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Surface modification of thermoplastics—towards the plastic biochip for high throughput screening devices†

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Microarrays have become one of the most convenient tools for high throughput screening, supporting major advances in genomics and proteomics. Other important applications can be found in medical diagnostics, detection of biothreats, drug discovery, *etc*. Integration of microarrays with microfluidic devices can be highly advantageous in terms of portability, shorter analysis time and lower consumption of expensive biological analytes. Since fabrication of microfluidic devices using traditional materials such as glass is rather expensive, there is great interest in employing polymeric materials as a low cost alternative that is suitable for mass production. A number of commercially available plastic materials were reviewed for this purpose and poly(methylmethacrylate) Zeonor 1060R and Zeonex E48R were identified as promising candidates, for which methods for surface modification and covalent immobilization of DNA oligonucleotides were developed. In addition, we present proof-of-concept plastic-based microarrays with and without integration with microfluidics.

Introduction

Rapid progress in life sciences relies strongly on improvements in the areas of genomics and proteomics. Such advances are made possible with high throughput screening systems, which are for the most part, based on microarray technologies. DNA microarrays, for example, play a vital role in the study of gene expression across the entire genome in a single experiment.[1,2] Recently, there has been an increased interest in combining microfluidics with microarrays since this approach is highly advantageous in terms of portability, shorter analysis time and lower consumption of precious biological analytes. This is particularly important for potential applications in not only genomics and proteomics, but also in medical diagnostics, biothreat detection, drug discovery, chemical catalysis, *etc*. Such applications have not only fuelled interest in academia but also in commercial companies such as Micronics, Biosite, Handylab, Spin-x Technologies, Gyros, and Agilent technologies.

Medical diagnostics is an area of great interest and this has recently fuelled an increased interest in the development of a disposable rapid medical theranostics (diagnostics and therapeutics) system for point-of-care use. However, commercialisation of microfluidic devices is still hindered by the nature of polymeric materials which should be amenable for industrial fabrication and yet possess excellent optical properties such as low intrinsic fluorescence and high transparency. In addition, the surface modification of these plastics needs to be simple, robust and industrially scalable. We present herein our contribution to the selection of the plastic support, surface modification and immobilization of biomolecules on plastic surfaces. Moreover, we present a proof-of-concept microfluidic device for rapid hybridisation of DNA arrays thereby setting the stage for the development of a medical diagnosis device that is based on the detection of pathogenic DNA.

Experimental

All chemical reagents were purchased from Aldrich, except for 5-(and-6)-((*N*-(5-aminopentyl) amino)carbonyl) tetramethyl rhodamine (also known as tetramethylrhodamine cadaverine) which was purchased from Molecular Probes. Oligomers of DNA were custom synthesized by the microarraying facilities at NRC's Biotechnology Research Institute (BRI). Zeonor and Zeonex resins were purchased from Zeon Chemicals. PMMA-VSUVT, -HT121 and -825 were obtained from Atoglas. PMMA-OptixCA41 was purchased from Plexiglas. Topas resins were obtained from Ticona.

Infrared spectra were collected with a Nicolet Magna 860 infrared spectrometer equipped with a single reflection ATR (Attenuated Total Reflectance) accessory. XPS spectra were

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† Electronic supplementary information (ESI) available: Optical transmission curves for the initial set of potential plastics (Fig. S1). Steady state emission fluorescence spectra from the plastics under consideration. Excitation was performed at 543 nm (Fig. S2). Steady state emission fluorescence spectra from the plastics under consideration. Excitation was performed at 633 nm (Fig. S3). Infrared spectra from Zeonor 1060R before and after oxidation with ozone and UV-ozone (Fig. S4). High-resolution carbon 1 s spectrum of pristine Zeonex E48R (Fig. S5). Fluorescent images of a hybridised array of two different strands of DNA on Zeonex E48R illustrating selective binding of each complementary strand (Fig. S6). See DOI: 10.1039/b700322f

collected with a PHI-5500 XPS spectrometer from Physical Electronics operated with a monochromatic Al X-ray source at 300 W. Sample charging was minimized with an electron flood-gun which was adjusted accordingly to minimize line widths. In all cases, the C 1 s aliphatic peak was set to 285 eV. Optical transmission spectra from 1 mm thick sheets of plastics were collected with a Beckman DU-640 spectrometer. Fluorescence spectra from solid rectangular prisms (10 × 6 × 40 mm) for each plastic were acquired with a SPEX fluorolog2-F111AI using 1.25 mm fixed slits.

