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Development of a versatile biotinylated material based on SU-8†

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The negative epoxy-based SU-8 photoresist has a wide variety of applications within the semiconductor industry, photonics and lab-on-a-chip devices, and it is emerging as an alternative to silicon-based devices for sensing purposes. In the present work, biotinylation of the SU-8 polymer surface promoted by light is reported. As a result, a novel, effective, and low-cost material, focusing on the immobilization of bioreceptors and consequent biosensing, is developed. This material allows the spatial discrimination depending on the irradiation of desired areas. The most salient feature is that the photobiotin may be directly incorporated into the SU-8 curing process, consequently reducing time and cost. The potential use of this substrate is demonstrated by the immunoanalytical detection of the synthetic steroid gestrinone, showing excellent performances. Moreover, the naked eye biodetection due to the transparent SU-8 substrate, and simple instrumental quantification are additional advantages.

Introduction

Optical biosensors are powerful analytical tools with application in healthcare, biomedical and pharmaceutical research, environmental monitoring, and security, among others.[1] Commonly used materials for the production of miniaturized sensors include silicon, silicon oxide, silicon nitride, and gold. These devices are made using standard microfabrication techniques, such as oxidation and chemical or physical vapor deposition, lithography, *etc.* This adds complexity, cost and production time to sensor fabrication. Polymeric materials are an alternative to silicon derivatives because of their low-cost, high-throughput production methods,[2] and greater chance of chemical functionalization on demand.

The SU-8 polymer (glycidyl ether of bisphenol A) is an epoxy-based negative photoresist with excellent mechanical, physical, and optical properties after polymerization.[3] SU-8 is a valuable material used in a wide range of applications such as optical waveguides for telecommunication;[4] microelectromechanical systems (MEMS)[5,6] including microfluidic structures,[7,8] probes for microscopy[9,10] and molds for microembossing;[11,12] and microfabrication-based sensors.[13,14]

Due to its chemical functionalization capability on demand and low cost, SU-8 is an attractive substrate candidate for the fabrication of bioanalytical micro and nano-devices. Although its surface hydrophobicity is a limitation, causing the high non-specific adsorption of probes and targets, and decreasing the surface wettability,[15] these drawbacks can be resolved by chemically tailoring the SU-8 surface properties.

When SU-8 is used for biosensing, probe immobilization can be done by covalent, electrostatic, or adsorptive interactions. SU-8 chemical surface modification can be accomplished to include at least one functional group, such as CHO, NH_2, or SH, which can be used for covalent binding of biologically active probes,[16–20] or for solid phase synthesis.[21,22] Direct covalent immobilization of aminated or thiolated moieties is possible as SU-8 epoxide rings can undergo S_N2 reactions.

The functionalization of SU-8 for biosensing is an upcoming field. Thus, the direct immobilization of aminoalkyl, thiophosphoryl and phosphonylated single strand DNA on SU-8 to set up microarray based hybridization assays is reported.[16,23] Blagoi's group described the SU-8 polymer treatment with 3-aminopropyltriethoxysilane and glutaraldehyde – this procedure is similar to the well known organosilane based silicon derivatization – to detect C-reactive protein by fluorescent sandwich immunoassay on SU-8 microwells.[17] Using a similar strategy, Joshi *et al.* immobilized human immunoglobulins on microfabricated SU-8 cantilevers although no immunoassay demonstration was reported.[18] In other work, Joshi *et al.* performed SU-8 microcantilever surface modification by grafting amine groups using pyrolytic dissociation of ammonia in a hotwire CVD setup; after glutaraldehyde treatment, human

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immunoglobulin was attached to the surface and recognized by a fluorescent labeled anti-human antibody[19] Using the same immunoreagents, Deepu *et al.* employed glycine and 11-mercaptoundecanoic acid to covalently attach the human immunoglobulins using carbodiimide bioconjugation chemistry.[20] Also, SU-8 encoded microparticles are chemically modified to attach oligonucleotides or proteins to perform multiplexed assays.[24,25] Recently, a swelling–deswelling method for the facile surface modification of 2D and 3D patterns of SU-8 has been described, demonstrating the oligonucleotide attachment.[26] However, this method involves the use of toluene, and this makes it inapplicable to the attachment of proteins due to the risk of denaturation. Regarding the SU-8 surface modification of microfabricated structures for label-free optical biosensing, there are few examples in the literature, and they are based on adsorption or covalent attachment after the fabrication of the microstructures.[13,27–31]

