability on the nitrogen functionalised PCPs, an MTT–ESTA assay[17] was used. This estimates the viable cell number, the assay having been previously shown to parallel increases in cell number for human keratinocytes.[18] Cells were incubated with 0.5 $mg\ ml^{-1}$ of MTT in PBS for 40 min. The stain was then eluted with acidified isopropyl alcohol. An optical density measurement was then made at 540 nm with a protein reference wavelength of 630 nm which was subtracted.

Results

Characterisation of PCPs

XP survey scan spectra of PCPs prepared from acrylic acid and octa-1,7-diene revealed only carbon and oxygen in the deposits. The O/C ratios were measured and are shown in Table 1. The O/C ratio was observed to increase as the molar fraction of acrylic acid in the monomer feed increased. If ideal gas behaviour is assumed then the molar fraction of acrylic acid is equal to the flow rate ratio F_{aa}/F_{tot}, where $F_{tot} = F_{aa} + F_{oct}$. The C 1s core level spectra of the PCPs were peak fitted for various oxygen-containing functionalities.[15] First, spectra were corrected for sample charging, 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 (COOH/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 Fig. 1(a). In the peak fit the FWHM of component peaks were kept equal and were in the range 1.4–1.6 eV. The Gaussian to Lorentzian ratios (G/L) of the component peaks were also kept constant and were in the range 0.8–0.9. While XPS cannot distinguish between carboxylic acid and ester groups, grazing angle IR spectroscopy of plasma polymerised acrylic acid has shown, that at the low powers employed in this study, the carboxylate peak in the XP spectra can be assigned to carboxylic acid rather than ester.[4] There is a direct relationship between the proportion of acrylic acid in the monomer feed and the concentration of acid groups in the PCPs. This can be seen in the data reported in Table 1. This relationship is described in more detail elsewhere.[5] Other carbon–oxygen functionalities present in the PCPs (besides carboxylic acid) include carbonyl and alcohol/ether. These arise 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 polymerisation) and atmospheric oxygen and water (after polymerisation) also contribute. The C—OH/R peak is thought to be predominantly ether. In the pure hydrocarbon PCP ($F_{aa}/F_{tot} = 0$), the O/C ratio determined from the C 1s and O 1s peak areas is 0.01. This is considerably lower than the O/C calculated from the peak fit (0.03). In the calculation of the O/C ratio from the

Table 1 Summary of XPS results for PCPs prepared from acrylic acid and octa-1,7-diene[a]

		% of functionality in the C 1s core level			
F_{aa}/F_{tot}	O/C ratio	C—C, C—H	C—OR	C=O	COOH/R
0	0.01	97.2	3.4	—	—
0.52	0.10	90.3	5.4	0.1	2.3
0.64	0.14	84.0	6.3	0.5	4.7
0.80	0.28	70.7	7.4	1.3	10.4
0.90	0.43	58.3	6.8	2.2	16.4
1.00	0.53	47.0	7.5	3.5	21.0

[a]A β shift (at +0.7 eV from the hydrocarbon) of equal magnitude to the carboxylate has been added to the peak fit. Conditions for polymerisation were $F_{tot} = 2.0$ sccm, power = 2 W, deposition time = 20 min.

