

Characterization of Air Plasma-Treated Polymer Surfaces by ESCA and Contact Angle Measurements for Optimization of Surface Stability and Cell Growth

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ABSTRACT: A radiofrequency air plasma has been used to incorporate new functionalities at the surface of cycloolefin polymers (Zeonex[®] and Topas[®]), polymethyl methacrylate (PMMA), styrene-acrylonitrile copolymer (SAN), and polystyrene (PS). The main goals with the plasma treatment of the different plastics were to hydrophilize the surfaces and to provide good cell culture properties. Surfaces treated at high RF power/gas flow ratios (50 to 100 W/sccm) became highly hydrophilic (water contact angles of about 5 degrees) and stable towards washing in 70% (v/v) ethanol. Those treated at lower power/gas flow ratios (3 to 10 W/sccm) were less hydrophilic and not wash-stable. Cell growth properties of HeLa cervix carcinoma cells as good as on commercial tissue-culture polystyrene could be obtained

for Zeonex, SAN, and PS, treated at relatively low RF power/gas flow ratios. However, no untreated plastics were suitable for culturing these cells. XPS spectra features show that ester, ether/alcohol, and ester/carboxyl groups are formed during the plasma treatments of the different plastics. Measurable amounts of carboxylic acid carbon after plasma treatment were only observed for PS and Topas. Furthermore, at high RF power/gas flow ratios fluorine, aluminium and silicon were incorporated in all investigated plastics surfaces due to ablation-deposition processes in the reaction chamber. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 86: 2618–2625, 2002

Key words: biomaterials; cold plasma; ESCA/XPS; surfaces

INTRODUCTION

With the ongoing trend to miniaturize most kinds of bioanalytical systems, more and more devices will have dimensions where capillary forces are of importance for the introduction of liquids into them. This applies in particular to disposable plastic devices, where the hydrophobic nature of most plastics complicates the introduction of aqueous solutions into narrow openings. An example of such a system is the type of microfabricated cell assay device described in ref. ¹, where microfluidic channels are shaped in a CD disk substrate and covered with a gas permeable silicone rubber lid to permit cell growth in the channels.

To overcome these problems, an attractive solution is to apply some form of hydrophilising surface treatment on the device. Many such processes for plastics have been described in the literature, including flame treatment,² UV/ozone treatment,³ corona treatment,^{4,5} and various types of plasma treatments.^{6–8} A general drawback of these methods is that the polymer molecules in the surface are degraded so that much of the

hydrophilicity results from the presence of low molecular weight oxidized material (LMWOM) on the surface. If the surface is subjected to washing, a major part of the hydrophilisation effect is lost due to dissolution of the LMWOM. Hence, such surfaces are not suitable for devices where more than one portion of liquid is to be introduced through the same opening.

Alternative techniques to produce wash-stable hydrophilic surfaces on plastics mostly involve the attachment of hydrophilic polymeric species to the surface, either by grafting⁹ or by plasma polymerization methods.¹⁰ These methods are generally more complex to use in production scale, as they often need long treatment times and/or handling of hazardous chemicals. Recently,^{11,12} we discovered that polycarbonate and other plastics treated with high-intensity oxygen, air, or argon plasmas could be made extremely hydrophilic, with good stability towards washing with either water or ethanol solutions.

In plasma treatment of polymers, the main factors thought to be responsible for the effects on the surface are (a) free radicals present in the plasma, (b) vacuum UV radiation in the chamber, and (c) impingement of ions on the surface.^{13–15} With plasmas derived from simple gases (oxygen, nitrogen, noble gases, etc.) the major modification effects can be described as: (a) ablation or etching, (b) degradation of polymer molecules, (c)

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crosslinking or branching of near-surface molecules, and (d) introduction of new functional groups.

These four effects occur concurrently and, depending on processing conditions and reactor design, one or more of them may predominate. In most cases, these processes affect only the top few molecular layers (about 100 Å).

In plasma polymerisations, the intensity of the plasma is often expressed by the composite variable P/FM , where P is the discharge power, F the monomer vapour flow rate, and M the monomer molecular weight.¹⁶ This variable has the dimension of W per unit mass of monomer, and is the most commonly used variable for scaling of polymerizing plasmas. For nonpolymerizing simple gas plasmas, the similar variable P/F has also been used as a plasma intensity measure to scale, for example, the ablation rate.¹⁷

When adherent mammalian cells are to be grown in plastic devices, it is generally also necessary to perform a surface modification to create a suitable substrate surface for the cells. Typically, corona treatments, plasma treatments, and UV treatments are used for this purpose.^{5,18} The treatments used in commercially available polystyrene cell culture plasticware have a certain hydrophilizing effect. Measurements we performed on commercial materials indicate that the equilibrium water contact angles are usually in the range of 50–60 degrees, which is lower than for native polystyrene (typically 90 degrees). In microfabricated cell culture devices these surfaces may not, however, be sufficiently hydrophilic. According to our experience, contact angles around 20° are needed for spontaneous introduction of aqueous liquids into microfabricated cell culture chambers covered with a hydrophobic silicone rubber lid.¹ Ref. ¹¹ describes a series of high-intensity air and oxygen plasma treatments of polycarbonate, where the growth properties of adherent mammalian cells on the treated surfaces are as good as on commercial cell culture plates, while the equilibrium water contact angles can be as low as 20 degrees. Contact angle data from the air plasma treatments in ref. ¹¹ are incorporated in this study.