The bioreceptor spatial localization on a surface is a powerful approach to generate molecular arrays for analytical or bioelectronic applications. In this sense, photoactivation of light-sensitive reagents on a solid surface through a photomask allows the generation of reactive intermediates, such as radicals, carbenes, and nitrenes, which react with the surface yielding coated domains of the reactive intermediates.[32] Until now, these approaches have been applied in materials like polystyrene, nitrocellulose, and silicon, but scarcely with SU-8.[15,33]

The derivative *N*-(4-azido-2-nitrophenyl)-*N*′-(3-biotinylaminopropyl)-*N*′-methyl-1,3-propanediamine, commonly called photobiotin (Phb), is a particularly suitable compound belonging to the generation of extremely photoreactive species. The specific binding of biotin to streptavidin presents a very high affinity ($K_a = 10^{15}$ M^{-1}),[34] and its tetravalency allows for a simultaneous binding to several biotins emerging from the surface *via* a biotin–streptavidin–biotin bridge.[35,36] Upon UV irradiation ($\lambda = 350$ nm), the photoactivatable group of Phb gives rise to a nitrene from an azide, which can be inserted readily into both C–H as well as other functional groups, such as OH, NH, NO_2, C=C, *etc.*[37] This is a well-established methodology for protein biotinylation.

In this paper, we propose a biotin-modified SU-8 material providing a generic biosurface for the effective immobilization of any biotinylated molecule, *via* a streptavidin intermediate. To this end, the SU-8 surface can be coated with photobiotin and irradiated through a photomask to yield the patterned surface. Also, we demonstrate the biotinylation during the SU-8 fabrication process as the wavelength used to attach the Phb overlap with the wavelength needed for the crosslinking of the photoresist.

The main advantages of the proposed method against the approaches already described in the literature[16–20,23,26–31] for the biofunctionalization of SU-8 include the spatial discrimination – obtained by irradiating only on the desired areas – , the suitability to be incorporated within the SU-8 polymer standard fabrication protocol – thus saving time and cost – , and the versatility of the surface obtained, as any biotinylated receptor can be attached to the surface by a streptavidine bridge.

Experimental

Chemicals

The SU-8 polymer and SU-8 developer are distributed through MicroChem Corp. (Newton, MA, USA) in several formats depending on their composition. In this paper, SU-8 2000.5 was employed attending to the desired layer thickness. Streptavidin, streptavidin-gold (~2.5 A_{520} units per mL), streptavidin-ATTO 655 from *Streptomyces avidinii*, gold-labeled goat anti-rabbit immunoglobulin (GAR-Au), anti-bovine albumin antibody produced in rabbit (a-BSA), silver enhancer solutions A and B, ovalbumin (OVA) lyophilized powder, photobiotin (Phb), mercaptoethanol, ethanolamine, hydrogen peroxide 35% w/w, and sulfuric acid 95–98% were purchased from Sigma-Aldrich Química (Madrid, Spain). The anti-biotin polyclonal antibody produced in rabbit (a-biotin) was provided by Abcam (Cambridge, United Kingdom). Dimethylsulfoxide (DMSO) was acquired from Acros Organics (Madrid, Spain). PBS (10 mmol L^{-1} sodium phosphate, 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, pH 7.4) and PBS-T (PBS containing 0.05% Tween 20) were used as buffers. The anti-gestrinone polyclonal antibody and the gestrinone hapten conjugate were obtained by our research group.[38]