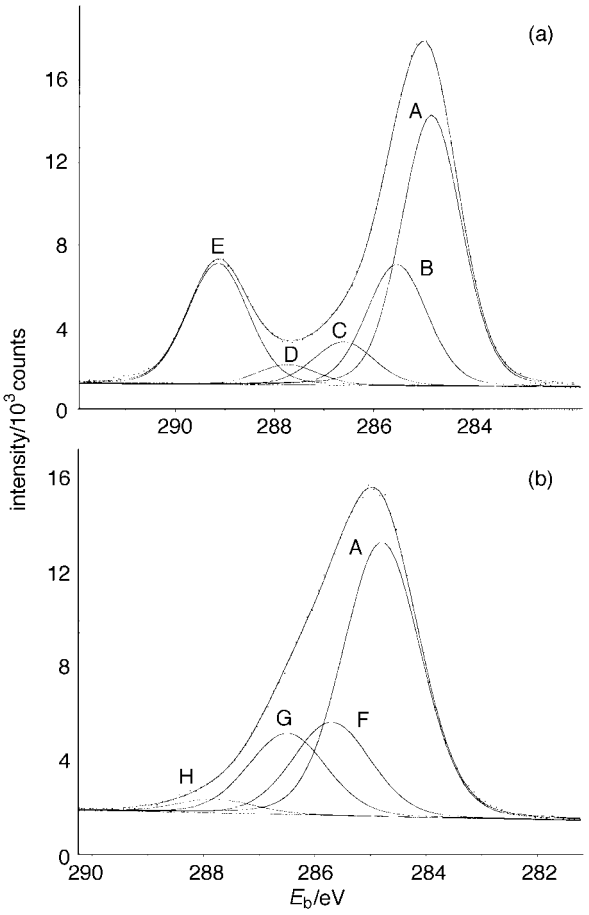

Fig. 1 Peak fitted C 1s core levels of PPs of (a) acrylic acid and (b) allyl amine. A: *C*—C, *C*—H, B: *C*—COOH/R, C: *C*—OR/H, D: *C*=O, E: *C*OOH/R, F: *C*—NR_2, G: *C*=N, H: *C*NO.

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 3.4 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. In the PCPs the number of *C*—OH/R environments is constant; between 5 and 7 per 100 carbons. In the hydrocarbon PP of octa-1,7-diene the number is a little lower (3.4 per 100 carbons).

XP survey scans were made of PCPs prepared from allyl amine and octa-1,7-diene. These deposits contained carbon, nitrogen and oxygen. The XPS data were quantified and the results are shown in Table 2. Small amounts of oxygen were detected in these PCPs. This oxygen was most likely incorporated on exposure to the laboratory atmosphere. However, oxygen may also have been incorporated within the plasma. Water is known to be constantly desorbing from the reactor vessel walls. The C 1s core level spectra of the PCPs were peak fitted for nitrogen-containing functionalities, using chemical shift values reported in the literature.[14,19] The functionalities fitted were: amine (*C*—NR_2) at +0.9 eV; imine (*C*=N) at +1.7 eV; and amide (*C*NO) at +3.0 eV. The results of these peak fits are shown in Table 2 and an example peak fit ($F_{aa}/F_{tot}=1$) is displayed in Fig. 1(b). The FWHM used were in the range 1.5–1.7 eV and the G/L ratios in the range 0.8–0.9. It is obvious from the peak fit results that there has been lower selectivity towards the amine functionality in allyl amine/octa-1,7-diene PCPs than might have been anticipated, particularly when compared with the acrylic acid/octa-1,7-diene PCPs. Imine and amide functionalities are present in the allyl amine/octa-1,7-diene PCPs. In addition to the low selectivity, the nature of the amine, *i.e.* primary, secondary or tertiary, is unknown.

Table 2 Summary of XPS results for PCPs prepared from allyl amine and octa-1,7-diene[a]

			% of functionality in the C 1s core level			
F_{aa}/F_{tot}	N/C ratio	O/C ratio	*C*—C, *C*—H	*C*—NR_2 (*C*—OR)	*C*=N	*C*NO
0	0	0.01	97.5	0 (3.5)	0	0
0.48	0.06	0.03	82.9	12.3	4.8	0.4
0.74	0.15	0.08	71.2	13.7	11.6	3.9
1.00	0.37	0.06	59.5	20.2	17.6	3.0

[a]XPS analysis of sample prepared at $F_{aa}/F_{tot}=0.74$ was carried out 11 days after preparation. Conditions for polymerisation were $F_{tot}=$ 2.0 sccm, power = 2 W, deposition time = 20 min.

Keratinocyte culture on PCPs containing carboxylic acid groups

The effect of carboxylic acid group concentration on keratinocyte attachment was investigated. Six well tissue culture plates were coated with PCPs. Freshly isolated keratinocytes were seeded at 6.2×10^5 cells ml^{-1} on these PCP surfaces. Three wells were used on each plate.