This article will discuss the low-pressure radiofrequency air plasma treatment of different transparent and rigid polymers (chosen to be potentially suitable for bioanalytical devices) and its effect on the polymer surfaces, as well as the results of that treatment on wettability, stability, and cell growth properties. The objective has been to find plasma conditions for the polymers to give optimal surfaces for cell growth.

EXPERIMENTAL

Materials

The following six different polymers were studied: (1) Zeonex® (cycloolefin polymer, Zeonex 480R from Nip-

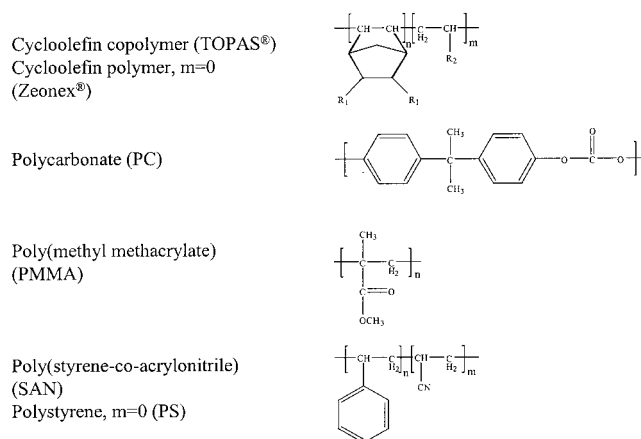


Figure 1 Chemical structures of the polymer materials used.

pon Zeon in Japan); (2) PMMA (polymethyl methyl methacrylate, VQ 105 from Röhm in Germany); (3) SAN (styrene-acrylonitrile copolymer, Luran® S KR2636 from BASF in Germany); (4) PS (polystyrene, 158 K from BASF AG in Germany); (5) Topas® (cycloolefin copolymer, Topas 5513X2 from Ticona in Germany); and (6) PC (polycarbonate of bisphenol A, Makrolon® DP1-1265 from Bayer in Germany). The chemical structures of the six polymers are depicted in Figure 1.

Preparation of surfaces

Injection-molded polycarbonate discs (1-mm thickness) were washed with isopropanol to remove any additives from the surface. They were then subjected to air plasmas (Air Liquide N40, an 80% nitrogen + 20% oxygen mixture of 99.99% purity) in a production-scale Plasma Science PS0500 reactor, equipped with an ENI ACG-5 radiofrequency (13.56 MHz) generator and one internal aluminium electrode (the aluminium reactor vessel acting as the counter electrode). Typical treatment conditions were: 200–500 W RF power, 5–100 sccm gas flow (standard cm³/min), and 5-min treatment time (Table I). These conditions were chosen on the basis of the experience from ref. 11.

Surface characterizations

Equilibrium contact angles of Milli-Q water were measured with the sessile drop method in a Ramé-Hart manual goniometer setup, both for the surfaces directly after plasma treatment and after a 2-min wash in 70% ethanol. Rinsing in 70% ethanol is a common sterilization procedure in cell culture work.

XPS (ESCA) spectra were recorded on a Physical Electronics spectrometer, using monochromatic Al K α X-rays for the excitation, a detector angle of 45° from the surface normal, and a spot size of 1 mm in diameter nominally. Survey spectra were acquired for 15

TABLE I
Experimental Parameters at Air Plasma Treatments for all Materials.

Plasma Condition	Gas Flow (sccm ^a)	Pressure ^b (mTorr)	Treatment Time (min)	Power of the Plasma Reactor (W)	Power/Gas Flow Ratio (W/sccm)
A	100	109–99	5	300	3
B	100	101–109	5	500	5
C	50	73–64	5	300	6
D	50	75–63	5	500	10
E	25	49–43	5	300	12
F	25	49–43	5	500	20
G	10	29–24	5	300	30
H	10	30–25	5	500	50
I	5	19–14	5	300	60
J	5	23–15	5	500	100

^a Scm=standard cubic centimeter per minute.

^b The total variation of the pressure.

min at a pass energy of 187.85 eV and a step size of 0.5 eV/channel. Detail spectra of the C1s region were collected for 15 min at 11.95 eV pass energy and 0.1 eV/channel. A low-energy flood gun set at 3 eV was used to control the charging. The chamber pressure was better than 10^{-8} Torr during measurements.

