Cell Patterning

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# Patterning Mammalian Cells for Modeling Three Types of Naturally Occurring Cell–Cell Interactions**

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Herein we report a method for the simulation of three types of cell–cell interactions in vitro on the same substrate by using selective modification of the surface by microfluidic channels. In vivo interactions between different types of cells include the following types: 1) those between two types of cells that are both immobilized and confined to isolated areas (for brevity, we call this kind of interaction "type I"), for example, epithelial cells and fibroblasts, and epithelial cells and polar cells during ovarian development;[1] 2) those between one cell type that is immobile and another that moves freely ("type II"), such as glial cells and neurons during the development of the nervous system and in various neurodegenerative disorders;[2] and 3) those between two or more types of cells that are both moving freely ("type III"), for example, hepatocytes and fibroblasts in the liver.[3] The development of an organism, the establishment of neural networks, and the formation of tumors involve all aforementioned types of interactions between different types of cells. None of the existing techniques can, however, dynamically control all of these behaviors and model all three types of cell–cell interactions with micrometer-scale precision.[4] Even though we have a large number of available methods to pattern biological molecules (such as proteins) on solid surfaces, two characteristics of the interactions between mammalian cells and solid surfaces make it difficult to pattern multiple types of cells: 1) Most adherent mammalian cells, when cultured in vitro, randomly move and scatter around; and 2) newly seeded cells tend to squeeze into even the most tightly formed monolayers of cells and adhere to the surface, resulting in nonhomogeneous populations of cells in patterns. These features make patterning of multiple types of cells, in other words, the ability to control the adhesion and migration of mammalian cells to allow all three types of cell–cell interactions, still quite difficult in comparison to the patterning of multiple types of proteins.

Several research groups have reported techniques for patterning multiple types of cells; some of these methods allow the confinement of two or more types of cells to specific locations on surfaces (to simulate type I),[5,6] as well as the movement of these different types of cells toward each other (to simulate type III).[5,7] To our knowledge, however, no published work demonstrates the simulation of type II cell–cell interactions though it is an important type of cell–cell interaction often encountered in normal and pathological physiology.[2,8]

Herein, we present a new method that achieves all three types of cell–cell interactions: "no release" (the confinement and segregation of multiple types of cells, type I), "partial release" (the selective release of one cell type for free motility while keeping the other cell type confined, type II), and "complete release" (the complete release of all types of cells, type III) of multiple types of cells (Figure 1). Our method uses an alkanethiol [$(HS(CH_2)_{11}(OCH_2CH_2)_6OH$, abbreviated as $EG_6$][9] to generate self-assembled monolayers (SAMs) that resist cell adhesion ("inert" areas), and the extracellular matrix protein fibronectin (FN) physically adsorbed from solution to generate surfaces that promote cell adhesion ("permissive" areas); we employ microchannels both to create areas with different surface properties (either permissive or inert) and to transport different types of cells to designated locations on the same surface. Our strategy is dramatically simpler than previously reported methods. We strongly believe that this strategy will find wide application in fundamental research in cell biology.

We first demonstrate the approach to achieving "partial release" of one type (out of two types) of cells (type II, Figure 1). We used a poly(dimethylsiloxane) (PDMS) stamp with embedded microfluidic channels for selective modification of the surface of the gold-coated glass substrate.[10] The PDMS stamp reversibly sealed with the gold substrate through physical contact.[5] To vary the distances between different types of cells on the same substrate, we made one of the microchannels long and serpentine. When we modified the gold-coated substrate with FN and $EG_6$ in PDMS microchannels, we noted that $EG_6$ could diffuse under the PDMS stamp to form inert SAMs, but FN could not. (We investigated the dynamics of this migration; see Figure S1 in