Carboxylic acid functional group concentration was found to have a significant effect on the level of cell attachment. The results are shown in Fig. 2(a). On PCP surfaces containing no carboxylic acid groups (PP of octa-1,7-diene), the level of attachment was found to be poor; comparable to that on TCPS. On PCP surfaces containing 2.3% carboxylic acid

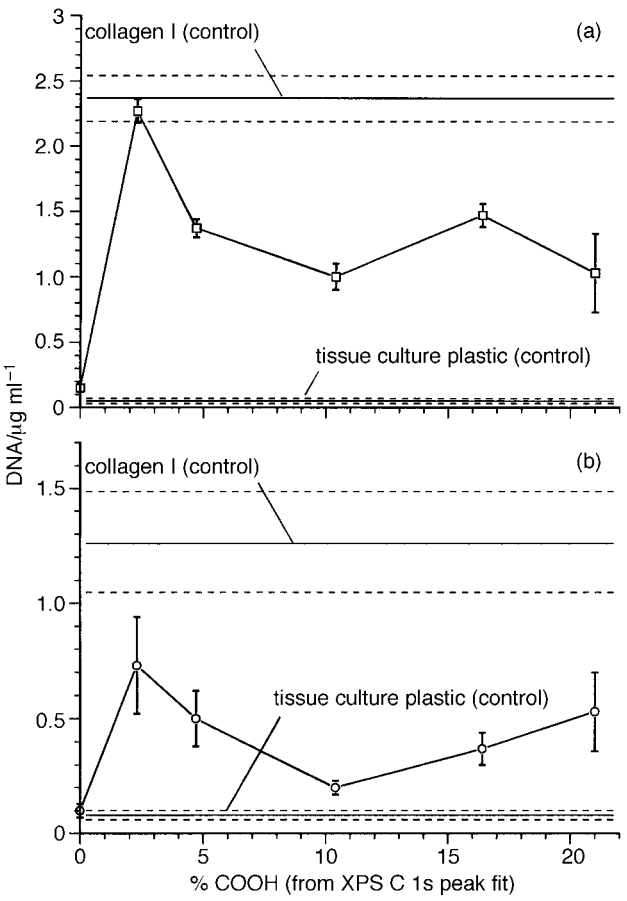

Fig. 2 Attachment of keratinocytes to PCP surfaces containing carboxylic acid groups. Attachment was estimated from the total DNA of cells attached to surface. (a) Freshly isolated cells (seeded at 6.2×10^5 cells ml^{-1}). (b) First passage keratinocytes (from another donor, seeded at 1.6×10^5 cells ml^{-1}). Results shown are the means ± s.e.m. of triplicate wells of cells (s.e.m. = standard error of the mean).

groups very high levels of cell attachment were measured. The level of attachment was comparable to that measured on collagen I. On PCPs containing $>2.3\%$ carboxylic acid groups, the level of attachment was observed to be reduced. However, the amount of attachment measured was still in excess of that measured on the PCP surfaces with no carboxylic acid chemistry.

Freshly isolated cells were examined by light microscopy, prior to washing with PBS. Optical micrographs are shown in Fig. 3. Cells on PCP surfaces of low concentrations of acid groups and on collagen I, Fig. 3(b) and (e) respectively, display morphological features consistent with good attachment and spreading. In contrast, cells seeded on the PCP surfaces containing no acid groups and on TCPS, Fig. 3(a) and (d), respectively, show poor attachment: the cells are rounded and lighter in appearance. Cells seeded on the PCP surfaces of higher acid group concentrations, Fig. 3(c), show a mixed response: some have attached well and spread, whilst others are poorly attached.

The attachment of passaged cells (from a different donor, first passage, seeded at 1.6×10^5 cells ml^{-1}) to PCP surfaces was also investigated, using the remaining three wells of the six well tissue culture plates. The results from the DNA assays are shown in Fig. 2(b). Significant enhancement in the level of attachment (over that observed upon PCP surfaces containing no carboxylic acid groups) was again seen on the PCP surfaces containing a low concentration (2.3%) of carboxylic acid groups. Enhanced attachment was also seen on surfaces containing higher acid group concentrations, although the level of attachment to these surfaces failed to reach that measured on collagen I.