Surface oxidation of Zeonor and Zeonex plastic slides was accomplished in 1 h with O_3 zomax OZO-2VTT ozone generator that produced 3% ozone. PMMA slides were modified by immersion in a saturated aqueous solution of sodium hydroxide for at least 4 h including 1 h sonication. The resultant Zeonor, Zeonex and PMMA slides were further activated with a freshly prepared solution of 8 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 2 mg *N*-hydroxysuccinimide (NHS) in 100 μL of phosphate buffered saline (PBS, pH = 7.4) solution for 1 h.

Proof-of-concept arraying of oligonucleotides on chemically modified slides was accomplished by dispensing *via* capillary action a 6 μM solution of an amino-modified single stranded oligonucleotide (15 mer) using a 0.5–10 μL eppendorf pipette mounted on an XYZ micro translator from ThorLabs (MT3). The arrayed oligonucleotide solutions were allowed to react with plastic surfaces for 1 h in a humid chamber at 37 °C. Unreacted oligonucleotide was removed by washing the slides with a solution of 0.1% sodium dodecyl sulfate (SDS) in PBS. Slides were further treated with a 1 M solution of ethanolamine in PBS (pH = 8.4) for 1 h.

Hybridisation buffer consisted of a solution containing 6 × SSPE buffer, 0.03% polyvinylpyrrolidone and 30% formamide. All hybridisation experiments were carried out at room temperature for 1 h using an equimolar mixture of the complementary and non-complementary strands at a concentration of 0.8 μM. In order to remove non-specifically bound DNA, all microarrays were washed 3 times with 0.1% SDS in PBS for 5 min each time. Fluorescent images of the hybridised oligonucleotides were acquired with a ScanArray Lite-ALCST01 from Perkin Elmer. Injection of solutions in the microfluidic experiments were carried out with a syringe pump from Kd Scientific Inc. (Kds210) using a flow rate of 1.4 μL min^{-1} for an equimolar mixture of complementary (target #1) and non-complementary (target #2) DNA (0.8 μM, 10 μL) and 8.3 μL min^{-1} for the washing solution (0.1% sodium dodecyl in PBS, 50 μL).

Results and discussion

Plastic support selection

Poly(dimethyl siloxane) is the most popular material that is employed in the fabrication of microfluidic devices. However, this substrate is not suitable for large-scale fabrication of devices. In addition, it has been recently demonstrated that PDMS exhibits a high degree of absorption of small molecules.[3] This issue introduces a number of difficulties in potential applications for drug screening and quantitative measurements. Finally, it has been reported by Cesaro-Tadic *et al.* that PDMS exhibits a significant fluorescence background at excitation and emission wavelengths near 530–570 nm.[4] Consequently, new materials are being sought for potential applications.

The ideal plastic needed for the fabrication of high throughput screening devices that are based on fluorescence detection should have the following properties: (i) provide rapid microfabricaton and scalable production that will ensure low-cost manufacturing, (ii) low intrinsic fluorescence background as well as low fluorescence due to non-specific-binding and (iii) allow a simple, scalable and environmentally friendly surface chemistry for the immobilization of biomolecules.

Evaluation of all properties would require proof-of-concept devices for a large number of plastics and this is prohibitively demanding in terms of resources and time. Consequently, plastics were pre-screened solely based on their performance in micromachining, namely hot embossing, optical transmission and intrinsic fluorescence. The microfabrication requirement can be met by restricting the potential plastics to thermoplastics since embossing is a suitable technique for industrial fabrication. Further refinement of the list of potential plastics was accomplished by excluding polymers containing aromatic or conjugated systems since they are expected to have a significant intrinsic fluorescence.

As a consequence, different commercial grades of poly(methylmethacrylate) (PMMA: VSUVT, HT121, V825 and OptixCA41) and a number of poly(cyclic olefins) available commercially under the trade names of Topas (5013 and 8007), Zeonor (480, 750R, 1020R, 1060R and 1600) and Zeonex E48R formed the initial set of potential polymeric resins.

Optical transmission curves are presented in Fig. S1† for the plastics under consideration. As expected the optical properties for any particular polymer are highly dependent on the commercial grade. For instance, the optical transparencies for the four different commercial grades of PMMA in Fig. S1 vary significantly. Defining 50% transmittance as the minimum acceptable transparency, PMMA-VSUVT has the widest transparency window for wavelengths as short as 275 nm. PMMA-V825 does not transmit in the UV region since it is not transparent below 365 nm. The other two grades of PMMA are the least transparent materials as they do not transmit efficiently below approximately 380 nm. The large variation in optical transparencies is most likely due the nature of additives such mould-releasing agents that are present in commercial resins. In fact, one could easily show that pure PMMA should be transparent to wavelengths as short as 255 nm based on the UV-visible spectrum of pure ethyl acetate.[5]

From the poly(cyclic olefins) that were studied, Zeonor 480 and 750R have the highest transparencies in the UV-visible region since they transmit at wavelengths as short as 285 and 270 nm, respectively. All other poly(cyclic olefins) reach 50% transmittance at 295 nm.