The samples were cut to a size of approx. 12×5 mm and clamped to the sample holder with metal clamps directly before introduction into the instrument.

The elemental compositions were calculated from the survey spectra using the Multipak software package supplied by the instrument vendor, applying Shirley background correction and sensitivity factors included in the software.

Prior to peak fitting, the C1s peaks were binding energy corrected to 284.8 eV and Shirley background corrected. All spectra from one polymer material were peak fitted in one run, with common settings for peak positions and full half widths. Three to four peaks were enough to obtain a good fit for all materials. The relative peak position and full width at half maximum of peak number 1 to 4 were set to obtain the best fit for all samples of that material. The settings were adjusted for each material.

Culture and microscopical evaluation of cells

Test pieces of the surface-treated samples were placed in multiwell tissue culture plates and sterilized in sealed polyethylene bags at STRIL AB (Kopparberg, Sweden), using a 9.7 MeV electron beam under standard sterilization conditions (25 kGray radiation dose). Commercial tissue culture plates (Costar[®] from Costar Corning Inc. and Nunclon[®] Delta from Nalge Nunc International) and nontreated materials served as positive and negative controls, respectively.

Cell cultures of human cervix carcinoma (HeLa) cells (European Cell Culture Collection) were used for the experiments. This cell line was regarded as the most suitable, because HeLa cells were the most sensitive to variation in polymer surface properties, in comparison

to MRC5 fibroblasts and Chang liver cells.¹⁰ For the experiments, 5000 cells/cm² in 45% Dulbecco's Modified Eagle Medium, 45% Ham's F-12, 10% fetal bovine serum, and 50 μ g/mL gentamicine (all medium components from Life Technologies, Paisley, UK) were seeded onto the test material and cultured for 2 days at 37°C, 7% CO₂, humidified atmosphere.

The amount of cells on each surface (approx. 4 cm²) and their degree of spread were semiquantitatively estimated by phase contrast microscopy at 100–400 \times magnification. A linear arbitrary scale of 0, 1, 2, 3, 4, and 5 was used where 0 represents no cells and 5 represents 100% of that cell number found on the positive control surface. With respect to spread, cells were assigned to different categories where 0 represents floating cells and 5 represents fully spread cells, comparable to cells on the positive control surface. Morphologic signs of cell deterioration such as increased numbers of cytoplasmic vacuoles, lysosomal granulae, membrane blebs, and altered nuclear shape and morphologic indications of motility such as membrane ruffles and dendrites, were evaluated on a qualitative basis. For simplicity, an overall grade was finally determined for each sample. The overall grade was thus based on the estimated value for the amount of cells, the value for the degree of spread and the impression of overall morphology.

RESULTS AND DISCUSSION

Air plasma treatments affect both the wettability and the elemental surface composition of all materials. The most obvious result of the plasma treatment was the improved wettability of the surfaces. Before air plasma treatment the surfaces of the materials Zeonex, PMMA, San, PS, and Topas had equilibrium water contact angles of 92°, 60°, 79°, 89°, and 97°, respectively. Tables II–IV show that air plasma treatment resulted in a decrease of the contact angles for all materials. As Figure 2(a) shows, the contact angles of the different materials decrease substantially already

TABLE II
ESCA and Contact Angle Results of Zeonex and PMMA Treated at Different Air Plasma Conditions

Material	Plasma Condition ^a	Contact Angle after Plasma Treatment (deg)	Contact Angle after Wash ^b (deg)	Elemental Composition (Atom %)					
				C	N	O	F	Al	Si
Zeonex	A	16	36	73.08	0.99	25.93	nd	nd	nd
Zeonex	B	16	29	74.59	1.25	23.85	nd	nd	0.32
Zeonex	C	15	26	74.30	1.39	23.94	nd	nd	0.36
Zeonex	D	9	6	70.05	1.09	28.18	nd	0.20	0.49
Zeonex	E	9	6	70.75	1.36	27.31	nd	nd	0.58
Zeonex	F	5	4	68.97	0.92	28.12	0.34	0.61	1.04
Zeonex	G	6	4	67.68	2.66	28.04	0.57	nd	1.05
Zeonex	H	5	4	68.77	2.12	25.94	0.69	0.69	1.79
Zeonex	I	5	4	64.65	4.07	26.79	2.05	1.18	1.26
Zeonex	J	8	4	60.37	3.63	28.90	3.10	1.86	2.13
PMMA	A	40	53	66.28	nd	33.72	nd	nd	nd
PMMA	B	40	53	66.80	0.88	32.33	nd	nd	nd
PMMA	C	39	53	66.13	1.07	32.28	nd	nd	0.51
PMMA	D	20	40	68.85	1.13	29.60	nd	nd	0.41
PMMA	E	26	44	69.21	1.14	29.16	nd	nd	0.48
PMMA	F	8	21	69.49	0.96	28.30	nd	0.49	0.76
PMMA	G	6	14	68.32	2.31	27.85	nd	0.74	0.78
PMMA	H	4	4	63.28	2.06	30.03	1.75	1.32	1.56
PMMA	I	5	3	63.63	3.57	28.53	1.63	1.38	1.27
PMMA	J	8	4	58.84	4.65	29.20	3.00	2.42	1.90

^a See Table I for details.

