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Fabrication of tunable micropatterned substrates for cell patterning via microcontact printing of polydopamine with poly(ethylene imine)-grafted copolymers

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ABSTRACT

Cell patterning is an important tool for biomedical research. In this work, we modified a technique combining mussel-inspired surface chemistry and microcontact printing (μCP) to modulate surface chemistry for cell patterning. Polymerized dopamine on poly(dimethylsiloxane) stamps was transferred to several cell-unfavorable substrates via μCP. Since cells only attached to the polydopamine (PDA)-imprinted areas, cell patterns were formed on a variety of cell-unfavorable surfaces. The stability of PDA imprints was proved under several harsh conditions. The cell affinity of PDA was modulated by co-deposition with several poly(ethylene imine) (PEI)-based copolymers, such as PEI, PEI-g-PEG (poly(ethylene glycol)) and PEI-g-galactose. The imprints of PDA/PEI-g-PEG provide the formation of cell patterns on cell-favorable substrates. Neuronal PC12 cells were patterned via imprinting of PDA/PEI, while HepG2/C3A cells were arranged on the imprint of PDA/PEI-g-galactose. Finally, co-culture of HepG2/C3A cells and L929 fibroblasts was accomplished by our micropatterning approach. This study demonstrated this simple and economic technique provides a powerful tool for development of functional patterned substrates for cell patterning. This technique should profit the preparation of cell patterns to study fundamental cell biology and to apply to biomedical engineering such as cell-based biosensors, diagnostic devices and tissue engineering.

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1. Introduction

Cell patterning on artificial substrates can be applied to the fundamental study of cell biology, as well as biomedical engineering applications, such as cell-based biosensors, diagnostic devices and tissue engineering [1]. The basic principle of cell patterning is to create cell-favorable and -unfavorable regions on substrates to control the spatial arrangement of cells. A variety of microfabrication techniques have been applied to create surface chemical or topographical patterns in order to regulate cell attachment and functions, as reviewed previously [2,3]. The most commonly used microfabrication techniques include photolithography [4–6], microcontact printing (μCP) [7,8], microfluid patterning [9] and transfer lithography [10–12]. A strategy of transfer photolithography has recently been developed to generate surface patterns that has the advantage of persistently localizing cells in the predesigned patterns, though at the cost of several fabrication procedures. In contrast, μCP is an easy, rapid and economic technique for cell patterning, though the cell patterns only persist for a relatively short time. This technique is based on contact transfer of the material of interest from a poly(dimethylsiloxane) (PDMS) stamp onto a surface only on the areas contacted by the stamp. A variety of molecules, such as alkanethiols [8], proteins [13], polymers [14] and nanoparticles [15], have been employed as the ink to create micropatterned surfaces via μCP. We suggest that an imprinting material that could adhere to a wide variety of substrates with adjustable cell affinity would have great advantages for patterning diverse cell types.

Recently, a technique based on the adhesive mechanism of marine mussels provides a simple and versatile tool for biomaterials surface modification [16]. Marine mussels bind tightly to the various surfaces on which they reside in an aqueous environment, a process which relies on the much repeated 3,4-dihydroxy-L-phenylalanine-lysine (DOPA-K) motif found in mussel adhesive proteins [17,18]. A dopamine molecule containing a catechol and an amine simulates the functional moieties of the DOPA-K motif. Dipping substrates in an alkaline dopamine solution (e.g. pH 8.5) creates a spontaneous coating of a thin adherent film via the oxidative polymerization of the dopamine [16]. An attractive character of polydopamine (PDA) for surface modification is its strong adhesive bonding to a wide range of organic and inorganic materials [16]. Furthermore, a thin PDA film is capable of promoting protein

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immobilization [19–21] and cell adhesion [22–25]. Another advantage of using a PDA coating is its reactivity towards nucleophiles such as thiols and amines [16], which can conjugate functional molecules to modulate surface functionality.

Because of the diverse characteristics of PDA, micropatterning of PDA could be a simple and versatile platform for the creation of a wide variety of micropatterned substrates for cell patterning. Several techniques, such as photolithography [16], microfluidic technique [24] and μCP [26,27] have been applied to create such PDA micropatterns on surfaces. For μCP of PDA, dopamine is polymerized directly onto PDMS stamps, then the developed PDA is transferred onto the target surface μCP (Fig. 1A). Recently, Sun et al. [26] showed that PDA could be microcontact printed onto a cell-unfavorable poly(ethylene glycol) substrate to create micropatterns of cells. However, the application of μCP of PDA has its limitation. First, because PDA mediates the adhesion of many types of cells, recent studies regarding cell patterning via μCP of PDA have focused on cell-unfavorable substrates, such as PEGylated surfaces. To create cell patterns on a cell-favorable surface with a defined chemistry, pure PDA ink cannot be used since cells attach to both areas. Second, PDA domains may not be suitable for studying cell physiology. Third, some cell types, such as neuron cells [28], attach to PDA poorly, so this strategy cannot be applied to every cell type.