It can be concluded that all these plastics are optically transparent in the region between 400 and 700 nm. This transparency window is sufficiently wide for a large number of fluorophores, particularly the most popular fluorescent dyes, Cy3 and Cy5, which have excitation wavelengths at 550 and 650 nm and emission wavelengths at 570 and 670 nm, respectively.

In addition to the optical transparencies, we have examined the bulk intrinsic fluorescence of these promising plastics. Steady state fluorescence emission spectra from plastic rectangular prisms were acquired using excitation wavelengths at 543 and 633 nm, which correspond to the ones employed for the detection of Cy3 and Cy5 in Perkin Elmer biochip scanners.

The emission spectra obtained using an excitation wavelength of 543 nm are presented in Fig. S2. Fluorescence emission from the plastics are compared to that of glass since the latter is the traditional material for DNA microarrays. It should be noted that the region of interest for fluorescence emission is between 570 and 590 nm for the detection of Cy3. Several conclusions can be drawn from this set of spectra. Firstly, Zeonor 480 has a strong fluorescent emission and therefore it is not a suitable substrate for the fabrication of devices that employ fluorescence detection in the Cy3 region. Secondly, three polymers, Topas 5013, PMMA-V825 and Zeonor 1600, have a higher intrinsic fluorescence when compared to other polymers. However, surface fluorescence measurements using a standard microarray scanner indicated that the background fluorescence from PMMA-V825 is acceptable since it is low compared to the signals observed with typical DNA microarrays. As a consequence, all these plastics with the exception of Zeonor 480 are suitable for fluorescence detection in the Cy3 region. However, Zeonor 1060R has the lowest fluorescent background in this region.

A second region of interest corresponds to that of Cy5 fluorophore since it is a standard fluorescent dye in DNA microarray technologies. Fluorescent spectra using an excitation wavelength of 633 nm were acquired in the same manner as before and the uncorrected spectra are presented in Fig. S3. This excitation wavelength corresponds to the laser employed in Perkin Elmer biochip scanners. It is clear from this figure that PMMA-HT121, Topas 8007 and PMMA-V825 have the highest fluorescence emission in the region near 670–680 nm. In fact, surface fluorescence measurements (Table 1) using a Perkin Elmer biochip scanner indicated that the background fluorescence for PMMA-HT121 and -V825 are 127 and 45 times higher than the intrinsic fluorescence of glass. Based on these data, these two PMMA resins and Topas 8007 may be excluded from the list of potential plastics. In addition, PMMA-OptixCA41 may also be excluded from the list of potential plastics since it has a fluorescence background that is 19 times higher than that of glass. It follows that Zeonor 1020R and Zeonor 480 should also be excluded since they have higher fluorescence backgrounds than PMMA-OptixCA41. As a consequence, PMMA-VSUVT, Zeonor 750R, Zeonor 1600, Topas 5013, Zeonex E48R and Zeonor 1060R are potential materials for fluorescence detection in the Cy5 region with Zeonor 1060R having the lowest fluorescence background.

Table 1 Relative surface fluorescence from selected plastics

Substrate	Cy3 region	Cy5 region
Glass	1 ± 0.3	1 ± 0.3
PMMA-VSUVT	1.8 ± 0.5	2.8 ± 0.5
PMMA-V825	3.9 ± 0.8	45 ± 4
PMMA-HT121	2.2 ± 0.5	127 ± 5
PMMA-OptixCA41	6 ± 1	19 ± 2
Zeonor 1060R	1.1 ± 0.5	1.8 ± 0.5
Zeonex E48R	1.4 ± 0.5	2.3 ± 0.5

Based on all the optical properties that have been presented, PMMA-VSUVT and Zeonor 1060R have been selected as the best polymeric materials for devices using fluorescence detection in the Cy3 and Cy5 fluorophore regions. It is also worth considering that fully integrated devices such as micro Total Analysis Systems (μTAS) based on fluorescence detection may be required to operate at temperatures close to the boiling point of water as it is the case for DNA amplification. For such situations, Zeonex E48R is the material of choice due to its excellent optical properties and its relatively high glass transition temperature (T_g = 139 °C) as compared to PMMA-VSUVT (T_g = 94 °C) or Zeonor 1060R (T_g = 100 °C).