^b Treated surface was immersed for 2 min in 70% (V/V) ethanol and then blown dry with nitrogen.
 nd = not detected.

at low power/flow ratios. However, the different materials are influenced to different degrees. If the materials are ranged according to the contact angle directly after the mildest plasma treatment (plasma condition A), the observed contact angles decrease in the order:

PMMA (40°) > Zeonex (16°) > Topas (15°) > SAN (11°) > PS (4°).

None of these surfaces are stable towards washing with 70% ethanol and the contact angles increase after the washing procedure. However, polymer surfaces

TABLE III
ESCA and Contact Angle Results of SAN and Polystyrene Treated at Different Air Plasma Conditions

Material	Plasma Condition ^a	Contact Angle after Plasma Treatment (deg)	Contact Angle after Wash ^b (deg)	Elemental Composition (Atom %)					
				C	N	O	F	Al	Si
SAN	A	11	23	73.14	4.16	22.70	nd	nd	nd
SAN	B	8	27	76.75	4.39	18.09	0.78	nd	nd
SAN	C	9	22	74.38	4.38	21.24	nd	nd	nd
SAN	D	8	7	70.49	3.53	24.95	nd	0.58	0.44
SAN	E	7	14	72.24	3.57	23.34	nd	0.29	0.56
SAN	F	5	7	68.85	2.93	26.00	0.54	0.83	0.86
SAN	G	5	5	68.36	3.99	25.17	0.58	0.84	1.06
SAN	H	3	5	65.13	3.33	27.75	1.14	1.16	1.48
SAN	I	5	3	67.51	4.38	24.50	1.45	1.14	1.03
SAN	J	4	2	60.46	3.99	28.86	2.43	2.23	2.03
PS	A	4	25	74.82	0.97	24.21	nd	nd	nd
PS	B	5	20	75.02	1.24	23.54	nd	nd	0.20
PS	C	4	19	75.65	1.53	22.61	nd	nd	0.22
PS	D	5	8	72.42	1.31	25.86	nd	nd	0.41
PS	E	4	8	72.50	1.85	24.90	nd	0.41	0.35
PS	F	4	6	69.37	1.37	27.16	0.65	0.80	0.64
PS	G	4	5	70.89	1.96	25.64	0.39	0.70	0.42
PS	H	3	5	64.06	1.61	28.80	2.47	1.58	0.90
PS	I	3	4	66.46	3.15	26.88	1.35	1.42	0.74
PS	J	4	4	63.82	2.87	28.34	2.01	2.01	0.94

^a See Table I for details.

^b Treated surface was immersed for 2 min in 70% (V/V) ethanol and then blown dry with nitrogen.
 nd = not detected.

TABLE IV
ESCA and Contact Angle Results of Topas-Treated at Different Air Plasma Conditions

Material	Plasma Condition ^a	Contact Angle after Plasma Treatment (deg)	Contact Angle after Wash ^b (deg)	Elemental Composition (atom %)					
				C	N	O	F	Al	Si
Topas	A	15	29	75.94	0.82	23.24	nd	nd	nd
Topas	B	10	20	74.58	1.73	23.46	nd	nd	0.23
Topas	C	10	24	76.75	1.83	21.42	nd	nd	nd
Topas	D	7	6	70.48	1.28	27.85	nd	nd	0.40
Topas	E	8	5	71.26	1.43	26.81	nd	0.51	nd
Topas	F	4	4	68.36	1.57	29.03	nd	0.64	0.40
Topas	G	3	4	67.97	2.49	27.73	0.52	0.88	0.40
Topas	H	3	6	64.34	2.55	29.42	1.08	1.55	1.05
Topas	I	4	4	62.93	3.70	28.30	1.90	2.22	0.64
Topas	J	5	4	60.55	3.34	30.02	2.31	2.54	1.24

^a See Table I for details.

^b Treated surface was immersed for 2 min in 70% (V/V) ethanol and then blown dry with nitrogen. nd = not detected.

stable to washing can be obtained for all materials as shown in Figure 2(b). Plasma treatment at a power/flow ratio of 10 W/sccm or larger (Tables II–IV) was required for all materials, except for PMMA and PC,

to receive stable surfaces. In case of PMMA and PC, the air plasma treatment had the least effect on the contact angle and the surface stability; therefore, a power/ratio of ca 30–50 W/sccm was required for wash stable surfaces. It can be noted that all surfaces that are wash stable have contact angles lower than 10° before washing.

