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# Heparin micropatterning onto fouling-release perfluoropolyether-based polymers via photobiotin activation

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ABSTRACT

A simple method for constructing versatile ordered biotin/avidin arrays on UV-curable perfluoropolyethers (PFPEs) is presented. The goal is the realization of a versatile platform where any biotinylated biological ligands can be further linked to the underlying biotin/avidin array. To this end, microcontact arrayer and microcontact printing technologies were developed for photobiotin direct printing on PFPEs. As attested by fluorescence images, we demonstrate that this photoactive form of biotin is capable of grafting onto PFPEs surfaces during irradiation. Bioaffinity conjugation of the biotin/avidin system was subsequently exploited for further self-assembly avidin family proteins onto photobiotin arrays. The excellent fouling release PFPEs surface properties enable performing avidin assembly step simply by arrays incubation without PFPEs surface passivation or chemical modification to avoid unspecific biomolecule adsorption. Finally, as a proof of principle biotinylated heparin was successfully grafted onto photobiotin/avidin arrays.



## 1. Introduction

The development of controlled immobilization of patterned arrays of biomolecules on solid surfaces is relevant to many areas of science and technology including biosensors, bioelectronics, bioanalysis and diagnostics [1–3]. Recent advances in biomolecule patterning have been widely exploited for cell sorting and for the realization of living cell arrays thus enabling high-resolution analysis of specific individual cellular processes [4–6]. By controlling the spatial localization of biochemical cues that affects cell adherence, it is possible to indirectly pattern and physically isolate large numbers of individual cells onto well-defined substrate areas. Depending on whether the printed biomolecules are more adhesive or less adhesive than the surrounding bare substrate, cells will preferentially localize onto or avoid the stamped regions, respectively. Therefore, this microarray technology relies on fidelity of affinity interactions between a surface-bound bait and its cell target. Actually, also the surface properties of the substrate play a critical role and the capture selectivity of the final discrete arrays is strikingly affected by the anti-fouling and fouling-release properties of their surrounding regions with respect to proteins and cells which are essential to prevent nonspecific adsorption and to trigger specific interactions exclusively [7–9]. In fact, microarray surfaces, usually immersed into aqueous biological fluids or cell culture media, must be capable of selectively immobilizing biomolecular baits in microscale patterned spots and hence, selectively capturing target cells, but also be efficient at avoiding the nonspecific adsorption on non-modified areas of the substrates

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[4,10,11]. The generally adopted approach to overcome biofouling problems is to create cytophobic regions often consisting of water-swelling polymers, typically containing poly(ethylene glycol) (PEG) chains. The efficacy of PEG as a biologically passivating surface is linked to both the presumed biological inertness of the polymer backbone and also to its solvated configuration, since a tightly bound water layer forms a physical and energetic barrier to prevent protein adsorption on the surface [12–15]. PEG deposition can be performed either by physical absorption [16,17] or by PEG covalent immobilization [18,19], with the latter considered to be more stable and, therefore preferred for biosensor applications [20]. However, conventional methods usually require complex or multistep procedures that deeply impact the overall costs and require long reaction times [21–23]. In addition, their implementation often requires harsh processing conditions (e.g. the use of organic solvents or ultrasonication steps) [14] which can affect the functionality of biomolecules.

