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Inkjet-printed polyaniline patterns for exocytosed molecule detection from live cells

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ABSTRACT

Polyaniline (PANi) patterns on flexible substrate are fabricated for biomolecule detection from live cells. PANi patterns are prepared by inkjet printing on polyethylene terephthalate film. Subsequently, arginine–glycine–aspartate (RGD) peptide is immobilized on the PANi pattern to selectively adhere cells. Rat pheochromocytoma PC12 cells are cultured on the RGD–immobilized PANi pattern, and patterned with high selectivity and growth. Additionally, the cells show focal adhesion on the RGD–immobilized PANi pattern, which are confirmed with vinculin staining and scanning electron microscopic images. To monitor dynamic biomolecular release from PC12 cells, RGD–immobilized PANi pattern is used for a real-time electrical signal detector. RGD–immobilized PANi patterning and sensing system represents outstanding ability to translate and amplify exocytosis molecules into a detectable signal as a transducer.

1. Introduction

Conducting polymers (CPs) have attracted considerable attention given their potential to produce flexible, biocompatible, and light-weight devices, as alternatives to inorganic semiconductors or metals [1–3]. Recent progress in the synthesis of CPs has overcome several interfacial problems in the field of organic bioelectronics such as the application of tissue–electrode interfaces for nerve regeneration, biorecognition, and the preparation of cell–electrode interfaces for multifunctional neuron probes [4–6]. Biocompatible CP electrodes have prompted to apply biointerfaces for enhancing cell–electrode communication, markedly in nervous systems for their patterning and recording functionalities. CPs are readily tailored by surface modification or doping method ranging from small ions to macromolecules [7–9].

Inkjet printing is an efficient alternative to conventional photolithography for the production of versatile CP–based micro/nanoelectronic devices owing to its low cost, high–speed patterning, flexibility, shape control, and applicability to various substrates [10–12]. Recently, inkjet printing has been reported as a promising candidate for bio-applications due to its facile and versatile micro-patterning of biomaterials, however, relatively little research has been carried out on biomedical fields [13,14].

Surface patterning is routinely used to immobilize bioactive molecules such as proteins, oligonucleotides and small ligands; to localize surface reactions for bioassays; and to provide cell and bacterial adhesion [15,16]. Such patterning is exploited for biochips, co–cultures, tissue engineering, cell–based biosensors, and studies of extracellular effects on cell behavior [17–19]. Cell adhesion plays a crucial role in many biological processes, including cell guidance, wound repair, and extracellular signal transduction [20,21]. When cells adhere to the extracellular matrix (ECM) *via* cell surface receptors, integrins mediate signal transduction pathways that influence a range of cellular processes [22]. Cell adhesion to substrate via transmembrane cell receptors has been mostly controlled by short and simple peptide sequences. The modification of substrates with ECM proteins provides cell-attachable surfaces [23]. As cell attachment to the substrate depends on the type of ECM protein and on the subset of integrin receptors present on a specific cell type, the choice of immobilized proteins provides selectivity for specific immobilization of certain cell types. The first peptide developed to the ECM protein fibronectin was the tripeptide Arg–Gly–Asp (RGD) in 1984 [24]. This tripeptide is the minimal recognition sequence for integrin receptors. Peptides containing the RGD sequence induce the adhesion of many cell types including fibroblasts, osteoblasts and macrophages [25]. Besides the RGD sequence other cell recognition motifs exist, but only some of them have been immobilized for cell attachment so far [26]. The RGD sequence is by far the most effective and most often employed peptide sequence for stimulated cell adhesion on synthetic surfaces.

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The recording of electrical signals from cells and tissues is central to areas ranging from fundamental biophysical studies (*e.g.*, heart and brain) to medical monitoring and intervention [27,28]. Over the past few decades, a large effort has been devoted to recording signals from electroactive cells [29,30]. Many studies have focused on the field effect transistors method, which uses inorganic nanowires and carbon nanotubes to produce recording with a high signal–to–noise ratio from electroactive cells [31,32]. However, these materials do not guarantee selective cell adhesion because of their intrinsic properties such as inert surface, cellular toxicity, modification difficulties [2]. The control of material–cell surface interactions is key for a wide range of biomedical applications [33]. To allow cells to adhere to the pattern, bioactive ligands must allow the cell to interact with extracellular matrix proteins or structurally related synthetic peptides [34].

Here we fabricated flexible inkjet-printed polyaniline (PANi) patterns for selective cellular patterning and the electrical detection of biomolecules. The PANi pattern was prepared by oxidant inkjet printing on poly(ethylene terephthalate) (PET) film with subsequent vapor deposition polymerization of aniline monomers. RGD peptide was then grafted onto the PANi pattern as a simple method for selectively binding live cells. The detection of electrical biomolecule from neurons was performed using an interdigitated microelectrode array. Several features of such a multifunctional PANi pattern make it particularly attractive for patterning and sensing neuronal cells, including: (1) the simplicity of fabricating precise micro–sized cell patterns; (2) facile modification for the grafting RGD peptide *via* covalent bonding with PANi; (3) focal adhesion between cells and the PANi pattern; (4) the flexibility of the substrate, which makes it applicable to various biomedical fields; and (5) the electrical detection of biomolecule secretion from live cells to allow studies at the subcellular level.