The most obvious explanation of the somewhat different behavior of PMMA and PC compared to the other polymers is that they are more prone to degradation during the plasma treatment. PMMA in particular is known to degrade via a depolymerization mechanism under plasma conditions,¹⁹ and is generally known as a “difficult” polymer in the plasma world.²⁰ In ref. 6, the lower wash stability of oxygen plasma-treated PC compared to PS is explained by the greater tendency of PC to form LMWOM on the surface due to degradation effects.

The elemental composition of the surface of most of the materials shows that the main effects of the plasma treatment are that the carbon content decreases and the nitrogen, oxygen, fluorine, aluminium, and silicon contents increase when the power/flow ratio increases (Tables II–IV). However, the nitrogen content of SAN and the carbon and oxygen content of PMMA did not follow this trend (Tables II and III). Because the molecular structures of untreated SAN and PMMA contain nitrogen and oxygen, respectively, this can probably explain the behavior of these materials.

The introduction of oxygen-containing functional groups in polymer surfaces treated with oxygen or air plasmas is a well-known phenomenon. It is believed that much of the oxidation is caused by radical reactions between the polymer chain backbones and atomic oxygen in the plasma,¹⁹ leading to various functionalities such as hydroperoxides, carbonyls, carboxylic acids, peracids, etc. In the case of sensitive polymers such as, for example, PMMA, the radical reactions can often cause depolymerization and evaporation of the formed monomer.

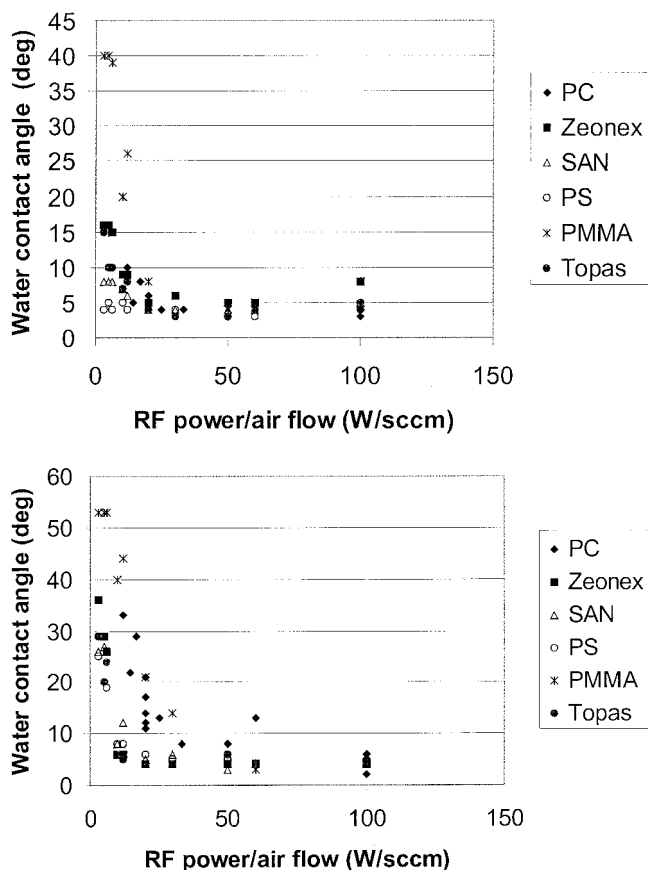


Figure 2 Water contact angle on plasma-treated Zeonex, PMMA, San, PS, and Topas vs. the ratio RF power/gas flow ratio in the plasma treatment. The contact angle measurements were made directly after the plasma treatment. PC data from ref. 7. Water contact angle on plasma-treated Zeonex, PMMA, San, PS, and Topas vs. the ratio RF power/gas flow ratio in the plasma treatment. The contact angle measurements were made after washing the surfaces in 70% ethanol. PC data from ref. 7.

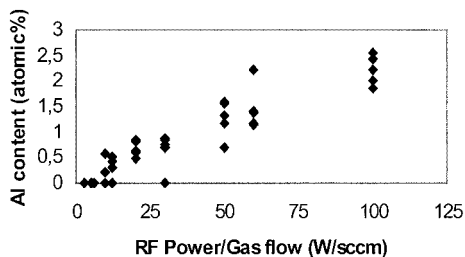


Figure 3 Aluminium content vs. the ratio RF power/gas flow in the plasma treatment for all materials.

The observation of fluorine, aluminium, and silicon on the different material surfaces after air plasma treatment at high power/gas flow ratios is probably a result of ablation–deposition processes in the reactor chamber. The sides of the chamber are covered with glass plates, and both the electrode and the chamber walls are made from aluminium. Figure 3 shows how the surface content of aluminium increases with the power/gas flow ratio for all materials. Silicon was detected at a lower power/flow ratio compared to aluminium and fluorine, whereas incorporation of fluorine on the surface of all investigated materials is only detected at more energetic plasma settings (Tables II–IV). The fluorine could originate from Viton O-rings in the reactor or possibly also from the per-fluorinated polyether vacuum oil in the pump system. These observations indicate that the results from plasma treatment with the same plasma settings but with different reactor chambers can significantly affect the obtained surface characteristics. This is further studied in ref. 21.

