Biomaterials 33 (2012) 624–633

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Patterning and transferring hydrogel-encapsulated bacterial cells for quantitative analysis of synthetically engineered genetic circuits

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ARTICLE INFO

Article history:
Received 30 August 2011
Accepted 26 September 2011
Available online 19 October 2011

Keywords:
Hydrogel patterning
Hydrogel transferring
Extracellular induction
Intercellular communication
Gene expression and regulation

ABSTRACT

We describe a hydrogel patterning and transferring (HPT) method that facilitates the quantitative analysis of synthetically engineered genetic circuits within bacterial cells. The HPT method encapsulates cells in the alginate hydrogel patterns by using polydimethylsiloxane (PDMS) template. Then, the hydrogel-encapsulated cell patterns are transferred onto an agarose hydrogel substrate that encapsulates inducer chemicals or bacterial cells. Using the HPT method, we demonstrate that inducers in the agarose hydrogel substrate regulate gene expression of the patterned cells for qualitative analysis by activating the promoters of fluorescence protein genes. In addition, we demonstrate that the HPT method can be used for the analysis of the cross-talk between genetic circuits and the concentration-dependent gene expression and regulation because the agarose hydrogel substrate can produce concentration gradients of inducers. Lastly, we demonstrate that the HPT method can be applied to investigating intercellular communication between neighboring cells with a wide range of cell densities. Since the HPT method is simple to deal with but versatile and powerful to quantitatively analyze genetic circuits in living cells in many controllable manners, we believe that the method can be widely used for the rapid advancement of synthetic, molecular, and systems biology.

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1. Introduction

Synthetic biology mainly deals with the design and assembly of robust, predictable, and controllable biological parts, called genetic circuits, so that system-level understanding of such biological parts is essential to engineer new tasks in biological systems [1–3]. For the advancement of synthetic biology, several quantitative analysis methods need to be developed in parallel for extracellular induction of genetic circuits (e.g., activation and inhibition of genes), intercellular communication and cross-talk between two genetic circuits, and quantitative analysis of gene expression and regulation [4–6]. Quantitative analysis methods seem to benefit from the microfabrication technology that has been offering unprecedented experimental tools for scientists and bioengineers to investigate many biosystems *in vivo* and *in vitro* [7–10]. In particular, many patterning methods based on the soft-lithography technology seem to have a high potential to analyze genetic circuits in cells because they allow the production of various spatial and temporal patterns of biomolecules [11,12] and even bacterial cells [13,14] on various substrates with high resolutions. Nevertheless, many synthetic biologists seem to depend more on manual pipetting or cell seeding methods rather than microfabrication-based cell patterning methods to investigate their synthetically engineered genetic circuits [15–17], and this seems to be attributed to the fact that the fabrication processes are somewhat complicated and the clean-room facility is unavailable.

First of all, we briefly review many documented cell patterning methods and roughly categorize them into four: 1) micro-contact printing/stamping, 2) microfabricated stencil/channel, 3) inkjet printing, and 4) microfabricated well array methods. These methods have been widely used in many biotechnological applications such as cellular differentiation, metapopulation dynamics, high throughput screening, drug screening, phenotypic variation screening, cell signaling molecule-driven pattern formation, cell-to-cell communication, and so on [9,18]. Firstly, the micro-contact printing/stamping methods use soft materials like PDMS to ink biomolecules or cells on the junctions and then write them onto another surface [9,14,19]. Various patterns are possible by using photomasks, printing/stamping areas are large enough to permit high throughput screening of drugs and cells, and patterning

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doi:10.1016/j.biomaterials.2011.09.069

placements can be precisely controlled [20]. However, the microcontact printing/stamping devices cannot easily adjust the concentrations of biomolecules or the number of cells to print and cannot be reused to avoid any undesired contaminations. Secondly, microfabricated stencil methods have been employed to pattern biomolecules and even adhere cells on a substrate [21–23]. These methods also provide a means to co-culture two different types of cells, allowing the study of intercellular interactions and communication. However, the stencil methods require a slightly complex fabrication process in order to make stencil features using biocompatible materials and hold a limitation to the intercellular communication study; it seems difficult to apply this to extracellular induction experiment. In other words, PDMS is not easily etched by using a dry or wet etching technology, although alternative methods were developed [22] and, these methods do not allow to investigate the interaction between inducers and cells. Thirdly, non-contact and direct printing methods are also widely used to pattern biomolecules and mammalian and bacterial cells onto a surface by using piezoelectric inkjet or bubble jet printing heads [24–26]. These methods have a unique advantage that they do not contaminate the substrate, so that they appear very promising to pattern many different biomolecules and cells spatially and temporally as a consumer printer does for photographs. However, a research-grade inkjet printer seems to be expensive, and a modified consumer printer requires some techniques to hack the printing controller and cartridge. Additionally, cells should be printed carefully. Otherwise, mechanical shear stresses caused by nozzles of a few tens μm diameter during the injection process can damage cells. Lastly, the multiple well array methods were used to pattern or compartmentalize cells and even provide millions of wells in a similar area as 96-well microplates, enabling extremely high throughput screening compared to conventional microplate readers [8,27,28]. However, the microfabricated well array methods seem inappropriate for the study of intercellular communication of heterogeneous cells, being limited to the study of extracellular induction of homogeneous cells.