Keratinocyte culture on PCPs containing nitrogen functional groups

The attachment of freshly isolated keratinocytes (seeded at 7.0×10^5 cells ml^{-1}) to PCP surfaces with different concentrations of nitrogen-containing functionalities was investigated. Nitrogen-containing functional groups were found to have a significant influence on the level of cell attachment. Cell attachment was estimated by both DNA and MTT–ESTA assays (three wells on the same tissue culture plate being used for each experiment). The results are shown in Fig. 4(a) and (b), respectively. In Fig. 4(a), the level of cell attachment is shown to increase with the number of nitrogen-containing functionalities. However, the overall level of attachment is lower than that observed upon TCPS. It should be noted here that we will subsequently argue that TCPS is not a good reference surface, because of possible variability in surface chemistry between samples. In our first experiment (with acid functional groups) there was a similar level of cell attachment to both TCPS and to the pure hydrocarbon plasma polymer surface. Also, in that experiment a marked difference was seen between TCPS and collagen. The pure hydrocarbon plasma polymer surface used in this experiment was identical to the one used before. Therefore, this surface should be considered as the negative control in both experiments.

The level of attachment as estimated by the MTT–ESTA assay, Fig. 4(b), mirrors the data shown by the DNA assay, Fig. 4(a). Enhanced cell attachment is observed at higher nitrogen functional group concentrations. However, even at the highest concentration, the level of attachment does not approach that achieved on collagen I.

Keratinocytes were examined by light microscopy prior to washing with PBS. Optical micrographs are shown in Fig. 5. Cells on the hydrocarbon PCP, Fig. 5(a), show poor attachment: the cells are round and light in appearance. The darker cells present on this micrograph are differentiated keratinocytes. Cells seeded upon both TCPS and collagen I,

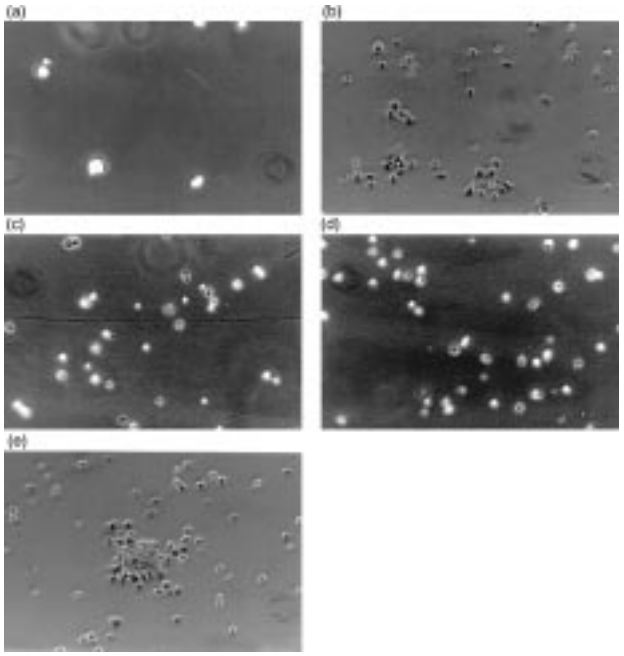

Fig. 3 Freshly isolated keratinocytes on PCPs containing carboxylic acid groups and on control substrates. Micrographs were taken 24 h post cell addition, prior to washing with phosphate buffer solution. (a) Hydrocarbon PCP (no carboxylic acid groups). (b) PCP surface containing 2.3% carboxylic acid groups. (c) PCP surface containing 21.0% carboxylic acid groups. (d) Tissue culture poly(styrene). (e) Collagen 1.