To check whether the amounts of nitrogen, oxygen, fluorine, aluminium, and silicon vary with the distance from the surface, angle-resolved ESCA was used. Figure 4 shows that all elements except carbon increased towards the surface when PMMA was air plasma treated.

To understand the effect of the plasma treatment more precisely the shape of the C 1s peak was evaluated. Air plasma treatment leads to significant widening of the C 1s peak, especially for the materials Zeonex, PS, and Topas. These materials do not inherently contain any oxygen or nitrogen atoms. From the curve fitting of the C 1s peak (Table V) the assignments were taken as carbon only bound to carbon and hydrogen (peak 1), carbon bound to hydroxyl, ether, or epoxide (peak 2), carbonyl carbon or carbon bound to double ether (peak 3), and carboxylic acid carbon (peak 4). A curve fit of the C 1s peak for plasma treated Zeonex is depicted in Figure 5. The materials Zeonex, PS and Topas resulted in a significant decrease of peak 1 relative to the other peaks when a more intense plasma condition was used (Table V). Peak 1 of PMMA increased relative to the other peaks when the plasma condition was changed from A to J. However, the C 1s peak of untreated PMMA gave a ratio be-

tween peak 1, peak 2, and peak 3 corresponding to 57, 23, and 20%, respectively. The ester carbon in the PMMA structure evidently appears in peak 3, which is consistent with the chemical shift of 4.03 V for ester carbons reported in ref. 22. It can, therefore, be concluded that the surface concentrations of the different carbon groups vary in a nonlinear way with the plasma intensity. Furthermore, no large differences between the fitted C 1s peaks could be observed for SAN after different air plasma treatments (Table V). Peak 4 was not observed on any of the untreated materials, and only the two materials PS and Topas showed any measurable amounts of carboxylic acid carbon after plasma treatment (Table V).

The main purpose of this investigation was to find whether it was possible to modify the different plastic surfaces in such a way that excellent cell growth properties could be obtained. The results were compared with commercially available tissue culture-treated plastics. For untreated and air plasma-treated materials the estimated grades for HeLa cell amount, spread, and overall grade are shown in Table VI. The estimates for cells cultured on untreated materials ranged between 2–3, and for cells cultured on the positive control surfaces (Costar and Nunclon) the estimates were invariably 5. Zeonex and PS surfaces treated at plasma conditions A, B, C, and D (only for PS) resulted in the best surfaces for growth of HeLa cells. For these surfaces the estimated grades were 5 for cell number, spread, and overall grades (Table VI) and contact angles (after wash) were in the range 8° to 36° (Tables II and III). It can also be noted that SAN treated at condition C resulted in an optimal surface for HeLa cells. For all materials, except PMMA, treatment under intense plasma conditions (plasma condition I and J) resulted in unsatisfactory cell culture results. The surfaces at these conditions have a higher content of oxygen, fluorine, aluminium, and silicon and a lower content of carbon compared to the surfaces obtained at milder plasma conditions (Tables II–IV) and the contact angles were lower than 5°. It can be concluded that only one polymer surface (PS treated at plasma condition D) with low contact angle (lower than 10°) and a wash stable surface has been

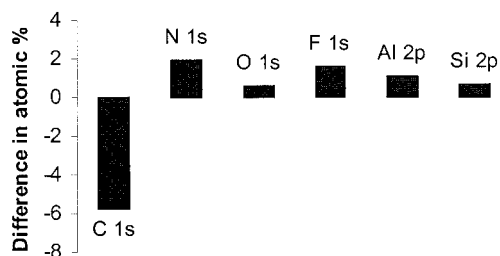


Figure 4 Angular variation in atomic % for air plasma-treated (plasma condition J according to Table I) PMMA. The difference in atomic % is expressed as (atomic % at an emission angle of 45°—atomic % at an emission of 25°).

TABLE V
Esca Results of Peak Fitted C 1s Spectrum from Different Air Plasma-Treated Materials

Material	Plasma Condition ^a	Peak Composition of the Deconvoluted C 1s Spectrum			
		Peak 1 ^b (%)	Peak 2 ^c (%)	Peak 3 ^d (%)	Peak 4 ^e (%)
Zeonex	A	71.76	17.03	11.21	nd
Zeonex	J	69.13	22.73	8.14	nd
PMMA	A	51.63	24.34	24.02	nd
PMMA	J	66.82	18.59	14.59	nd
SAN	A	60.89	27.78	11.33	nd
SAN	I	62.71	25.61	11.69	nd
PS	A	72.27	11.19	7.83	8.72
PS	J	64.75	19.21	10.45	5.59
Topas	A	80.67	7.45	5.81	6.06
Topas	J	73.51	13.70	11.06	1.73

^a See Table I.