Surface chemistry and immobilization of DNA on poly(cyclic olefins)

Generally poly(cyclic olefins) are obtained from the copolymerisation of ethene and a cyclic olefin. For instance, Topas (Ticona Inc.) and APEL (Mitsui Chemical) are synthesized *via* chain copolymerisation of monomers such as 8,9,10-trinorborn-2-ene (norbornene) or 1,2,3,3,4,4a,5,8,8a-octahydro-1,4:5,8-dimethanonaphthalene (tetracyclo dodecene) with ethene yielding a saturated hydrocarbon. On the other hand, Zeonex and Zeonor resins are produced by ring-opening metathesis polymerisation of a number of cyclic olefinic monomers, followed by a hydrogenation step. The hydrogenation step is necessary for increasing the thermal stability of the plastic by removing carbon–carbon double bonds that are necessarily formed during the polymerisation process.[6–8]

Although there exist poly(cyclic olefins) that contain ethers, esters and aromatic groups, a number of commercial poly(cyclic olefins) are saturated hydrocarbons. Consequently, it is difficult to introduce suitable functional groups at the surface of these chemically inert plastics which are expected to have a low intrinsic fluorescence background.

Several approaches are presented in the literature for the chemical modification of poly(cyclic olefins). For instance, plasma treatment has been employed successfully to increase the hydrophilicity of the plastics.[9–13] This is a rather aggressive method that generates a large number of polar groups such as hydroxyl groups. However, this technique is difficult to implement on an industrial scale since it requires expensive high vacuum systems. A second approach involves reacting the polymeric surface with highly reactive intermediates[14,15] such as free radicals,[16–18] nitrenes[19–21] or carbenes.[22–24]

We present a simple, economical and effective method for the surface modification of poly(cyclic olefins). To our knowledge, this is the first time this approach has been applied to saturated poly(cyclic olefins) in conjunction with the immobilization of biomolecules. The method involves the oxidation of the surface with ozone in order to generate a number of polar groups. It is known from previous literature reports that ozone oxidation of other plastics generates hydroxyl groups, esters, ketones and carboxylic acids.[25–27] Although the mechanism for the oxidation process is not known with certainty, some studies indicate that hydroperoxides and peroxyl radicals are involved.[28] However, there

is considerable evidence for the formation of hydrotrioxides which have been observed at low temperatures and which are formed *via* a direct insertion of ozone in a C–H bond.[29] These reactive intermediates are the precursors for the formation alcohols and carbonyl-containing species. Indeed, we have studied the rapid oxidation of poly(cyclic olefins) with ozone in the presence and absence of UV light (Fig. 1).

Ozone oxidation in the absence of UV light is an effective method for the generation of chemically reactive groups at the surface without increasing the fluorescence background of the modified plastic. On the other hand, our studies indicate that the fluorescence background of the plastics does increase by 2 orders of magnitude upon UV photolysis of ozone during a period of 1 h. Examination of the infrared spectrum of ozone oxidized Zeonex E48R (Fig. 1) indicates that a broad band that corresponds to the OH stretch is observed in the region between 3600 and 2100 cm^{-1} with a maximum at 3400 cm^{-1}. Such a broad OH stretch band is characteristic of carboxylic acids. However, the maximum at 3400 cm^{-1} does indicate that alcohols are also contributing to this broad signal. This is in agreement with the literature since the formation of alcohols and carboxylic acids during ozonation has been reported for other polymeric systems.[25,27]

The second most significant feature of the infrared spectrum is the appearance of a strong signal at 1708 cm^{-1} that corresponds to a C=O stretch from species with carbonyl or carboxyl groups. It is very likely that this signal is due to ketones, carboxylic acids and esters. Typically, unconjugated ketones appear at 1715 cm^{-1} (cyclohexanone). However, the resonance frequency of ketones does vary significantly depending on the chemical environment.[30] Since the oxidation process of poly(cyclic olefins) produces highly polar surfaces, it is expected that the ketones which are formed will have their resonance frequencies lowered by the polar environment. The formation of ketones has been reported previously for the ozone oxidation of other polymeric systems.[25]

Closer inspection of the peak at 1708 cm^{-1} indicates that it has a strong overlapping band (2/3 the height) at 1730 cm^{-1} which is attributed to the formation of carboxylic acids and esters. The presence of carboxylic acids has also been corroborated by observing the characteristic resonances for carboxylate after reaction with ammonia (data not shown).

