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Biopatterning of antibodies on poly(pyrrole)-nanowires using nanocontact printing: Surface characterization

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ABSTRACT

A highly performant patterning of antibodies using poly(pyrrole) nanowires (PPy-NWs) was devised on thermoplastic surfaces based on silane derivatives. The PPy-NWs were fabricated employing nanocontact printing and controlled chemical polymerization (nCP-CCP) on poly(ethylene terephthalate), cyclic olefin copolymer, poly(ethylene 2,6-naphthalate), and polyimide. The technique used a commercial compact disk as a template (mold) to produce nanopatterned polydimethylsiloxane stamps. The nanopatterned stamp was then employed to print PPy-NWs. The printing technique permits to control PPy-NW size and shape. The dimensions of the printed PPy-NWs were: 785 ± 1.5 nm (width), 174 ± 2.1 nm (height), and a separation between wires of 540 ± 1.2 nm. The printing process and the surface properties of the PPy-NWs pattern were successfully characterized by scanning electron microscopy and atomic force microscopy. Biopatterning was completed by the chemical immobilization of the specific anti-human interleukin-10 monoclonal antibody on PPy-NW using gluteraldehyde. The biocomposite was tested using qualitative immunocytokine bioassay, which is of great importance for early stage cancer detection. For that purpose, fluorescent imaging was used to certify the immunodetection of the recombinant human interleukin-10. The biopatterning technology provides a simple, low cost and one step procedure. Undoubtedly, this new technology will impact and provide an alternative to the current techniques applied for bioengineering and nanopatterning.

1. Introduction

Patterned surfaces having sub-micron scale resolution are of great importance in many fields of life sciences and biomedicine [1]. Different techniques have been proposed for surface patterning at the nanoscale. There is a huge variety of systems explored, such as single-walled carbon nanotubes (SWCNTs) [2], silicon nanowires (Si-NWs) [3,4], and gold nanowires (Au-NWs) [5]. These materials are expensive and difficult to produce [6]. Fabrication of those materials involves time consuming and long protocols such as chemical vapor deposition [7,8], laser beam ablation [9], ion beam etching, and photolithography techniques [10,11], which are commonly no adapted for biological applications. Alignment of those structures generally is performed by complex electrophoresis and microfluidic systems. Besides, one of the main challenges using nano-engineered tools is to address and biofunctionalize individually structures.

To overcome those problems, conducting polymer nanowires (CP-NWs) [12–14] have been successfully employed as alternative to fabricate nanostructures and to address it individually in sensing devices. From all CP-NWs, poly(pyrrole) nanowires (PPy-NWs) have many advantages when compared to SWCNTs and Si-NWs, such as easy synthesis and biofunctionalization. As a result, PPy-NWs are one of the most promising materials used to construct NWs with a high technological potential.

Detection based on NWs has revolutionized our ability to provide label-free and real-time detection with higher sensitivity and selectivity over a wide range of chemical and biological species [15]. However, most of the NWs fabrication techniques present some limitations regarding the patterned area size and the fabrication. Unfortunately, individually NWs for sensing devices involves tedious time-consuming methods such as photolithography and microfluidics [16,17]. On the other hand, usually, biosensing devices based on PPy-NWs are synthesized by electrochemical methods [18,19], in lithographic patterned channels [20], hard template assisted [21–24], free template assisted

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[25,26], or electrospinning methods [27]. Also, PPy can be synthesized by chemical polymerization [28,29], while PPy-NWs are commonly fabricated by combining hard template [30,31] or soft template methods [32–34].

Due to the complicated NWs fabrication and difficult manipulation, alternative fabrication methods based on soft lithography methodologies have been successfully introduced, for e.g., micro punching [35], direct ink-jet printing [36], contact printing method [37], edge transfer printing [38], transfer-printing [39], and nano transfer printing lithography [40]. Especially for sensing devices based on NWs printing, techniques like nanoimprint lithography (NIL) [41], hot embossing lithography (HEL) [42], and microcontact printing (μCP) [43,44] have been successfully employed for CP-NW fabrication; however, these techniques are still complex due to a multi-step fabrication process.

Micro/nanocontact printing (μ/nCP) is the most common soft lithography-based technique for surface patterning at the nanoscale. The μ/nCP technique is an alternative solution due to its low cost, simplicity, and the absence of special laboratory requirements. Nanostructured polydimethylsiloxane (PDMS) stamps are replicated from micro- and nanopatterned melds, silicon molds, and commercially available digital compact disks (CDs). This is an easy, direct, effective, and low cost methodology for molecule patterning immobilization, which is of interest in those areas that require nanoscale structures over large areas, for e.g., tissue engineering or biosensor applications.

The μCP technique is a flexible and non-photolithographic method that allows the patterning of molecules at submicron dimensions on a surface through contact pattern transfer. A PDMS elastomeric stamp is used to transfer molecules onto the surface of the substrate by conformal contact [45]. The μCP and nCP techniques require silicon master molds, where different methodologies to fabricate master molds have been proposed, for e.g., silicon patterning using electronic microscopy and e-beam lithography [46,47]. As previously mentioned, most of these techniques require expensive instruments and they are time consuming for creating large area (cm^2) patterned nano molds. To overcome this problem, commercially available blank CDs are good alternatives as they can be used as nanoscale master molds [48]. This technique is an easy way to fabricate PDMS stamps for nanopatterning by nCP [49].