Instruments

The spin-coating process was performed with a WS-400BX-6MPP/LITE spin-coater (Laurell Technologies Corp., North Wales, PA, USA). Microarray printing was carried out with a low volume non-contact dispensing system from Biodot (Irvine, CA, USA), model AD1500. SU-8 and SU-8–photobiotin curing was done with a laser workstation from Optec, Inc. (Lowell, MI, USA), model ML-100, using a high power diode-pumped solid state (DPSS) Q-switched laser of Nd:YVO4 from Spectra Physics (Irvine, CA, USA), model HIPPO 355-5, with an output at 350 nm wavelength. Glass slides were purchased from Menzel-Gläser (Braunschweig, Germany). UV-Vis spectra measurements were obtained with a spectrophotometer from Agilent Technologies (Santa Clara, CA, USA), model 8453. The hot-plate model Agimatic E-C was acquired from JP-Selecta (Barcelona, Spain). Signal measurement of the Ag complex was carried out with a document scanner from Seiko Epson Corp. (Nagano, Japan), model EU-34, and images were processed with Adobe Photoshop CS software from Adobe Systems Inc. (San Jose, CA, USA). For standard microarray image analysis, GenePix Pro 6.0 software from Molecular Devices, Inc. (Sunnyvale, CA, USA) was employed. Contact angle system OCA20 equipped with SCA20 software was from Dataphysics Instruments GmbH (Filderstadt, Germany). A Bruker Tensor 27 equipped with an ATR dura SamplIR accessory was used to acquire FTIR surface spectra.

Deposition of SU-8 and SU-8–Phb layers on glass slides

In order to obtain maximum process reliability, the substrate was cleaned and dried prior to applying SU-8 2000.5 resist. First, a glass chip of 1 mm thickness was treated with piranha solution ($H_2O_2 : H_2SO_4$) wet etching, followed by rinsing with deionized water (DI)-H_2O and ethanol and drying with N_2. After a

preheating process at 80 °C for 1 min applied to the chip, the SU-8 or SU-8–Phb mixture in the appropriate volumetric proportion was dispensed (1 mL resist/5 cm^2 substrate). To prepare the mixture, Phb was previously dissolved in DMSO at 100 μg mL^{-1}. The sample was spun at 4000 rpm for 50 seconds, and soft baked at 80 °C for 1 min on a hotplate with good thermal control and uniformity. Next it was exposed to the DPSS laser radiation at 350 nm wavelength, defocused to 55% of maximum power for 2 min, baked at 80 °C for 1 min, and dried with N_2. Finally, the glass slides were cut into suitable sizes with a tungsten awl.

Photobiotin SU-8 coating

The SU-8 chip surface was biotinylated after fabrication as follows: 20 μL of Phb (100 μg mL^{-1}) in water were dispensed on the surface of a chip and spread out using a coverslip. After 2 h in darkness, the surface was blown with N_2. The chip was irradiated at 350 nm wavelength with a UV lamp for 20 min, rinsed with (DI)-H_2O and dried with N_2 again.

The procedure to perform the patterning was the same but using the adequate photomask instead of a coverslip.

Anti-biotin immunoassay development

Different concentrations of anti-BSA and anti-biotin antibodies, ranging from 1 μg mL^{-1} to 10 μg mL^{-1}, in PBS-T buffer (40 nL), were printed with a non-contact automatic arrayer on SU-8 or SU-8–Phb surfaces. After incubation for 20 min at room temperature in a wet and dark environment, the samples were rinsed with PBS-T and (DI)-H_2O, and blown with N_2. Then, 20 μL of OVA 1% in PBS were spread out on the microarray and incubated for 1 h as before. After washing with PBS-T and (DI)-H_2O, the microarrays were incubated with a gold-labeled secondary antibody (GAR-Au, 25 μg mL^{-1} in PBS-T) for 5 min, followed by washing and drying as before. A mixture of 10 μL of silver enhancer solution type A and 10 μL of silver enhancer solution type B was added onto the microarray and spread out with a coverslip. After 20 min, the chips were washed and dried.

To quantify microarray signals, a high definition color image of the chip was acquired by an Epson EU-34 office scanner. The image was transformed to grey scale and 16-bit format for its data treatment with GenePix software.

Conjugation of streptavidin to gestrinone hapten

The gestrinone oxime hapten 18α-Homo-pregna-4,9,11-trien-17β-ol-20-yn-3-carboxymethyl oxime (GH) and the streptavidin–GH conjugate were synthesized and purified as previously described.[38] The concentration of streptavidin–GH conjugate and the streptavidin/GH molecular ratio (3) was done by UV-Vis spectrophotometry.