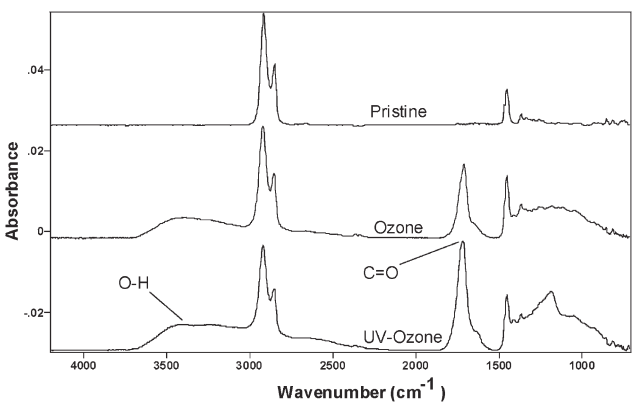

Fig. 1 Infrared spectra from Zeonex E48R before and after oxidation with ozone and UV-ozone.

The resonance at 1730 cm^{-1} indicates that the carboxylic acids are engaged in extensive hydrogen bonding. Of course, conjugation to carbon–carbon double bonds that might be formed can significantly lower the resonance frequency as well. The formation of esters cannot be confirmed or ruled out based on the present data. However, their formation has been reported during ozone oxidation of other plastics.[25]

A rather weak and broad signal at 1630 cm^{-1} is also observed to overlap with the 1708 cm^{-1} band. It is very likely that this band might be due to the formation of carbon–carbon double bonds. Indeed, the formation of double bonds has been reported during the oxidation of polypropylene.[31]

The presence of ethers cannot be confirmed or ruled out with the present data since their expected C–O stretch in the region of 1150–1085 is obscured by a broad signal that extends from 1430 to 700 cm^{-1}. The broad signal might be due to significant overlap of infrared bands from chemically different ketones, carboxylic acids, *etc*. Similar spectra are observed for the oxidation of Zeonor 1060R (Fig. S4).

On the other hand, UV-ozone oxidation is a much more aggressive treatment since ozone is directly photolysed with UV light at 254 nm to give a molecule of oxygen and an oxygen atom. Consequently, the infrared spectrum of UV-ozone oxidized Zeonex E48R (Fig. 1) exhibits more intense bands for the OH and C=O stretch. It should be noted that the position of the carbonyl signal increases by 8 cm^{-1} compared to the corresponding signal that is obtained from the sample that was oxidized in the absence of UV light. The shift can be attributed to the formation of a higher proportion of carboxylic acids and perhaps esters.

Besides the bands related to the OH and C=O stretch, a new broad band is observed at 1180 cm^{-1} during the photolysis of ozone. This band may arise from the formation of peroxides. In fact, formation of peroxides has been reported during the oxidation of other plastics.[31,32] Unfortunately, the photolysis of ozone induces a slightly blue colour and a high fluorescence background in the oxidized plastics. Consequently, this method is not suitable for the surface modification of plastics devices that are based on fluorescence detection. Therefore, all the surface modifications that are reported in the present article were carried out in the absence of UV light.

In addition to infrared spectroscopy, XPS was employed for the characterization of the oxidation process in Zeonex E48R. The XPS spectrum from a pristine sample of Zeonex E48R contained a single peak at 285.0 eV which corresponds to the C 1 s and it is characteristic of a saturated hydrocarbon (Fig. S5), as expected. Line deconvolution indicates that there is a very small peak at 285.8 eV which is most likely due to a vibrational component that is normally observed in aliphatic polymers.

The survey XPS spectrum of the oxidized Zeonex E48R indicated that a high degree of oxidation had occurred since 28% of atomic oxygen is present on the sample. This indicates that the degree of oxidation at the surface, strictly speaking, should be significantly higher since XPS samples a depth of approximately 5 nm. One would expect that the degree of oxidation should decrease with depth due to limited diffusion of ozone into the polymeric matrix. Line deconvolution of the C 1 s peak in the high resolution spectrum was found to

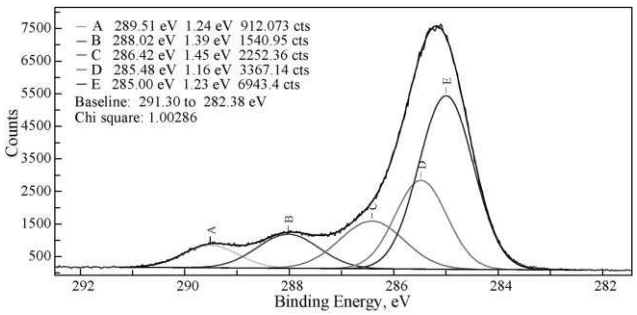

Fig. 2 High-resolution carbon 1 s spectrum of Zeonex E48R after oxidation with ozone.