Current limitations at nanoscale for biosensing applications is the lack of techniques that allow to easily control and to simply address biostructures, which is still a challenge [50]. Most of the nano scale fabrication techniques include complicated and time consuming protocols, expensive and special materials, and sophisticated laboratory equipment is required. The aim of this study is to overcome these problems. Therefore, we describe a simple process to fabricate PPy-NWs supported on thermoplastics films via nCP techniques. We have previously reported PPy micro- and nanopatterning techniques on gold substrates by using thiol chemistry [51,52]. Also, we have recently developed a new PPy micropatterning technique by μCP and chemical polymerization [53] on glass and PETE. Here, we present a new printing technology, which has allowed us to pattern PPy on non-conductive and conductive substrates at the nanoscale.

In this work, we show the possibility of PPy nanopatterning by combining nCP with controlled catalytic polymerization (nCP-CCP). The nCP technique provides a valuable tool for printing over large areas and easy fabrication of sub-micrometer conducting polymeric patterns that would be useful in polymer based electronics, microchips, lab-on-chip, and biosensors. This method offers the opportunity to grow PPy-NWs in-situ at the desired position and also between two electrodes (for e.g., interdigitated electrodes).

In the present work, we have printed PPy-NWs on different surfaces (poly(ethylene terephthalate (PETE), poly(ethylene 2,6-naphthalate (PEN), polyimide (PI), and cyclic olefin copolymer (COC)) and we have studied the printing process and the surface properties to understand the different physical and mechanical characteristics of the PPy patterns. As a final point, we focus on the analysis of the unique PPy-NWs properties, such as electrical conductivity and biocompatibility.

2. Materials and methods

Chemicals and reagents: *N*-(3-trimethoxysilyl-propyl)pyrrole (Py-silane) was purchased from ABCR GmbH & Co. KG, Germany. Sulphuric acid (H_2SO_4) (30%), sodium dodecyl sulphate (SDS), iron (III) chloride, and pyrrole were purchased from Sigma Aldrich, France. Hydrogen peroxide (H_2O_2) (35% wt.) and potassium hydroxide (KOH) were obtained from Acros Organics, France.

Interleukin 10, recombinant human IL-10: Recombinant human IL-10 was provided by R&D systems, USA. Reconstitution at 0.5 mg/mL in PBS. Cytokine that plays important roles either immune stimulatory or immunosuppressive effects on a variety of cell types (hematopoietic, hepatic stellate, Keratinocytes, and placental cytotrophoblasts). It is produced predominantly by lymphoid cells. Immunogen (*Spodoptera frugiperda*) Sf 21-(baculovirus)-derived rhIL-10, mouse IgG1 class. It is a critical molecule in the control of autoimmune inflammation.

Human IL-10 antibody: Sf 21-derived recombinant human IL-10, mouse IgG1 class. Monoclonal antibody was provided by R&D systems, USA. Reconstitution at 0.5 mg/mL in PBS.

Polymers: PDMS (Sylgard 184) was purchased from Dow Corning, France. The applied thermoplastic films were: PI (HN, 125 μm, DuPont), PETE (125 μm, Goodfellow), PEN (125 μm, Goodfellow), and COC (188 μm, ChipShop, Germany).

Microcontact printer: Semi-automatic Micro-Contact-Printing System μ-CP 3.0 from GeSiM Gesellschaft fuer Silizium-Mikrosysteme mbH.

Microscopy: Scanning electron microscope (SEM) images were obtained with a Hitachi SEM S800, France. Finally, atomic force microscopy (AFM) images were obtained with a Nano observer (CSI Company) (AFM specifications can be found in the Supplementary materials).

Four-point probe resistivity: The electrical conductivity was measured by a four-point probe measurement system Pro4 (Microworld) and a Keithley 2400/2600 probe head. Samples size and thickness were 100 mm and 8 μm, respectively, measurements performed at 23 °C.

2.1. Substrate surface activation

The thermoplastics films were cleaned by sonication and rinsed first with propanol and then with distilled water. The PET, PEN, PI, and COC films were activated in KOH (3M) solution for 5 min (COC was previously treated with a 60% HNO_3 solution for 10 min). The substrates were thoroughly rinsed with distilled water and then dried with nitrogen. Finally, the surface was exposed to ozone (UV/O_3 Procleaner ™, Bioforces Nanosciences) for 5 min.

2.2. Stamp fabrication

The nanostructured master was obtained from a commercially available blank compact disk (CD, 700 MB, 52×, Maxell). The CD consisting of a base part of polycarbonate (PC), and a thin metallic foil portion (aluminium, silver, gold, etc.) and polymeric protective resin (acrylics). The PC nano-patterned layer consists of wire arrays with a typical depth and width of ~180 nm and 800 nm, respectively. The periodicity is ~500 nm depending upon the employed CD [54]. Samples of 1 cm^2 of CD were first ultrasonically cleaned in acetone for 5 min. The metallic reflective layer and the polymeric protective layer were separated from the nanostructured plastic substrate by submerging the CD samples in nitric acid (60%) for 5 min. The resulting PC substrates were then rinsed in Milli-Q ultrapure water and ethanol 96%, and finally dried under nitrogen. The PC part was used as the mold to produce a PDMS stamps (Fig. S1).