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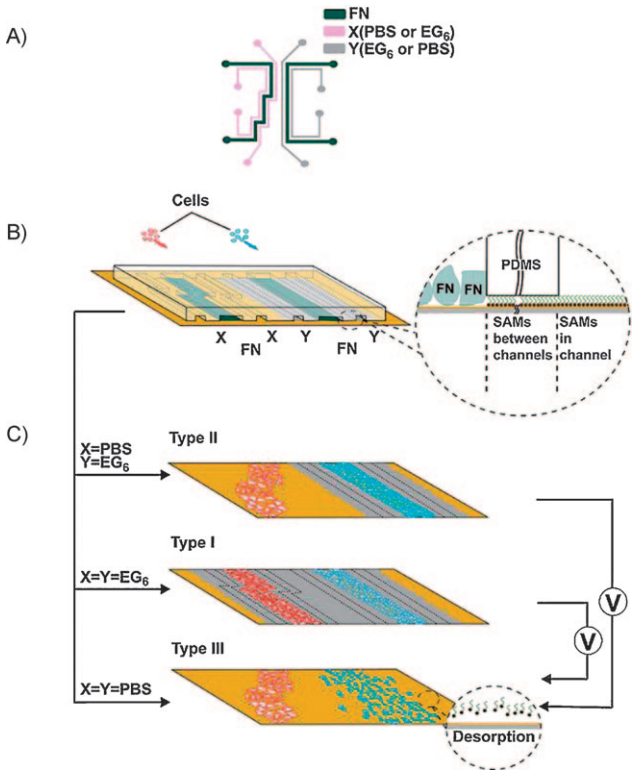


***Figure 1.*** The strategy for patterning multiple types of cells on the same substrate that allows three types of naturally occurring cell–cell interactions. A) Representation of the microchannels filled with different reagents for patterning the surface to produce the substrate for type II cell–cell interactions. For type I cell–cell interactions, only channels that introduce FN and $EG_6$ are necessary (no need for PBS). For type III cell–cell interactions, only channels that introduce FN are necessary (no need for $EG_6$). B) A PDMS stamp with an embedded microfluidic system came into contact with the gold slide to form microchannels that introduced different solutions. Adsorption of FN inside the microchannels (green areas) and formation of SAMs of $EG_6$ flanking microchannels (gray areas) were followed by the adhesion of different types of cells on the floors of the channels coated with FN. Solutions of $EG_6$ could migrate under the PDMS stamp to form "inert" SAMs (gray areas) to constrain one type of cells on the FN-coated parts of the surface (green areas). After incubation, we used PBS to replace the solution of $EG_6$ and introduced different types of cells into different channels. C) After the PDMS stamp was removed, a pattern of different types of cells formed. For type I, all types of cells were well confined. For Type II, one cell type can move freely while the other was confined. For type III, all cells can move freely. With electrochemical desorption of $EG_6$ from substrate in type I and type II cell–cell interactions, cells initially confined by $EG_6$ were released and migrated freely to result in type III cell–cell interactions.

the Supporting Information.) We designed a microfluidic system where two channels introduce phosphate-buffered saline (PBS, pH 7.4) solutions or were left empty to leave sufficient space for the unconfined type of cells to freely migrate (Figure 1A).

Our method for fabrication is as follows: We first passed solutions of FN into the microchannels designated to be permissive for cell adhesion. After incubation for 50 minutes, we introduced a solution of $EG_6$ into the microchannels that flanked the channels that contained FN, to generate areas inert to cell adhesion. Incubation of $EG_6$ allowed the formation of sufficiently well-ordered SAMs. These inert areas would keep cells confined (3T6 cells in Figure 2). We either passed PBS solutions into the channels flanking the channels that introduce NIH 3T3 cells or left them empty (to

