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ParaStamp and Its Applications to Cell Patterning, Drug Synergy Screening, and Rewritable Devices for Droplet Storage

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Abstract

Engineered materials have been employed as versatile tools to explore the fundamental cell biology/drug development as well as to approach the intelligent device, thereby becoming the key components in modern technology. Herein, a ParaStamp technique has been revealed to possess applications for cell patterning, drug screening, and rewritable functional patterning. The ParaStamp includes a micropatterned PDMS master and a liquid-phased paraffin oil generated at high temperature, which can transfer the patterned paramembrane onto varied material surfaces, such as glass, polystyrene, and flexible foil. This technique is simple and cost-effective to meet the high-throughput requirement for industries. Taken together, our findings herein should have general insights in cell biology, biodetection, and development of smart hydrophobic surface.

Graphical abstract

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

A novel ParaStamp technique developed enables not only for deterministic cell patterning but also for synergy drug screening and rewritable functional device. This technique provides a simple and cost-effective solution to meet the high-throughput requirement for industries, igniting a new direction for coating such the transparent and hydrophobic material onto varied material surfaces.

Keywords

microarray; cell patterning; drug screening; droplet storage

In the past decade, microengineered materials have opened new doors to a wide range of biomedical and smart device issues.^[1–3] For example, high-throughput and high-content approaches by microsystems advance the systematic insights into the cellular functions stimulated by their surrounding microenvironments, including extracellular matrices, chemical factors, and physical stresses.^[4, 5] In addition, smart drophobic devices have emerged as invaluable tools due to their wide applications, such as liquid droplet transportation/storage^[3, 6] and water/oil separation.^[7] These materials have been employed as versatile tools to explore the fundamental cell biology/drug development as well as to approach the intelligent device, thereby becoming the key components in modern technology.

To simulate and recapitulate the *in vivo* cellular condition, biomaterial-based surfaces – capable of generating cellular patterning on demand and of controlling microenvironmental stimuli in microsystems – are crucial. The ability to direct cells onto a targeted area of a surface has been demonstrated by varied techniques, including microcontact printing,^[5] microstructure-based approach,^[8] active control of cell attachment,^[9, 10] and surface wettability.^[11] Nevertheless, challenges still remain. For example, the use of external systems, e.g. electric power or syringe pump, would raise the complexity and is less attractive than more simplistic approaches.^[9, 12] Given that the control of surface wettability can advance the one-step cell patterning as well as the high-throughput drug screening simultaneously, yet the evaluated half maximal effective concentration of drug is not

comparable with the control derived from a commercial 96-well plate.^[11, 13] In addition, the wettability-guided assembly of cells using the effect of reversible PDMS bonding^[14] might restrict the potential to be applicable for varied surfaces, e.g. polystyrene or flexible substrate that is commonly utilized in current technology.

Herein, we present a simple and cost-effective approach that can directly dominate the surface wettability of varied materials by a widely accessible polydimethylsiloxane (PDMS)-paraffin stamping process (Figure 1a and “Preparation of the ParaStamp” in the Supporting Information). PDMS is well known for its lipophilic nature and has a hydrophobic property with a water contact angle (WCA) of approximately 99°, thereby being able to immobilize hydrophobic organic materials, such as paraffin wax. Paraffin wax, which contains a mixture of hydrocarbon component and has a melting point about 46 to 68 °C, is widely conducted to prevent the absorption of small hydrophobic molecules into PDMS microchannels.^[15] Upon the advantages of using PDMS combined with paraffin wax, we first fabricated a PDMS master with designed micropatterns using conventional soft lithography technique. We then sandwiched the whole set, including the PDMS master, a commercial Parafilm, and a foil as the carrier, and placed it on a hotplate at 120 °C for 30 seconds. Meanwhile, the solid paraffin was turned into a liquid-phased paraffin oil and stably mobilized on the PDMS surface due to PDMS’s lipophilic property, in which we called it as the ParaStamp. The stamped and transferred patterns, with a layer of paramembrane, on a desired substrate were achieved following the 30-second stamping at room temperature and peeling off the ParaStamp. Note that the paraffin would be less mobile on PDMS while the temperature is reduced to room temperature.

The paramembrane stamped on a glass substrate is hydrophobic with a WCA of 112.3°, whereas the bare glass utilized in this work has a WCA of 44.7° (Figure 1b). The optical transmittances measured from the bare and stamped glasses are identical while compared with the commercial Parafilm used (Figure 1c). Due to the property of mobile paraffin on PDMS at high temperature, we further demonstrated the self-healing ability of the ParaStamp. Results show that the artificial crack on the ParaStamp could be recovered and healed while heated at 120 °C for 1 minute, whereas it cannot recover the cracked paramembrane while coated on the glass substrate (Figure 1d and Figure S1, Supporting Information). Intrinsically, this suggests that the ParaStamp may behave in a good damage tolerance and could be applicable for a wide range of applications.

Moreover, we successfully employed the ParaStamp to transfer micropatterns onto different substrates, including cover glass, polystyrene, PDMS, and flexible foil sheet (Figure 1e and Figure S2, Supporting Information). At the paramembrane-substrate interface, it contributes a pinned boundary condition for improving the sessile droplets in relatively hydrophilic regions, even on intrinsically hydrophobic substrates, i.e. polystyrene and PDMS. Our technique can be simply utilized to fabricate varied micropatterns. For example, the cross-sectional and circular patterns were performed on a glass substrate, in which the patterns were detected from the fluorescent dyes, which were pinned in hydrophilic regions, after withdrawing the dye initially fulfilled on the glass (Figure 1f). This wettability patterns result in pinning of aqueous solutions to hydrophilic regions and repulsion of the solutions from hydrophobic regions. In addition, the paramembrane can prevent the absorption of

small hydrophobic molecules than PDMS used and no auto-fluorescence is detected (Figure S3, Supporting Information), indicating the advantage of using the ParaStamp technique for cell studies.