Gestrinone immunoassay development

The biotinylated surface was treated with a solution of streptavidin–GH (991 μg mL^{-1} in PBS-T) for 20 min. The chip was washed with PBS-T and (DI)-H_2O, rinsed with N_2, and the surface was blocked with OVA 1% in PBS-T for 1 h. Then, 20 μL of rabbit serum (dilution 1/4000 in PBS-T) and 20 μL of gestrinone at different concentrations (0.2, 2, 10, and 20 ng mL^{-1}) were mixed and printed as described above, including a control blank. After incubation for 15 min, the surface was washed and dried. Finally, GAR-Au incubation, silver development and microarray quantification were performed as described above.

SU-8 and SU-8–Phb nanopillars fabrication

Structures based on arrays of nanopillars were fabricated using SU-8 or a mixture of SU-8 and Phb (100 μg mL^{-1} in DMSO) in a 1/1 volumetric ratio. In both cases, the procedure was that previously described.[28,29]

Results and discussion

For the biotinylation of SU-8, the epoxy moieties could be used for covalent binding of amino groups like amino-biotin, but this method would not lead to the spatial selectivity provided by photoactivation.

As far as we know, there are only two reported SU-8 surface functionalization approaches allowing this spatial selectivity that can be achieved by using photoactivation. The approach described by Blagoi *et al.*,[33] which consists of the micropatterning of SU-8 surfaces with antraquinone derivatives and further covalent attachment of aminated biotin or other proteins; and the micropatterning approach developed by Wang *et al.*[15] based on UV mediated graft polymerization of an acrylic acid derivative. However, none of them is demonstrated for biosensing.

Phb has been widely used in the literature for surface patterning of glass, silicon, and PDMS mainly. The mechanism of insertion occurs through a nitrene radical, allowing the insertion on OH, NH and CH bonds. Thus, the biotinylation of SU-8 can take place as it is represented in Fig. 1. However, it is demonstrated that Phb has a considerable affinity towards the insertion into C–H bonds and this must be the preferred insertion site.

To proceed with the Phb tethering, it was necessary to perform a previous step to open the SU-8 epoxy moieties transforming them into hydroxyl groups, which prevents the bioreceptor attachment through its amine groups. On the other

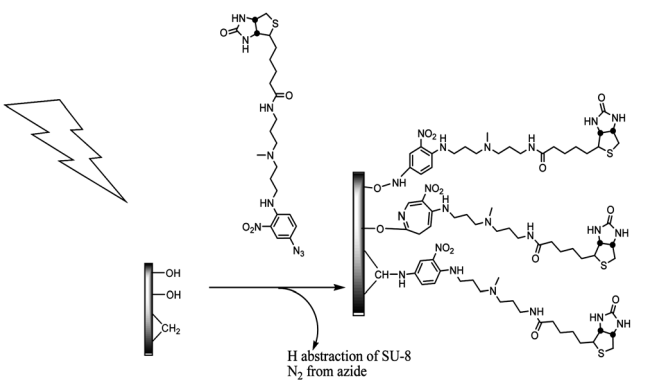

Fig. 1 Photobiotin insertion on the SU-8 polymer under 350 nm light irradiation.

hand, this change on the surface increases its hydrophilicity, minimizing the protein adsorption and improving the surface wettability. For the opening procedure, an acidic pre-treatment of the surface was performed, and a buffer containing surfactant (Tween 20) was added to the protein solutions in order to prevent non-specific adsorption.

According to that, the epoxy-ring opening was done through acidic conditions, which were applied by immersing the chips in 1 M H_2SO_4 for one minute. After that, the insertion of biotin was done by spreading out an aqueous solution of Phb 100 μg mL^{-1} over the chip surface, and irradiating for 20 min at 350 nm. In order to assess the photobiotin immobilization, microarrays including several streptavidin-Au and GAR-Au concentrations were printed onto the biotinylated chips, with or without previous cleavage of epoxy rings using 1 M H_2SO_4. After incubation and further washings, the arrays were developed with the silver reagent, showing a black precipitate when a gold labeled protein is present on the surface. Results could be observed by the naked eye because the substrate is transparent, while quantitative monitoring was accomplished with a document scanner.