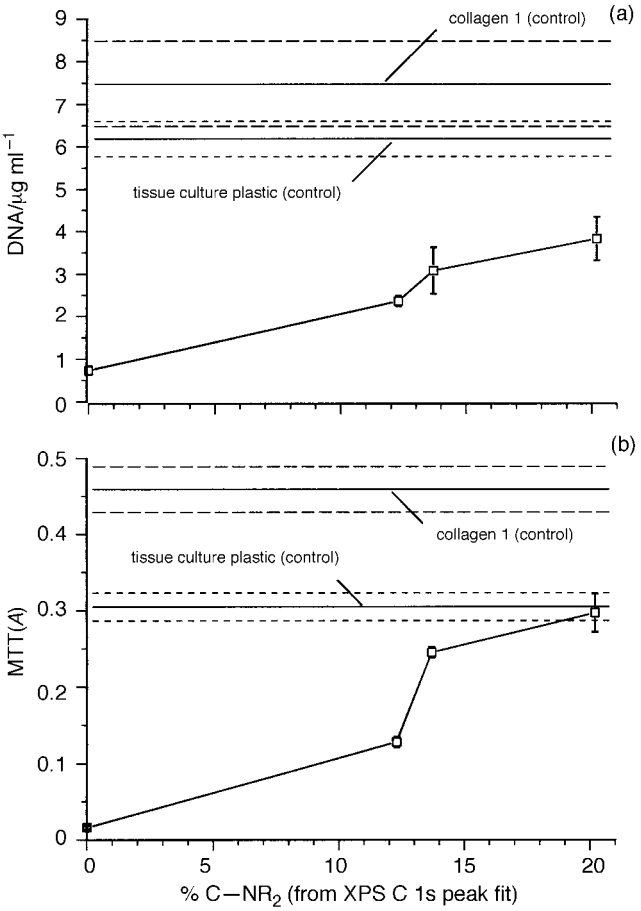

Fig. 4 Attachment of keratinocytes to PCPs containing amine groups and to control surfaces. The level of attachment was estimated from (a) the total DNA content of cells and (b) the MTT–ESTA assay (which assesses cell viability). All cells were freshly isolated (seeded at 7.0×10^5 cells ml^{-1}). Results shown are the means $\pm$ s.e.m. of triplicate wells of cells.

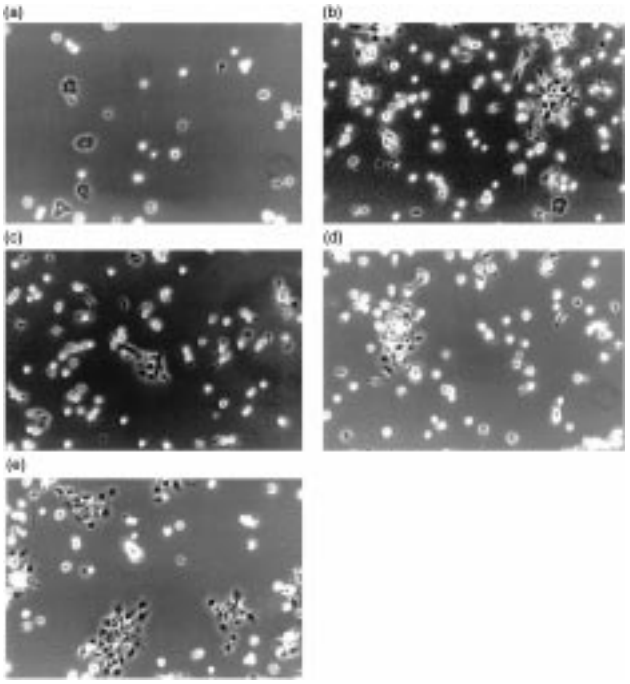

Fig. 5 Freshly isolated keratinocytes on PCPs containing amine groups and on control substrates. Micrographs were taken 24 h post cell addition, prior to washing with phosphate buffer solution. (a) Hydrocarbon PCP (no amine groups). (b) PCP surface containing 13.7% amine groups. (c) PCP surface containing 20.2% amine groups. (d) Tissue culture poly(styrene). (e) Collagen I.

Fig. 5(d) and (e), respectively, display morphological features indicative of good attachment and spreading. In these micrographs, more attached and well spread cells (those darker in appearance) are observed upon collagen I, *cf*. TCPS, consistent with the cell attachment assay results. The cells seeded upon PCPs containing nitrogen functionalities, Fig. 5(b) and (c), show a mixture of good attachment/spreading and poor attachment.