In our previous study, we demonstrated that functionalized PDA patterns could be easily imprinted from PDMS stamps to a wide variety of substrates μCP, and at a low cost [27]. In this study, we have further modified the technique of PDA μCP to increase its applicability to create more types of cell patterns. The applicability of μCP of PDA to several cell-unfavorable substrates and the stabil-

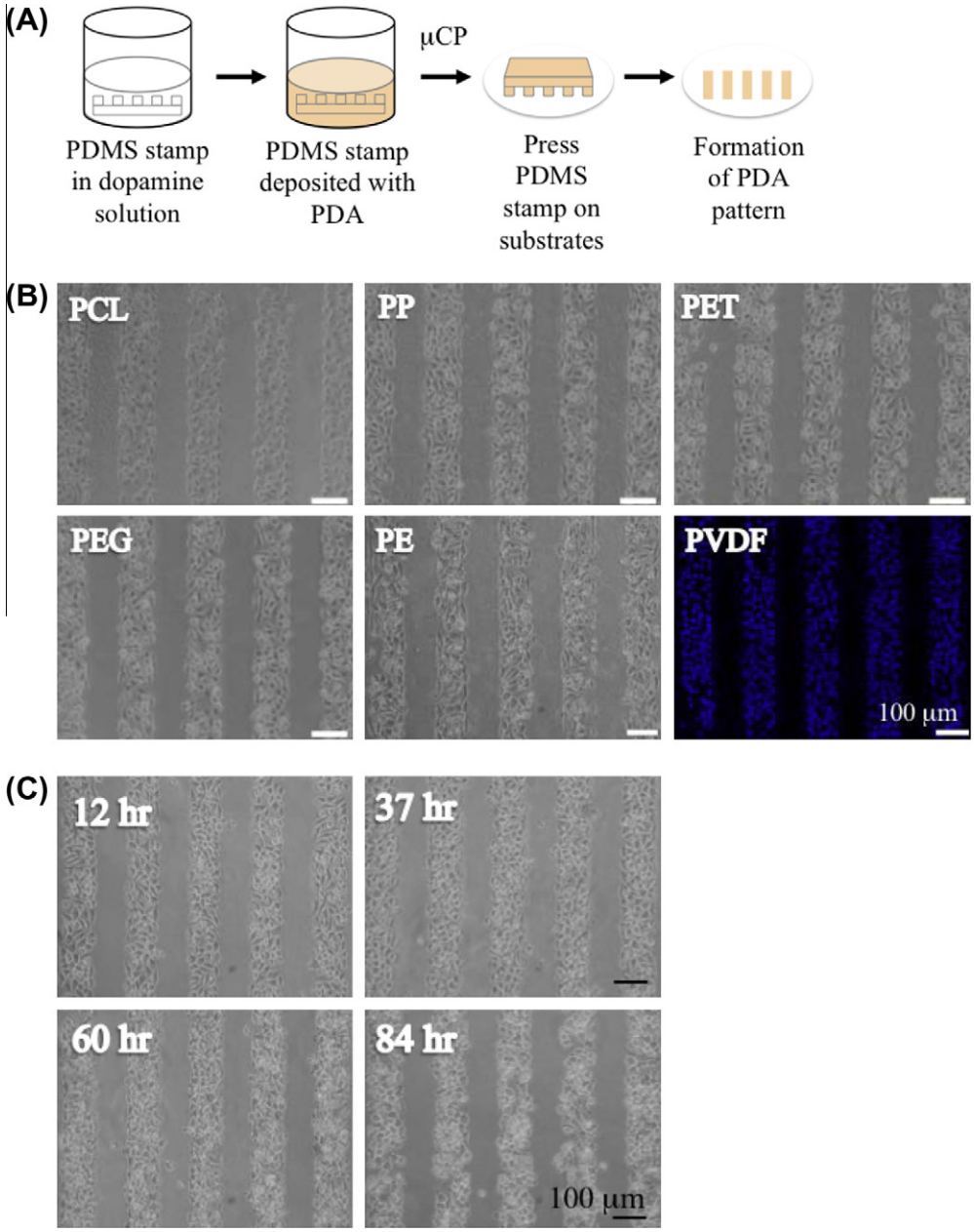

Fig. 1. (A) Schematic illustration of μCP of PDA patterns on other substrates. A PDMS stamp is immersed in dopamine solution, then the PDA developed on the PDMS stamp is transferred to another surface via μCP. (B) PDMS stamps were incubated in dopamine solution for 60 min and then imprinted on various cell-unfavorable substrates. L929 cells were cultured on the PDA-imprinted surfaces for 12 h. All images were taken by a phase contrast microscope except the PVDF one, which was taken by a confocal microscope. (C) L929 cells were cultured for 12–84 h on a PS substrate imprinted by a PDMS stamp that had been incubated in dopamine solution for 60 min. Magnification ×100.

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