It is obvious that all these patterning methods hold their own advantages, and they also show some disadvantages. To address the weaknesses and enhance the strengths of previous cell patterning methods, we describe a hydrogel-based cell patterning and transferring (HPT) method that is particularly useful in quantitatively analyzing the operation of engineered genetic circuits in living bacterial cells; detailed comparisons are discussed later. As illustrated in Fig. 1A, the method encapsulates bacterial cells in sodium alginate hydrogel with various patterns made using a PDMS template. In parallel, we prepare another hydrogel substrate in a similar manner by using a thin PDMS frame on a glass surface to pour a hydrogel solution and then make the solution gel, which can contain either inducers or cells. The hydrogel patterns are then transferred onto the hydrogel substrate; the hydrogel cell patterns in the PDMS template are laid onto the hydrogel substrate carefully and then peeled off gently. Since the HPT method facilitates the formation and transferring of various hydrogel-encapsulated cell patterns, in this work we apply the method to quantitative analyses

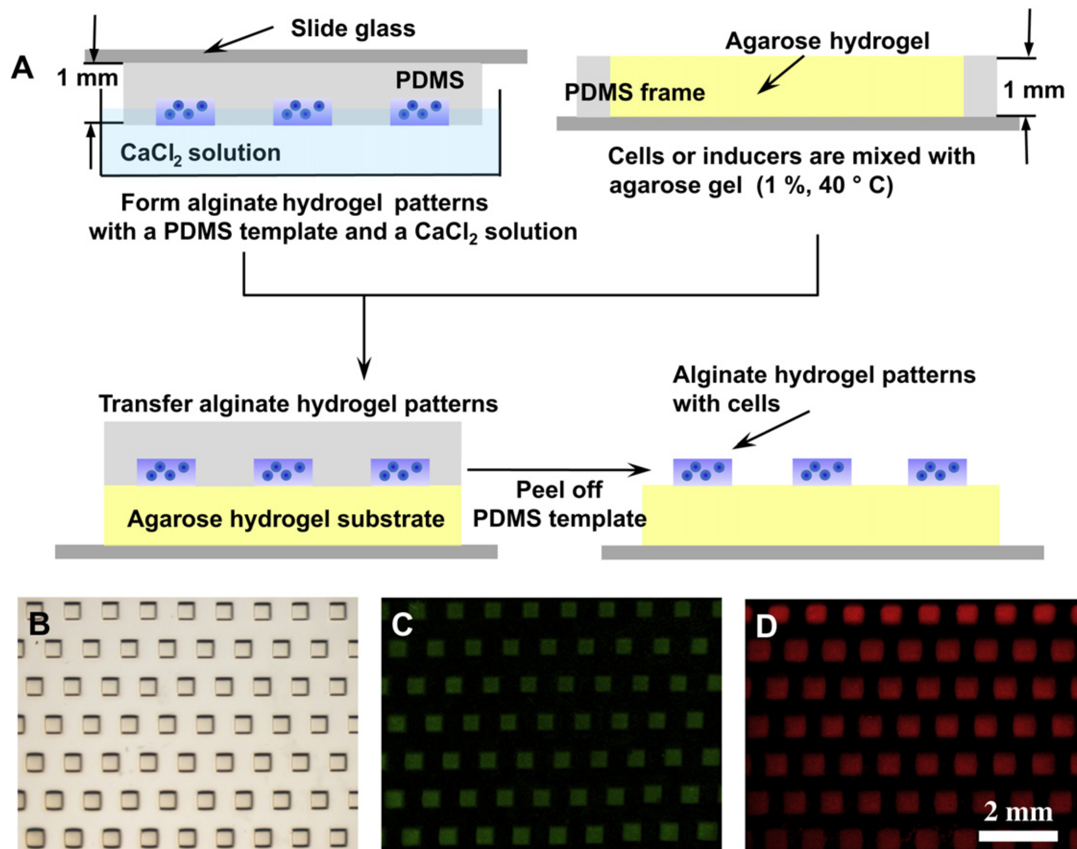

Fig. 1. (A) Microfabrication processes for the hydrogel patterning and transferring method. An alginate solution with bacterial cells and necessary nutrients is patterned using a PDMS template and solidified using a $CaCl_2$ solution. Then, the alginate hydrogel patterns are transferred onto an agarose hydrogel substrate that contains inducers or cells. (B) A bright light micrograph of the alginate hydrogel patterns. Each square is 500 μm by 500 μm and 140 μm in height. (C) A fluorescent image of the alginate hydrogel patterns encapsulating bacterial cells that constitutively express green fluorescent reporter gene (pTKU4-2). (D) In the same manner, bacterial cells are patterned on the agarose hydrogel substrate that contains 400 μM arabinose as an inducer and activated to express the red fluorescent reporter gene (pZBRG). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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