Recently, perfluoropolyethers (PFPEs) have also emerged as promising materials to be exploited for their anti-biofouling properties that are related to a combination of both surface energy and mechanical properties [24–27]. Actually, due to their physico-chemical properties, PFPEs are already widely used as low surface tension materials in the marine industry [28,29] for ship hull coatings. Conversely, in the biomedical field, UV curable perfluoropolyether-dimethacrylates (PFPE-DMAs) have gained increasing attention for the fabrication of optically transparent bio-microfluidic protein resistant devices [30,31]. Finally, our group recently published on the optimization of a single-step photolithographic process for selectively micropatterning hyaluronic acid (HA) onto UV-curable PFPEs aimed at selectively capture cancer cells [32,33]. Reported results attested the effectiveness of PFPEs as high-performing protein resistant substrates for discrete biomolecule patterning applications where passivation steps or chemical modification can be avoided. In the present work we sought to combine standard patterning processes to obtain a versatile biomolecular bait array on UV-curable PFPEs surfaces. Studies from literature reveal that depending on the type of target cells, different kinds of biological ligands (peptides, antibodies, nucleic acids, proteoglycans) [34–36] can be grafted as baits for specific biomolecular recognition and several approaches have been introduced to achieve biomarker patterning [37–40]. Among these, photochemical activation of light sensitive molecules has been recognized as a versatile patterning method. In particular, several studies have reported the combination of photolithographic processes for the selective patterning of a UV-sensitive biotin and its highly specific affinity with avidin family proteins to develop biotin-conjugated ligand patterns [3,41–43]. Generally, photoactivable forms of biotin are heterobifunctional molecules carrying a biotin head, a linker arm and a photoreactive aryl azide moiety [44] capable of covalently attaching to C—H, N—H and C=C bonds of the underlying surface upon exposure to UV-light [45]. Instead, the biotin head can be further conjugated to avidin family proteins whose tetravalent molecular structures consist of two pairs of biotin-binding sites on opposite sides. One pair could be used for complexation with the surface-bound biotin, while leaving the other pair available for binding of biotinylated molecules via a biotin-avidin-biotin bridge. Here, the photobiotin/avidin system was developed to enable binding of biotin-conjugated ligands onto PFPE-based polymers. By simply switching the biotinylated baits to be conjugated to the underlying photobiotin/avidin array the final goal of obtaining a versatile experimental platform for the immobilization of different specific cell populations can be achieved. The key step was the direct printing of UV-sensitive biotin onto photocurable PFPE substrates. As cost-effective alternatives to commonly used photolithography, microcontact arrayer and microcontact printing processes were both explored for the high-resolution spatially controlled delivery of photobiotin. While the latter consists in the transfer of biological inks from an elastomeric stamp [46], the spot-arraying technology is equipped with a robotic spotter to deliver few nanoliters of protein solutions on different regions of the substrates [39,47]. To the best of our knowledge, biotin immobilization onto PFPEs using these technologies has not been reported to date. Due to the absence of harsh processing conditions, photobiotin is not denaturated and its functionality is subsequently exploited by simply depositing avidin onto the printed photobiotin array. In fact, the high-performing anti-fouling PFPEs substrates spatially confined avidin binding where biotin was grafted. Onto this biomolecular array other biotinylated ligands could be bound carrying selected specific binding moieties designed to capture a specific cell population. Here, as an example, biotinylated-heparin was grafted onto PFPE substrates. Since heparin has been demonstrated to bind *Plasmodium*-infected red blood cells (pRBCs) [48–50] with high specificity and affinity, the availability of heparin in well-defined positions may open perspectives in the engineering of devices for molecular biology and nanomedicine. In particular, this specific interactions can be foreseeingly exploited for the design of new rapid diagnostic tests (RDTs) for malaria based on heparin-pRBC recognition. Such device would have several advantages relative to current antibody-based RDTs [51]: (i) Heparin is sturdier than antibodies in front of the harsh environmental conditions found in malaria endemic regions; likely, a heparin-based RDT will not require cold storage and can last for months at temperatures close to 40 °C if adequately kept dry and in the dark. (ii) Heparin production is more economically affordable than that of antibodies. (iii) Heparin has been found to bind cells infected by different *Plasmodium* species and different stages in the parasite's life cycle [52], both in the human and in the mosquito vector [53,54]; thus, a heparin-based RDT might be used for pan-malaria diagnosis and would not be so susceptible to the antigenic variability of the parasite as antibody-based devices based on the recognition of small antigenic determinants.

## 2. Experimental

### 2.1. Perfluoropolyether substrates

Two photocurable dimethacryloxy-functionalized perfluoropolyethers (PFPE-DMAs) differing for molecular weight were used in the present work. PFPE urethane dimethacrylate oligomer with molecular weight $M_n$ = 1980 g/mol (PFPE-DMA 2000) was kindly provided by Solvay Specialty Polymers (commercial name Fluorolink$^{TM}$ MD700). A higher molecular weight PFPE-DMA 4000, $M_n$ = 4127 g/mol, was synthesized starting from the commercial PFPE macrodiol (Fluorolink$^{TM}$ D4000, Solvay) and end-capping by the isocyanatoethyl methacrylate (IEM, Alfa Aesar) to form a NCO terminated polymer with polyacrylic functionality [55–57]. A PFPE-DMAs blend, indicated as Blend for brevity, composed of 50% by weight of the two was also considered. Subsequent photocuring of the PFPEs was accomplished through blending with 1% wt of 2-hydroxyl-2-methyl-1-phenyl-propan-1-one (Darocur® 1173, Ciba) as photoinitiator with the exception of PFPE-DMA 2000 that was prepared by adding 4% w/w of the same photoinitiator. PFPE-DMAs surfaces for patterning were prepared by spincoating (900 rpm, 1 min; ws400 6npp lite, Laurell TechnologiesCorp) on glass slides previously functionalized with 3-acriloxypropyltrimethoxysilane (AOPTMS, BCR, 2% v/v in ethanol, pH 4.5/5) and exposing to UV light for crosslinking. UV irradiation was made under vacuum with a bromograph (MF 1030, Nuova Delta Elettronica, Italy), equipped with 4 UV lamps (λ = 365 nm, 2 mW/cm$^2$). Polymerization kinetics was determined by photo-differential scanning calorimetry (UV-DSC, Mettler-Toledo DSC 823e) by evaluating the conversion