^b Peak 1 corresponds to a binding energy 284.8 eV.

^c Peak 2 corresponds to a binding energy in the interval 286.3–286.8 eV.

^d Peak 3 corresponds to a binding energy in the interval 287.8–288.9 eV.

^e Peak 4 corresponds to a binding energy in the interval 289.2–289.4 eV.

nd = not detected.

found to be a good cell-culture surface. Most of the optimal cell culture surfaces have contact angles in range 19–36° and, consequently, they were not entirely stable to washing. However, polymer surfaces with contact angles in that range do not always result in good growth of HeLa cells. This is illustrated by the results from Topas and PMMA. A tentative explanation of why these two materials are less cell-friendly could be that some cytotoxic substance is leached out from the surfaces (as, e.g., methyl methacrylate monomer in the PMMA case). The difference between the chemically closely related materials Zeonex and Topas is striking, and might possibly indicate a negative effect from some kind of additive in the Topas material. Comparing the ESCA results of the two at higher

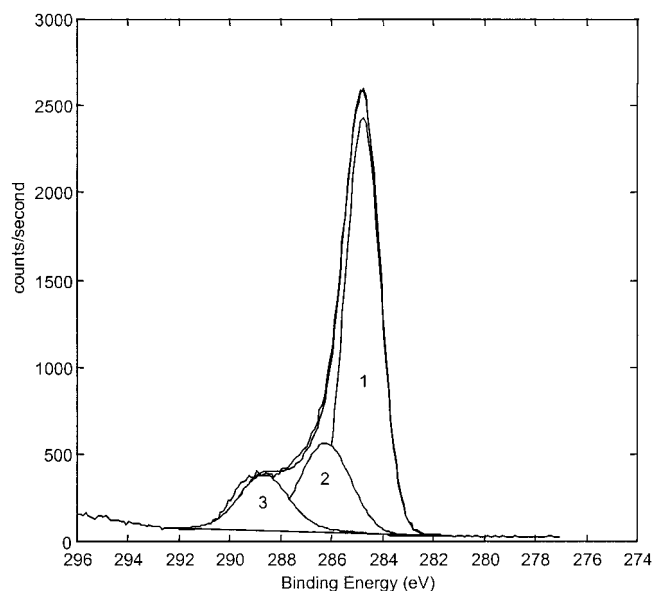


Figure 5 C 1s peak fit of air plasma-treated Zeonex at the lowest power/flow ratio (3 W/sccm).

TABLE VI
Cell Culture Results for HeLa Cells on Different Untreated and Air Plasma-Treated Plastic Surfaces

Material	Plasma Condition ^a	Cell Number	Cell Spread	Overall Grade
Zeonex	Untreated	3	2	3
Zeonex	A	5	5	5
Zeonex	B	5	5	5
Zeonex	C	5	5	5
Zeonex	D	3	3	3
Zeonex	I	4	3	4
Zeonex	J	4	3	4
PMMA	Untreated	2	2	2
PMMA	A	3	2	3
PMMA	B	3	2	3
PMMA	C	3	1	2
PMMA	D	3	2	3
PMMA	I	3	3	3
PMMA	J	4	4	4
SAN	Untreated	2	2	2
SAN	A	5	4	4
SAN	B	5	4	4
SAN	C	5	5	5
SAN	D	4	4	4
SAN	I	3	3	3
SAN	J	4	3	3
PS	Untreated	3	3	3
PS	A	5	5	5
PS	B	5	5	5
PS	C	5	5	5
PS	D	5	5	5
PS	I	4	2	3
PS	J	3	3	3
Topas	Untreated	2	2	2
Topas	A	4	2	3
Topas	B	5	3	4
Topas	C	4	4	4
Topas	D	3	2	3
Topas	I	4	3	3
Topas	J	2	1	2

^a See Table I for details.

plasma intensities, two differences were also noticed. First, that more oxygen was incorporated into the Topas surface, and second, that the added oxygen was found in curve fit peaks 2 and 3, while it was mainly found in peak 2 for the Zeonex. Apparently the two materials were different enough for the plasma treatment to affect the two materials differently.

For the used cell line (HeLa cells) it seems that the cell spread properties are most influenced by the different plasma treatments (Table VI).

CONCLUSIONS

Low-pressure radiofrequency air plasma treatments of five polymers (Zeonex, PMMA, SAN, PS, and Topas) at different RF power/gas flow ratios (3–100 W/sccm) have shown that plasma conditions for all polymers can be found, that result in surfaces that are hydrophilic and stable towards washing with 70% ethanol. A considerable decrease in contact angle (below 20°) was observed even after treatment at the least intense plasma condition for all polymers except PMMA. However, it can be noted that wash stable surfaces were only obtained when the equilibrium water contact angle before washing was lower than 10°. Furthermore, polymers not containing heteroatoms (Zeonex, Topas, and PS) could be transformed to hydrophilic and stable surfaces at lower RF power/gas flow ratio treatments compared to PMMA and SAN. For Zeonex, Topas, and PS the plasma treatment resulted in a decrease of carbon content and an increase in nitrogen and oxygen contents at the surface when the RF power/flow ratio increased. In addition, the surface concentration of fluorine, aluminium, and silicon increased for all investigated polymers with the power/flow ratio.