Qualitative results are presented in Fig. S1 (ESI†), showing that photobiotin covalent immobilization on the SU-8 chain is conditioned to the exposure with UV radiation and only the streptavidin remains on the surface, without non-specific adsorption. With and without previous epoxy hydrolysis, a specific immobilization of streptavidin was observed, while GAR was not retained on the surface. This fact indicated the success in the surface selective biotin photoattachment. When UV irradiation was avoided (after photobiotin surface application) no signal was observed with streptavidin nor with GAR if previous epoxy acidic hydrolysis was performed. When no epoxy hydrolysis was carried out, both streptavidin and GAR were immobilized on the surface. This may be explained by the covalent attachment of both proteins to the surface by means of a nucleophilic attack from protein amine groups to the epoxy moieties. It is in agreement with that found in the literature, where different authors use the epoxy hydrolysis to reduce or avoid the unwanted protein immobilization on the SU-8 surface, or demonstrate the covalent nature of the protein attachment, using cerium ammonium nitrate,[17] sulfochromic solution,[13,18] chrome etch,[16] or PEG grafting.[15]

The surface characterization of biotin modified chips was performed using X-ray photoelectron spectroscopy (XPS) and contact angle measurements. Results showed the presence of S and N atoms in the treated chips, whereas these chemical elements were not found in bare SU-8 chips. Contact angle values showed an expected decrease associated with an increase in the surface hydrophilic character, due to the acidic ring opening and further biotin incorporation. For SU-8 chips, the contact angle value was 82°, whereas the value was 69° for the hydrolyzed SU-8 chip, and 67° for the biotinylated polymer (Fig. 2).

In order to ensure the availability of this new surface to develop immunoassays, the immunoreaction between biotin and a specific antibiotin polyclonal antibody was studied. For that, two microarrays (dimension 3 × 3) were printed on the

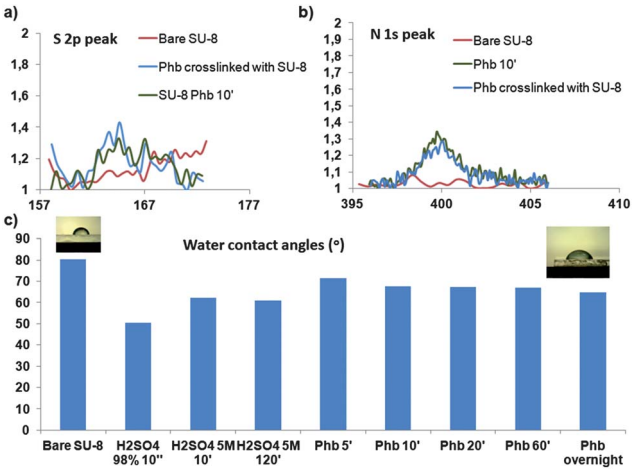

Fig. 2 XPS analysis of (a) the S 2p peak and (b) the N 1s peak for the bare SU-8 surface, the SU-8 polymer coated with photobiotin, and the biotinylated SU-8 surface employing the SU-8 and photobiotin polymerization mixture. (c) Contact angle values for both SU-8, bare and under different hydrolysis and biotinylation conditions.

biotinylated material, one of a-BSA antibody as a control, and the other one using the specific antibody a-biotin. A gold labelled secondary antibody (GAR-Au) was added to detect signals derived from biotin–a-biotin interaction. To avoid GAR-Au non-specific adsorption, several blocking agents such as OVA, ethanolamine, and mercaptoethanol were tested. OVA provided the best results probably because mercaptoethanol and ethanolamine interact with photobiotin to form intermolecular H-bonds. Also, the blocking step was assayed before and after the primary antibody incubation step. Blocking after the incubation with a primary antibody provided much better results in terms of specificity (Fig. 3). This effect may be explained by the OVA size, which is larger than photobiotin, so the coating totally loses its specificity. It can be observed through a similar intensity for a-biotin and a-BSA microarrays. Even without OVA blocking, the immunoreaction was possible;