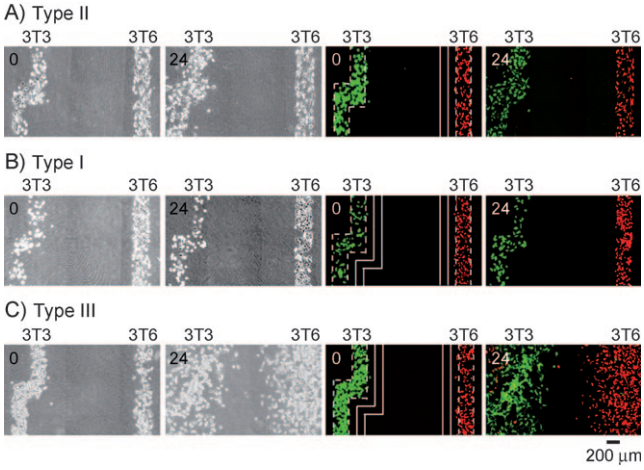


***Figure 2.*** Time-lapse phase-contrast and fluorescence micrographs for the three types of cell–cell interactions between 3T6 and NIH 3T3 cells. The numbers at the upper left of the images indicate the time (in hours) after the PDMS stamp had been peeled off. The areas between two neighboring dashed lines are the channels that deliver FN to the surface; areas between two neighboring solid lines are channels that deliver $EG_6$ to the surface. A) Type II cell–cell interactions: NIH 3T3 cells moved freely on the surface while 3T6 cells were confined in the stripe. B) Type I cell–cell interactions: all cells were well confined. C) Type III cell–cell interactions: both types of cells moved freely on the surface.

allow these cells to move freely once the PDMS stamp is removed; see Figure 2A). We used cell culture medium to replace the solution of FN before introducing NIH 3T3 and 3T6 cells into their respective microchannels (Figure 1B). After allowing cells to attach for approximately one hour, we peeled off the PDMS stamp and immersed the substrate with the attached cells into cell culture medium (in a regular petri dish) (Figure 1C). After the removal of the PDMS stamp, we noted that the cells had formed a sharp boundary at edge of the channel containing FN, indicating that $EG_6$ had diffused under the PDMS stamp to form inert SAMs. We discuss this phenomenon in detail in the Supporting Information. This protocol effectively confined 3T6 cells to their original areas while allowing NIH 3T3 cells to move freely. 3T6 cells were well confined after co-culture for at least 24 hours (Figure 2A). The type II cell–cell interaction or "partial release" was achieved.

We also achieved "no release" and "complete release" with slight modifications of the protocols. We first introduced the solution of FN into select microchannels and allowed it to fill the microchannels before we passed the solution of $EG_6$ into all the flanking microchannels. After incubation of FN and $EG_6$ in microchannels for one hour, we washed the microchannels and introduced 3T6 and NIH 3T3 cells into the microchannels coated with FN. After the attachment of cells,

  

we peeled the PDMS stamp off and immersed the substrate into normal culture medium. Both types of cells were well confined (Figure 2B). Neither cell type grew out of their designated areas or migrated outside their original stripes of confinement. This protocol realized "no release" for the patterned cells.

There are two ways to achieve complete release (type III) of both types of cells. The first method is simply to deliver cells directly to different channels; removal of the stamp will allow all cells to migrate freely on surfaces. The second approach is to start with type I or type II cell–cell interaction. After peeling the PDMS stamp off, we can apply a cathodic voltage pulse (ca. 1.2 V for 30 s) to the gold surface (Figure 1C). Upon the application of the pulse, $EG_6$ desorbs from the substrate, activating the inert areas for cell adhesion.[5,11] Both types of cells initially confined by $EG_6$ are released to migrate freely on the surface and eventually contact each other (Figure 2C). This method, therefore, achieves both type I and type III cell–cell interactions, and the temporal control of converting "no release" or "partial release" to "all release" of both types of cells.

In conclusion, we have established a versatile technique for patterning multiple types of cells on the same substrate to simulate three types of cell–cell interactions. "Partial release" of one type of cells can be achieved. "No release" and "complete release" can both be readily extended to the application of more than two types of cells. Electrochemistry allows the temporal conversion between "no release" and "complete release", but the ability to sequentially release several types of cells (i.e., releasing one type of cell at one time) while keeping other types of cells confined, at this point, remains a challenge. The simplification in implementation, and the improved capabilities in patterning described in this report, is likely to make co-culturing of different types of cells dramatically more accessible to biologists.[5–7,12] We also believe that the sharp boundary as a result of the migration of thiols under the PDMS stamp when we selectively modify the surface with microchannels at the micrometer resolution may be useful in a broad array of applications, such as surface engineering, biological detection, and microfabrication.

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