Table 2 Line deconvolution of C 1 s from oxidized Zeonex E48R

Peak	BE/eV	FWHM/eV	Area (%)
A	289.51	1.24	6.1%
B	288.02	1.39	10.3%
C	286.42	1.45	15.0%
D	285.48	1.16	22.4%
E	285.00	1.23	46.2%

contain 5 lines (Fig. 2). Results from line deconvolution are presented in Table 2.

Peak A at 289.5 eV is typical of carboxylic carbons in carboxylic acids or esters.[33] Peak B at 288.0 eV is indicative of carbons that are double bonded to oxygen (C=O) or bonded to two different oxygen atoms (O–C–O).[33] This is consistent with the reported formation of ketones. However the existence of the formation of acetals in small concentrations cannot be discarded. Peak C at 286.4 eV is consistent with carbon atoms bonded to one oxygen atom (C–O) as for the case of ethers and alcohols.[33] Formation of alcohols is consistent with the infrared data. However, it is very likely that ethers are also formed. Peak D at 285.5 eV is associated with carbon atoms that are not directly bonded to oxygen (C*–C=O) and to carbon atoms linked to ester groups (C(=O)–O–C*).[33] The major peak E at 285.0 eV is due to the unoxidized aliphatic carbons.[33]

In addition to spectroscopic evidence for the surface modification of polycyclic olefins, sessile contact angles were obtained for Zeonor 1060R and Zeonex E48R in their pristine and oxidized state as obtained with ozone in the presence and absence of UV light (Table 3). Oxidation in the absence of UV light causes the contact angles for Zeonor 1060R and Zeonex E48R to change from 95° to 56° and 65°, respectively. This is indicative of the formation of highly polar groups such as ethers, ketones, carboxylic acids. In fact, it has been argued in the literature that ether groups are the main contributors to the hydrophilicity on oxidized surfaces.[31]

Table 3 Steady state contact angles for PMMA-VSUVT, Zeonor 1060R and Zeonex E48R before and after various surface treatments

Substrate	Water	0.2 M NaOH	0.2 M HCl
VSUVT	67° ± 2°	67° ± 2°	67° ± 1°
VSUVT-hydrolysed	57° ± 2°	48° ± 2°	58° ± 2°
Zeonor 1060R	95° ± 2°	95° ± 2°	91° ± 1°
Zeonor 1060R-O_3	56° ± 2°	22° ± 1°	55° ± 1°
Zeonor 1060R-UV/O_3	13° ± 2°	7° ± 2°	15° ± 2°
Zeonex E48R	95° ± 1°	95° ± 1°	95° ± 1°
Zeonex E48R-O_3	65° ± 1°	38° ± 2°	64° ± 1°
Zeonex E48R-UV/O_3	15° ± 2°	8° ± 2°	17° ± 2°

Oxidation in the presence of UV light yields highly hydrophilic surfaces with contact angles of 13° and 15° for Zeonor 1060R and Zeonex E48R, as expected for a more aggressive oxidation. Evidence for the presence of a high concentration of carboxylic acid groups was obtained from contact angle measurements using a 0.2 M NaOH solution. In this case, the contact angles decreased further to 7° and 8° for Zeonor 1060R and Zeonex E48R as would be expected for an acidic functionality. It should also be noted that washing the samples with water after oxidation increases the contact angles from 13° and 15° to 46° and 43°, respectively. This is consistent with the removal of a highly oxidized polymeric material or low molecular weight residue produced by chain scission.

Further evidence for the presence of carboxylic acids was obtained by immobilizing fluorescent dyes using chemical reactions that are specific to carboxylic acids. That is, the carboxylic acids are activated *via* a reaction with EDC and NHS to form an NHS ester which is subsequently reacted with a fluorescent dye that possesses a primary amino group such as tetramethylrhodamine cadaverine. Control experiments indicate that indeed ozone is required for the successful immobilization.