Discussion

Human keratinocytes have been successfully cultured on PCP surfaces containing carboxylic acid and nitrogen functional groups. The use of co-polymerisation has allowed the effect of functional group concentration to be studied.

Keratinocytes show high levels of attachment to PCP surfaces containing low levels of carboxylic acid functionalities (2.3%). The number of cells attached to these surfaces is comparable to the number of cells attaching to collagen I, a preferred substratum material for the culturing of keratinocytes. Optical microscopy of cells cultured on these two surfaces reveals morphologies characteristic of both attachment and spreading. Enhanced attachment was also observed at higher acid group concentrations. The same trend was found for both freshly isolated cells and passaged cells. Hydrocarbon plasma polymer surfaces did not promote attachment.

The attachment of keratinocytes to PCPs of allyl amine and octa-1,7-diene was greatest at high functional group concentrations. Optical microscopy revealed a mixed response on these surfaces: some cells were attached and well spread, others were poorly attached. These PCP surfaces do not contain one unique nitrogen functionality. The original objective of this study was to prepare amine-containing surfaces, however, the selectivity towards amine functionalities in these PCPs was lower than anticipated. The concentration of other nitrogen functionalities (imine and amide) increased disproportionally as the amount of allyl amine in the monomer feed increased. This confuses the interpretation of our data. The observed increase in cell attachment may result from an increase in the concentration of one specific nitrogen functionality, *e.g*. amine. Alternatively, cell attachment may have increased as a result of the increase in the total amount of nitrogen surface functionality. Significantly, the level of attachment on theses PCP surfaces was lower than on collagen I. Again, the hydrocarbon plasma polymer surface did not promote attachment.

A serious problem with one of the controls, TCPS, was identified. In the experiment with acid functionalised PCP surfaces, the level of attachment to the TCPS was comparable to the hydrocarbon plasma polymer surface. In the experiment with nitrogen functionalised PCP surfaces the TCPS performed significantly better than the hydrocarbon plasma polymer surface. Given that cells from different donors were used in these two experiments, it is important to have confidence in the controls. In these two experiments, collagen I and hydrocarbon plasma polymer surfaces were also used. In both experiments, there were approximately 20 times more cells attaching to the collagen I than to the hydrocarbon plasma polymer surface. The hydrocarbon plasma polymer surface can thus be considered the negative control, which does not promote attachment, and collagen I the positive control. We can use the results on these two surfaces to 'normalise' our data. The variable performance of the TCPS must still be explained. Keratinocytes from different donors do display considerable variation in culture as we have previously noted.[10] However we suspect that in this case the cause of this variation is the TCPS. TCPS is known to receive a propriety treatment, probably corona or plasma discharge. The surface of the TCPS was examined by XPS and it was found to contain oxygen. The surface O/C ratio was approximately 0.25. Peak fitting revealed a range of different oxygen-containing functional groups. In a separate study we have investigated the surface oxidation of PS.[20] We have shown that above an O/C ratio of 0.18, PS surfaces are not 'stable' to aqueous solutions. That is, low molecular mass material can be extracted. It is also uncertain whether different batches of TCPS would have received exactly the same level of surface treatment, or whether in any one batch, the surface treatment is stable to ageing. Based on this, we suggest that TCPS surfaces do not make for good controls.

Other methods have been used to prepare surfaces of well defined chemistry, on which cellular attachment has then been measured. Recent reports have appeared describing the preparation of such surfaces using self-assembly monolayers (SAMs). SAMs are typically formed by the adsorption of alkylthiols onto gold supports[21] or by the chemisorption of alkylsilanes onto silica surfaces.[22] Recent papers describe the culturing of cells on SAMs with a variety of different surface chemistries.[23–25] 3T3 murine fibroblasts have been successfully cultured on SAMs formed by the adsorption of carboxylic acid- and methyl-functionalised alkylthiols on gold surfaces.[12] A strong preference was exhibited for the COOH terminated monolayer. On this surface there was a greater number of attached cells, and these cells exhibited a more well spread morphology. On the methyl-terminated surface, cells were more rounded and clumped together. Human osteoblasts have also demonstrated a preference for COOH terminated surfaces.[25] Endothelial cells have shown a strong preference for COOH surfaces, and also for surfaces containing mixtures of OH and COOH.[26]