2. Materials and methods

2.1. Patterning of polyaniline (PANi)

Ammonium persulfate (APS; 98%), purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA), was used as an oxidant. Aniline monomer (99%) was obtained from Sigma-Aldrich Co. Ltd. and used without further purification. PET (3M) film was used as a substrate for inkjet printing. Commercial office inkjet printer (Canon Pixma IP1300) was purchased and modified for this work. The ink cartridge (printer head) was disassembled and washed several times with ethanol and distilled water after removal of the ink. An oxidant solution containing 1.18 M APS in distilled water was injected into the modified ink cartridge. The completely sealed cartridge was subsequently placed in the printer body and prepared for use. For PANi patterning, the complexly patterned architectures were designed in advance using a computer software package (Microsoft PowerPoint 2003). The oxidant APS solution was printed onto PET film in the desired pattern using the modified inkjet printer. The printed film was then cut and placed in a vapor phase polymerization (VDP) chamber (70 °C) containing aniline monomer (4 mL) and 0.1 M hydrochloric acid solution (1 mL) for 15 min. Emeraldine salt form of PANi patterns were formed in the VDP chamber at 70 °C. Fig. S1 shows a schematic diagram of our inkjet printing procedure.

2.2. RGD peptide immobilization

The printed patterns were washed with distilled water twice to remove residual APS. The printed patterns were treated with 0.5% glutaraldehyde solution for 2 h. The patterns were then thoroughly washed with distilled water and immersed in pure ethanol to remove adsorbed molecules from the substrate. The aldehyde presenting pattern was incubated with 0.5 mM amine terminated GRGDS peptide solution in PBS buffer for 1 h at 37 °C. After incubation, the pattern was washed three times with 0.1 M PBS. The patterns were reacted with EDC for 2 h and then incubated with NHS and 0.5 mM amine terminated GRGDS peptide solution in PBS buffer for 1 h at 37 °C. After incubation, the pattern was washed three times with 0.1 M PBS. As a control, poly(ethylene glycol) (PEG, molecular weight $Mw \sim 5{,}000$) modification was conducted on the PANi pattern. 1 mM PEG-succinimidyl ester (NANOCS Inc, MA, USA) solution was added in the PANi pattern for 1 h at 37 °C. After incubation, the pattern was washed three times with 0.1 M PBS.

2.3. Cell culture

PC–12 cells obtained from the American Type Culture Collection (Manassas, VA, USA) were maintained in RPMI-1640 with 10% fetal bovine serum, 1% penicillin–streptomycin solution, 25 mM sodium bicarbonate, 300 mg L^{-1} L–glutamine, and 25 mM 4–(2-hydroxyethyl)–1–piperazine ethansulfonic acid (HEPES) at 37 °C in a 5% CO_2 atmosphere in 75–cm^2 flasks. The cells were subcultured three times per week. All experiments were performed in a clean atmosphere.

2.4. Observation of live PC–12 cells on RGD-immobilized PANi pattern

PC-12 cells (2×10^4) were cultured on an RGD-immobilized PANi substrate in 24-well plates (Nunc, Thermo Fisher Scientific, Rochester, NY, USA). After 48 h, the medium was removed and the cells were washed twice with 0.1 M PBS. The cells were then stained with 1 μM cell tracker probe solution (Molecular Probes, Invitrogen, Carlsbad, CA, USA) for 30 min at 37 °C. The probe solution was then replaced with pre-warmed medium and the cells were incubated for another 30 min at 37 °C. The cells were then washed twice with 0.1 M PBS and fixed in 3.7% paraformaldehyde for 15 min at 25 °C. The cells fixed on the RGD-patterned PANi substrates were analyzed with a Delta Vision® RT imaging system (Applied Precision, Issaquah, WA, USA) under 5% CO_2 at 37 °C. To obtain images, a Cascade II electron multiplying charge-coupled device (EMCCD) camera was used.

2.5. Immunofluorescence staining of PC–12 cells

PC–12 cells, grown under the conditions described above, were fixed in 4% paraformaldehyde for 10 min, permeabilized in 0.5% Triton X–100 for 5 min, and then blocked in PBS containing 4% bovine serum albumin, and 0.01% sodium azide for 15 min. To detect focal adhesion, FITC–tagged monoclonal anti–vinculin antibodies (Sigma-Aldrich Co. Ltd.) was used. To characterize the cytoskeleton morphology, rhodamine–phalloidin (Cytoskeleton) labeling (100 nM in PBS from a 1 mg mL^{-1} methanol stock solution) was used. After washing, the nuclei were stained and the samples were mounted on a glass coverslip using Fluoroshield (Sigma) and characterized using a Delta Vision® RT imaging system (Applied Precision). The samples were observed at 40 × using the 1.515 N.A. oil immersion objective.

2.6. Imaging of PC-12 cells on the RGD–immobilized PANi pattern by SEM

The morphology of cells on the RGD-immobilized PANi pattern was determined by SEM (SUPRA 55VP, Carl Zeiss, Jena, Germany). Cells (2×10^4 mL^{-1}) were cultured on RGD-immobilized PANi patterns for 48 h, then washed twice with cold 0.1 M PBS and