The cell (HeLa) growth properties on the treated polymer surfaces show that the growth results could not be solely rationalized from the contact angle, wash stability, or elemental composition of the surfaces. Only three of the polymers (Zeonex, PS, and SAN) were possible to modify with air plasma to obtain cell growth properties as good as commercial tissue culture plastics. One result these polymers have in common is that good cell growth surfaces were only obtained when the less intense plasma treatments (3–60 W/sccm) were used. These surfaces were somewhat less hydrophilic (contact angles in several cases above 20°). Furthermore, good cell growth properties were not possible to obtain with air plasma treatment of PMMA and Topas. Because Topas and Zeonex are

both cycloolefin polymers, the results indicate that not only the bulk polymer structure and plasma treatment conditions influence the cell culture properties. For example, nonspecified additives in the polymers such as antistatic agents, antioxidants, and lubricants may affect the results.

References

1. Thomas, N.; Ocklind, A.; Blikstad, I.; Griffiths, S.; Kenrick, M.; Derand, H.; Ekstrand, G.; Ellström, G.; Larsson, A.; Andersson, P. In *Micro Total Analysis Systems 2000*; van den Berg, A., et al., Eds.; Kluwer Academic Publishers: The Netherlands, 2000; pp 249.
2. Strobel, M.; Walzak, M. J.; Hill, J. M.; Lin, A.; Karbasheski, E.; Lyons, C. S. *J Adhes Sci Technol* 1995, 9, 365.
3. Teare, D. O. H.; Thon-That, C.; Bradley, R. H. *Surf Interface Anal* 2000, 29, 276.
4. Strobel, M.; Dunatov, C.; Strobel, J. M.; Lyons, C. S.; Perron, S. J.; Morgen, M. C. *J Adhes Sci Technol* 1989, 3, 321.
5. Onyiriuka, E. C.; Hersh, L. S.; Hertl, W. *J Coll Interface Sci* 1991, 144, 98.
6. Murakami, T.; Kuroda, S.; Osawa, Z. *J Coll Interface Sci* 1998, 202, 37.
7. Morra, M.; Occhiello, E.; Garbassi, F. *Angew Makromol Chem* 1991, 189, 125.
8. Greenwood, O. D.; Hopkins, J.; Badyal, J. P. S. *Macromolecules* 1997, 30, 1091.
9. Loh, F. C.; Tan, K. L.; Kang, E. T.; Neoh, K. G.; Pun, M. Y. *Eur Polym J* 1995, 31, 481.
10. Yasuda, H. *Plasma Polymerization*; Academic Press: New York, 1985; p 344.
11. Larsson, A.; Ocklind, A. In *Proceedings of the Second International Symposium on Polymer Surface Modification*; Mittal, K. L., Ed.; VSP: Utrecht, 2000; p 121.
12. PCT WO 00/56808.
13. Denes, F.; Young, R. A.; Sarmadi, M. *J Photopolym Sci Technol* 1997, 10, 91.
14. Fozza, A. C.; Klemberg-Sapieha, J. E.; Wertheimer, M. R. *Plasmas Polym* 1999, 4, 183.
15. Zabeida, O.; Klemberg-Sapieha, J. E.; Martinu, L.; Morton, D. In *Plasma Deposition and Treatment of Polymers*; Lee, W. W.; d'Agostino, R.; Wertheimer, M. R., Eds.; Materials Research Society: Warrendale, PA, 1999; p 233.
16. Yasuda, H. *Plasma Polymerization*; Academic Press: New York, 1985; p 301.
17. Yasuda, H. *Plasma Polymerization*; Academic Press: New York, 1985; p 186.
18. Chinn, J. A.; Horbett, T. A.; Ratner, B. D. *J Tissue Culture Methods* 1994, 16, 155.
19. Lianos, L.; Parrat, D.; Hoc, T. Q.; Duc, T. M. *J Vac Sci Technol* 1994, a12, 2491.
20. Klemberg-Sapieha, J. E.; Martinu, L.; Yamasaki, N. L. S.; Lantman, C. W. In *Plasma Deposition and Treatment of Polymers*; Lee, W. W.; d'Agostino, R.; Wertheimer, M. R., Eds.; Materials Research Society: Warrendale, PA, 1999; p 277.
21. Larsson, A.; Derand, H. *J Coll Interface Sci* 2002, 246, 214.
22. Beamson, G.; Briggs, D. *High Resolution XPS of Polymers*, The Scienta ESCA 300 Database; Exeter, UK, 1992.