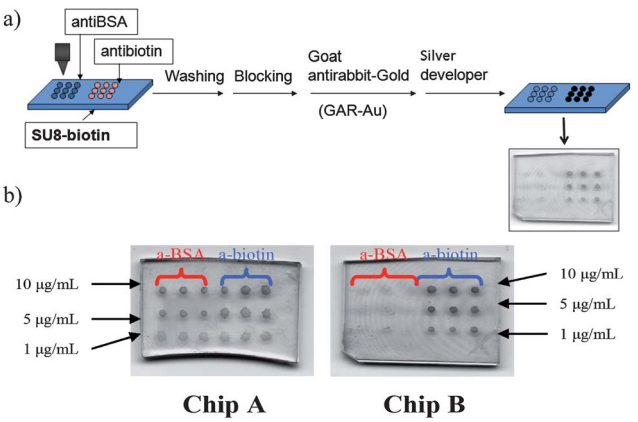

Fig. 3 (a) Scheme of the immunoassay designed to evaluate the bioavailability of the biotin immobilized on the biotinylated SU-8. (b) Image of the resulted microarray, chip A is not coated with photobiotin and chip B has a photobiotinylated surface.

hence immobilisation and recognition steps were performed under conditions (using an appropriate surfactant in the buffer) in which non-specific adsorption was almost eliminated. In any case, the use of OVA blocking helped reducing the background signal.

To prove the capability of the developed approach for surface patterning, Phb 100 μg mL^{-1} was deposited over the hydrolyzed SU-8 surface. The irradiation at 350 nm was performed during 10 min through a photomask as it is represented in Fig. 4. After washings, streptavidin-ATTO 655 50 μg mL^{-1} in PBS-T was incubated over the surface for 5 min at room temperature, and was washed with PBS-T and water. The fluorescence was registered showing a patterned surface.

Once the effectivity of the procedure for the selective SU-8 biotinylation while maintaining the bioavailability was demonstrated, the following step was to analyze the photobiotinylation of SU-8 in the UV crosslinking step carried out during the polymer fabrication process. The inclusion of additives, such as nanoparticles in the SU-8 to modify its electrical (or magnetic) properties while maintaining its photopatterning behaviour has been already demonstrated.[39] Thus, the biotinylation of the material simultaneous to the photoresist polymerization process is highly interesting, in particular for the construction of label-free nanobiosensing devices, as it offers the possibility to include the biofunctionalization process in the nano-structured material fabrication without any additional steps. This would provide a general biosurface using a very simple and elegant fabrication method, where any biotinylated compound could be anchored through streptavidin/biotin tandem.

To optimize the new material preparation, at first the appropriate SU-8–Phb ratio was studied. Initial mixtures of SU-8 2000.5 and photobiotin solution (100 μg mL^{-1}) in DMSO were tested involving several SU-8/Phb volumetric ratios: 1/0.5, 1/1, 1/4, and 1/10. These mixtures were spin coated on glass slides and photoresist crosslinking was performed as usual. For this biotinylated material, XPS and contact angle measurements were done. Again, contact angle (Table 1) showed a decrease (68°) with regard to the non-modified SU-8 (82°) because of the lower hydrophobicity of the new surface. XPS global analysis also showed the presence of N and S atoms, indicating the biotin incorporation into the SU-8 (Fig. 2a and b).

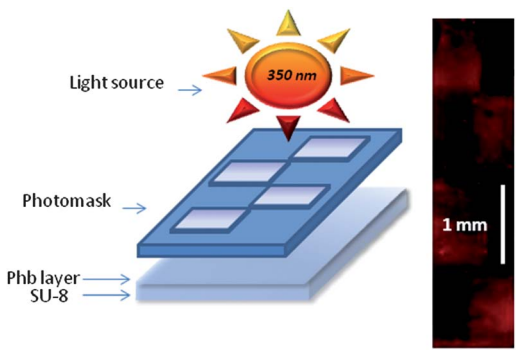

Fig. 4 Fluorescence image obtained after irradiation through a photomask.