The use of SAMs, as opposed to plasma polymers, for this type of study offers real advantages. First, there is a greater level of control over substratum chemistry. Even in the carboxylic acid PCPs some other oxygen-containing functional groups have been introduced. This problem does not arise with SAMs. Further, structural order can be introduced into SAMs.

Plasma polymerisation also offers advantages, particularly when it comes to device fabrication. PPs can be deposited

directly onto most surfaces. Objects of complicated geometry can be coated. In PPs, the distribution of functional groups, because of the nature of the process, will be uniform across the surface. The surface concentration of functional groups can be controlled and surfaces containing more than one functional group should, in principle, be readily prepared. PPs can be engineered with specific hydration properties, through the control of cross-links.

These two approaches should be considered complementary. The preference exhibited here by keratinocytes for PCPs containing the carboxylic acid functional groups (over TCPS or hydrocarbon surfaces) confirms other findings with fibroblasts,[12] osteoblasts[25] and endothelial cells.[26] Co-polymerisation has allowed control over the surface carboxylic acid concentration. The data show keratinocyte preference for surfaces with low amounts of this functionality. The dependence of cell attachment on functional group concentration has yet to be properly explored. SAMs have been prepared from mixtures of COOH and OH terminated alkanethiols.[26] The level of attachment to binary mixtures of COOH and OH is greater than to single component COOH terminated SAMs. Enhanced attachment of cells to surfaces containing mixtures of functionalities, has also been observed on fluorinated surfaces.[27] In ref. 27, PPs (containing both CF_2 and CF_3 groups) were observed to support the growth of bovine aortic endothelial cells. Single component SAMs (terminated with either CF_2 or CF_3 groups) did not support cell growth.

There is only a limited literature pertaining to cellular behaviour on surfaces containing nitrogen functional groups. The attachment and growth of human endothelial cells and fibroblasts on PPs containing amine and amide functional groups have been previously explored.[28] In this work, cell attachment followed nitrogen content within the films. It was suggested that the amide functionality was the main promoter of cell attachment. These results are certainly in agreement with those reported here for keratinocytes.

The study of cell–surface interactions, whether using SAMs or PPs, is very much in its infancy. Yet, general trends are already emerging. 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. In particular, vitronectin and fibronectin have been implicated in cellular attachment.[29] Understanding of the nature of the adsorbed protein layer will be critical in understanding cellular interactions with substrates and will form the basis of future work from this laboratory.

Conclusions

Plasma co-polymerisation was used to prepare surfaces on which keratinocyte attachment was investigated. Enhanced attachment was observed to surfaces containing low concentrations of carboxylic acid groups and to surfaces with high concentrations of nitrogen functional groups.

Plasma co-polymerisation of acrylic acid and octa-1,7-diene gave surfaces containing predominantly (but not exclusively) carboxylic acid functional groups. The attachment of keratinocytes to PCP surfaces containing concentrations of 2.3% acid groups was found to be comparable to the level of attachment observed to collagen I, the latter being a preferred surface for the culturing of keratinocytes. Optical microscopy revealed that on these low acid content surfaces, and on collagen I, keratinocytes were well attached and spread. On PCP surfaces of higher acid content, the level of attachment was still significantly above that seen on the negative control (a hydrocarbon PP), but well below the optimum (collagen I).

Plasma co-polymerisation of allyl amine and octa-1,7-diene produced surfaces containing a mixture of nitrogen functional groups, predominantly amine and imine. The attachment of keratinocytes to PCP surfaces containing nitrogen functional groups increased with the nitrogen content of these surfaces, although cells never attained the level of attachment seen on collagen I.

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Paper 7/05098D; *Received* 16*th July*, 1997