In addition to fluorescent dyes, amino modified DNA was immobilized using the same strategy. To this end, two amino-modified single strands of DNA (probe #1 and probe #2, Table 4) were arrayed and allowed to react with the NHS activated surface of Zeonor 1060R. The immobilized oligonucleotides were hybridised in a competitive manner with an equimolar mixture of their respective complementary strands (target #1 and target #2). Molecular recognition is observed as illustrated by the proof-of-concept fluorescent images of the hybridised oligonucleotide arrays on Zeonor 1060R which are presented in Fig. 3.

Similar results are observed for DNA oligonucleotide on Zeonex E48R and the corresponding fluorescent images are presented in the ESI (Fig. S6).

Surface chemistry and immobilization of DNA on PMMA

It has been reported in the literature that oligonucleotides may be covalently immobilized on the surface of PMMA by reacting an amino-modified DNA strand directly with a pristine surface of PMMA under highly basic conditions (pH = 11.5).[34] In this case, the methyl ester groups at the surface of PMMA undergo a nucleophilic attack by the amine groups and this gives directly an amide linkage upon elimination of methanol. Although this method is simple, the authors not only indicate a high variability in their results, but

Table 4 DNA oligonucleotide sequences and their respective modifications

Name	Sequence	Modification
Probe #1	5′-CCGCTCGCCAGCTCC-3′	5′-$(CH_2CH_2O)_6$-NH_2
Probe #2	5′-ATTATGAGTGTCCTA-3′	5′-$(CH_2CH_2O)_6$-NH_2
Target #1	5′-GGAGCTGGCGAGCGG-3′	5′-Cy3
Target #2	5′-TAGGACACTCATAAT-3′	5′-Cy5

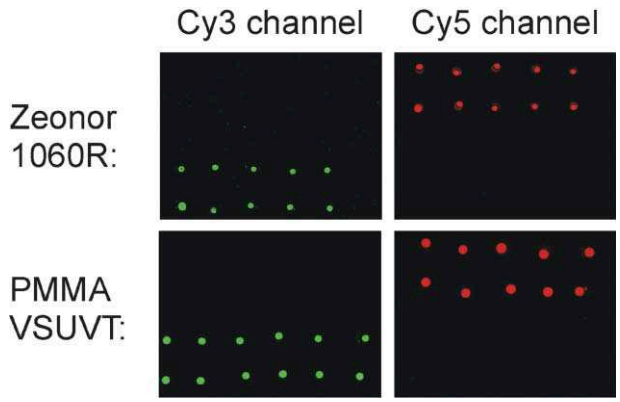

Fig. 3 Fluorescent images that were obtained after hybridising an array of probes #1 and #2 on Zeonor 1060R (upper images) and PMMA VSUVT (lower images). Hybridisation was carried out under competitive binding conditions using a mixture of their fluorescently labelled complementary strands, targets #1 and #2.

also report that the "coupling reactions fail occasionally" and this may be due to the high pH employed in the reaction as DNA may undergo hydrolysis.

Our approach to the immobilization of DNA on PMMA surfaces is based on the high reactivity of methyl ester groups towards hydrolysis under acid or base catalysis and it involves an initial treatment of the plastic with an aqueous solution of NaOH. This hydrolysis process yields sodium carboxylate groups which can be protonated in the presence of an acid to yield carboxylic acid groups. However, poly(methyl methacrylic acid) is water soluble, particularly the corresponding sodium salt. As a result, the treatment with NaOH has two main effects. Firstly, it serves as a cleaning step since a thin layer of the plastic is removed. Secondly, it generates a surface containing a small amount of carboxylic acid as the fully hydrolysed polymer is removed during rinsing with water.

The presence of a small amount of carboxylic acid groups was confirmed by a series of contact angle measurements using water, 0.2 M NaOH and 0.2 HCl solutions (Table 3). Indeed, average contact angles of pristine PMMA-VSUVT were found to be $67^\circ \pm 2^\circ$ irrespective of the nature of the solution employed in the measurement. This is consistent with a surface containing methyl ester groups. On the other hand, the average contact angles of the hydrolysed PMMA-VSUVT surface were found to be $58^\circ \pm 2^\circ$ and $48^\circ \pm 2^\circ$ using a 0.2 M solution of HCl and NaOH, respectively. The change in contact angles is rather small, indicating that a small concentration of free carboxylic acid is retained after hydrolysis. Nevertheless, the change is fully reversible and reproducible and it is consistent with acidic groups at the surface.

Further evidence for the presence of a small but significant amount of carboxylic acid groups at the surface of PMMA was obtained by immobilizing tetramethylrhodamine cadaverine in the same manner as before. Moreover, it was demonstrated that indeed EDC and NHS are required to activate the surface of PMMA-VSUVT as it would be expected for a surface with free carboxylic acid groups.