Table 1 Water contact angle values (°) for SU8–Phb mixed surfaces fabricated with different volume ratios

SU8–Phb 1/0 (v/v)	SU8–Phb 1/0.5 (v/v)	SU8–Phb 1/1 (v/v)	SU8–Phb 1/4 (v/v)	SU8–Phb 1/10 (v/v)
80.3 ± 0.8	68.0 ± 0.5	64.0 ± 0.2	61.6 ± 0.6	58.2 ± 0.3

Further, immunoassays with a-BSA and a-biotin were carried out, in a similar manner than onto biotin coated surfaces, to test the performance of this new technical procedure (Fig. 5). Quantitative results obtained are given in Table S3 (ESI†), where the net signal, the net signal/background ratio, and the standard deviation for a-biotin concentration employed in chips are compared. The net signal reached maximum and comparable values for SU-8–photobiotin 1/1 and 1/0.5 chips but the signal/background ratio was much better for 1/1 (background signal for 1/0.5 chips was too high even after OVA blocking). Samples corresponding to 1/4 and 1/10 did not provide better values. For 1/1 chips, a calibration curve was performed. To this end, we tried to determine the biotin density on the surface. Thus, streptavidin-ATTO microarrays with concentrations ranging from 0.05 to 100 μg mL^{-1} reacted specifically with the surface and the fluorescence intensity was measured before and after the washing step. The first measurement was used for the calibration curve, and the second one to determine the amount of streptavidin remaining on the surface after recognition. The highest values of fluorescence after washings were achieved for 50 μg mL^{-1} streptavidin-ATTO. Taking into account the dimensions of the spot (400 μm diameter), a coating density of 0.772 pmol cm^{-2} of streptavidin was obtained, which means an immobilization yield of 10%. This data is in agreement with other studies already reported in the literature showing that when biotin–streptavidin–biotin bridge chemistry is used for DNA assembly, low surface coverage (*e.g.* 10% biotin residue) is appreciated and leads to the best results, while a larger amount of streptavidin adsorption does not lead to a larger amount of biotinylated-DNA binding.[40] We concluded that the best performances for the new biotinylated material were achieved employing polymerization of a SU-8–photobiotin solution 1/1 (v/v).

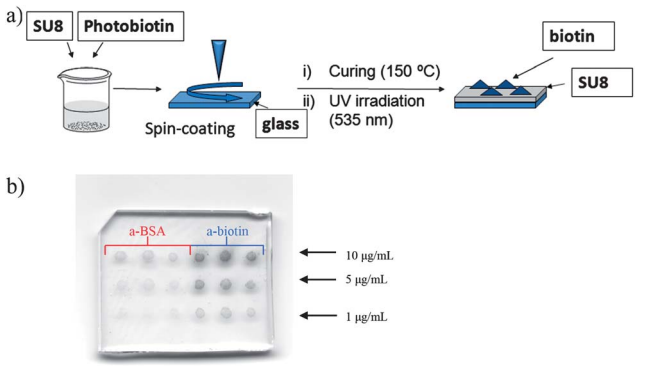

Fig. 5 (a) Scheme of biotinylation procedure incorporated into the SU-8 polymer fabrication. (b) a-BSA and a-biotin microarrays on SU-8–photobiotin 1/1 (v/v) chip. Concentration disposition is in accordance with Fig. 4.

The new material was employed in the detection of the synthetic steroid gestrinone. It was used as a model system to demonstrate the applicability of the platform in screening assays such as residue detection in sport doping samples or pharmacological excretion residues. Specifically, a SU-8–Phb 1/1 (v/v) chip was used as an assay platform and a competitive indirect format immunoassay was selected. Thus, the gestrinone hapten was conjugated to streptavidin (see Experimental), and the best streptavidin–hapten concentration was identified and fixed at 996 μg mL^{-1}. After the blocking step, a microarray with a mixture of rabbit polyclonal sera solution (1/4000) and gestrinone at different concentrations (0.2 to 20 ng mL^{-1}) was created (several replicates of each condition). Then GAR-Au was employed to develop the array (see Experimental). Spots could be observed by the naked eye. From the gestrinone dose–response curve (Fig. 6) the IC_{50} value was 1 ng mL^{-1}, and the LOD was estimated at 0.26 ng mL^{-1} of gestrinone.

It was demonstrated that the new biotin-ended material obtained from the reaction between SU-8 and photobiotin through coating or direct photopolymerization may be used to attach biotinylated probes, which can be further used to selectively detect targets by bioanalytical methods reaching high sensitivity. The potential of this new surface for the development of micro and nanofabricated based biosensors is of high interest, as the own biofunctionalization process may be incorporated into the material fabrication for micro- and nano-structures, reducing the number of steps and associated cost. To prove this concept, arrays of nanopillars and nanostrips were fabricated using SU-8 and with a mixture of SU-8 and Phb (1/1 v/v ratio). The consistency of such structures was confirmed by confocal microscopy. The bioavailability of biotin moieties on the nanopillars surface was demonstrated by incubating the chips, after the hydrolysis with sulfuric acid, with a solution of 50 μg mL^{-1} streptavidin-ATTO in PBS-T for 10 minutes. Fluorescence confocal microscopy images showed the presence of streptavidin only on those structures fabricated employing a mixture of SU-8–Phb (Fig. 7). This assay demonstrated the suitability of the methodology to be employed for the construction of biosensors performing simultaneously the biofunctionalization and the structure fabrication, saving time and costs.

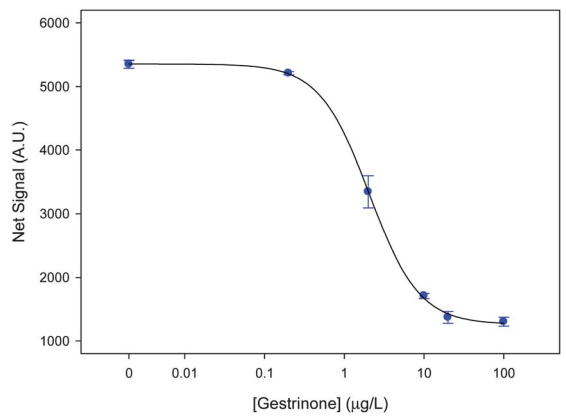

Fig. 6 Dose–response curve obtained for gestrinone competitive immunoassay.

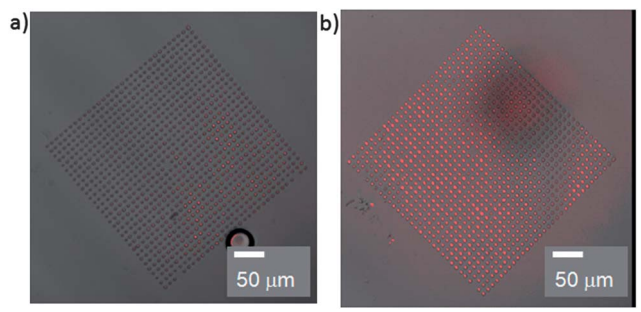

Fig. 7 Confocal image and fluorescence confocal image superposed for an array of nanopillars fabricated in (a) SU-8 and (b) SU-8–Phb 1/1 and incubated with ATTO-streptavidin for 10 minutes.

Conclusions

We have developed a new procedure for the chemical modification of SU-8 through the photobiotinylation promoted by UV radiation. The photobiotin coating of the previously cured SU-8 surface was demonstrated, and its activity through different bioaffinity assays was tested. Chemical behaviour of the new surface material was evaluated under distinct working conditions. Excellent results led to the development of a synthetic method for this novel material, which is based on the SU-8 curing process, and photobiotin mixture solution prepared with a range of volume ratios and promoted by 350 nm radiation. This procedure simultaneously facilitates SU-8 polymerization and photobiotin incorporation into the polymer structure.

Immunorecognition assays showed excellent specificity and reproducibility, even when a competitive assay for the gestrinone hormone determination was developed. Direct visualization of results with the naked eye is an advantage because it allows for immediate immunoassay monitoring without complex instrumentation. Furthermore, signal measurements can be performed with the assistance of a document scanner, reaching also a high sensitivity (LOD 0.26 ng mL^{-1}) in a not fully optimized proof-of-concept immunoassay. The reached sensitivity compares with other well-established immunoassay techniques as ELISA, where LODs of 0.09 and 0.14 ng mL^{-1} were obtained for gestrinone detection using direct and indirect ELISA plate formats respectively. Therefore, this biosurface can be used for the immobilization of any biotinylated compound, while it is an interesting alternative to other materials in high-throughput biosensor development. The photopatterning of the surfaces has been demonstrated. Also, the inclusion of the biotinylation step within the polymer fabrication process opens the possibility to employ it in the construction of low cost high density microarrays,[41] as well as in the fabrication of integrated optical label free biosensors.

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