A proof-of-concept oligonucleotide array is demonstrated in the presented work. Two single-stranded amino-modified oligonucleotides (probe #1 and #2) were immobilized on the surface of a NHS activated PMMA slide followed by a hybridisation step under competitive binding conditions in the same manner as illustrated for the case of poly(cyclic olefins). Full molecular recognition is demonstrated as illustrated by the fluorescent images in Fig. 3. It should also be pointed out that the background fluorescence due to non-specific binding is lower than the one observed for poly(cyclic olefins).

Although it has been illustrated that proof-of-concept oligonucleotide arrays can be successfully hybridised by simply incubating (passive method) the array with their complementary strands for 1 h, it is extremely important to demonstrate rapid hybridisation under microfluidic conditions at room temperature since this method could readily be implemented in an integrated medical diagnostic device. To this end, a microfluidic device was fabricated from PDMS using a silicon mould that had been micropatterned with SU8 photoresist *via* standard photolithographic techniques.

As illustrated in Fig. 4, the microfluidic device consisted of a 100 μm wide and 17 μm deep microchannel and a hexagonal hybridisation chamber whose widest dimension was 2.4 mm. The microfluidic device was brought into conformal contact with a PMMA slide that contained an array of a single strand of DNA oligonucleotide (probe #1) ensuring that the DNA array was enclosed by the hybridisation chamber. Rapid hybridisation was carried out using the hybridisation buffer reported by Peytavi *et al.*[35]

A volume of 10 μL of an equimolar mixture of its complementary (target #1) and the non-complementary strand (target #2) labelled with Cy3 and Cy5 was injected at a flow rate of 1 μL min^{-1} using a syringe pump. This ensured a

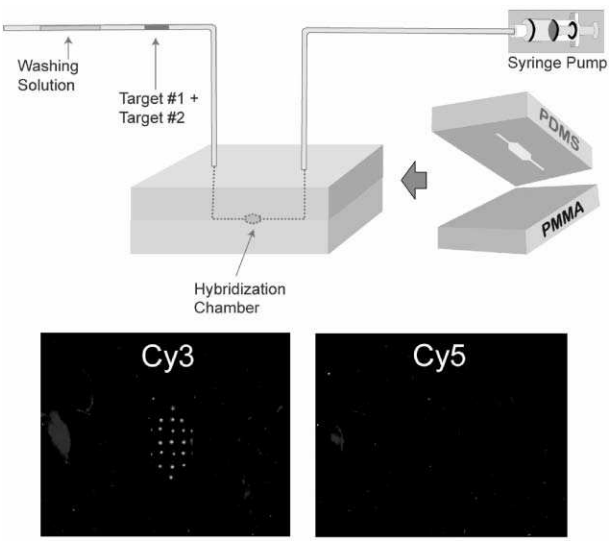

Fig. 4 Proof-of-concept hybridisation of DNA oligonucleotide under microfluidic conditions was achieved by immobilizing probe #1 in an array format on the surface of a PMMA slide. The array was enclosed by a PDMS device that possessed a microchannel and a hexagonal hybridisation chamber (upper drawing). Hybridisation was performed in 10 min by flowing a mixture of fluorescently labelled complementary and non-complementary strands followed by a washing solution in the form of plugs. The lower part of the figure illustrates the fluorescent image obtained after detaching the PDMS devise.

contact time of 10 min for the competitive hybridisation event at room temperature. This was followed by the injection of 50 μL of a 0.1% SDS solution in PBS at a rate of 10 μL min^{-1}. Analysis of the hybridised array was performed by simply detaching the PDMS microfluidic device and acquiring the fluorescent image of the plastic slide in a conventional microarray scanner (Fig. 4). It can be observed that hybridisation took place with the complementary strand under microfluidic conditions at room temperature within minutes.

Conclusions

Commercially available plastics have been identified for potential applications in high throughput screening devices that rely on fluorescence detection. These plastics are PMMA-OptixCA41 and Zeonor 1060R; however, Zeonex E48R has been identified as an excellent replacement for Zeonor 1060R in applications that require high temperatures. Moreover, strategies for their surface modification are presented using economical, industrially scalable and environmentally friendly methods. In addition, the covalent immobilization of DNA oligonucleotides is demonstrated using proof-of-concept DNA microarrays on the selected plastics. Rapid hybridisation under passive and microfluidic conditions was demonstrated at room temperature. Full molecular recognition is observed during competitive binding of complementary and non-complementary DNA strands.

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