Particular features of the ParaStamp enabled deterministic patterning of cells are shown in Figure 2a (see also “Configurable cell patterning” in the Supporting Information). The cell patterning device was fabricated as Figure 1a and the substrate conducted was a cover glass. Briefly, cell suspension was loaded on the device and incubated for 30 minutes, allowing cell sedimentation and adherence onto the substrate. Afterwards, followed by washing away unadherent cells, cell patterning was then detected after a 1-day culture. The transferred paramembrane on glass has a uniform thickness of 4.5 μm measured from the cross-sectional observation of the device (Figure 2b and Figure S4, Supporting Information). Figure 2c to 2e show this device enables the configurable cellular patterns, including single cell, circular shape, “9” and “0” characters, and “+” and “n” symbols, through the design of location of micropatterns and the suitable surface patterning *via* the ParaStamp technique. Furthermore, we demonstrate that the interfacial geometry of patterned cells could modulate the cytoskeleton reorganization, as revealed from the comparison of F-actin staining between cells patterned on edged and non-edged regions (Figure 2f). Cells cultured along with the edge of patterned boundary possess an enriched expression of F-actin in whole cells, whereas F-actin is only highly expressed at the cell-cell interactions while cultured on non-edged regions. Our finding resembles other research groups’ demonstrations, showing the interfacial geometry and biophysical parameters would cause the cytoskeleton reorganization and therefore affect tumor progression, in terms of epithelial to mesenchymal transition (EMT), cancer stem cell (CSC) characteristics, and tumorigenicity.^[16, 17]

It has been revealed with a potential to improve the therapeutically relevant selectivity by synergistic drug combinations.^[18, 19] Our developed drug screening microwell array device, fabricated by the ParaStamp technique (Figure 1a), also advances the synergistic drug screening from the proof-of-conceptual experiment (Figure 3a, see also “Drug synergy screening” in the Supporting Information). We treated MCF7 cells with two commonly-used chemotherapeutic drugs – cisplatin and paclitaxel – of varied concentrations during a 2-day incubation. Note that the aqueous drugs were pre-dispensed onto the microwell array device and then resulted in dry powders accordingly prior to the loading of cells. The wettability wells enabled to stabilize the dispensed cell drops due to the hydrophobic/hydrophilic interface with a thickness of 4.5 μm (Figure 2b). The cell viability was detected and evaluated using a CellTiter-Blue assay,^[20] and the representative images were shown in Figure 3b. Notably, no significant difference was observed in dose responses conducted from our array device and the conventional 96-well plate, in which the IC_{50} of paclitaxel on MCF7 cells derived from device is 26.4 $\mu\text{g/ml}$ and 26.0 $\mu\text{g/ml}$ from 96-well plate (Figure S5, Supporting Information). Both the two surface densities of cells are similar, i.e. 222 cells/ mm^2 in device and 188 cells/ mm^2 in plate, although the volume densities are distinct (400 cells/ μl in device and 60 cells/ μl in plate). We furthermore demonstrated the optimized drug combination searched from the array device is 25 $\mu\text{g/ml}$ cisplatin combined with 25 $\mu\text{g/ml}$ paclitaxel (Figure 3c and Figure S6, Supporting Information). It may efficiently reduce the dose of single drug used from cisplatin and paclitaxel down to 15 and 6 times, respectively, under the 81% cell inhibition (Figure 3d).

The ParaStamp technique developed in this work could be potentially applied for many applications, such as microdroplet-associated functional devices for droplet transportation, transfer, mixing, and collection. Although such the functional devices have been fabricated by creating chemical or physical patterns on engineered surfaces,^[6, 21, 22] almost the devices lack adaptability, contain only a single function, and are complicated to be reconfigurable. Based on these, we therefore present an alternative approach by our ParaStamp technique to the rewritable functional device for droplet storage. Figure 4a shows how we perform the rewritable device upon cycled patterning and removal of paramembranes (see also “Preparation of the rewritable device for droplet storage” in the Supporting Information). Briefly, to remove the stamped hydrophobic paramembrane, a PDMS eraser combined with ethanol was utilized to advance the removal. The *polar* ethanol would suggest to improve the detachment of liquid-phased paraffin from the glass substrate at high temperature; meanwhile, the detached paraffin oil could be absorbed onto the PDMS due to PDMS’s lipophilic nature. We further demonstrate the capability of repeated patterning/removal processes from the cycled measurements of water contact angles, indicating that such the approach is reproducible and capable of being a rewritable device (Figure 4b and Figure S7, Supporting Information). It is noteworthy that different patterns composed of wettability regions can be designed on the identical glass substrate. Figure 4c and 4d show the photos of droplets pinned on the wettability surfaces with patterns like letters “N”, “T”, “U”, and “U”, “T”, “S”, “W”, indicating that different programs can be realized on the same substrate. Moreover, Figure 4e shows the ability of one-step pinning of water droplets in a pattern like a reverse “Z” letter, even after 10 cycled operations (see also Movie S1 in the Supporting Information). The minimum volume of pinned droplet on the device is around 220 nl (Figure S8, Supporting Information). Although the demonstration herein is the functional device for droplet storage, it is believed that such the surface patterning by the ParaStamp can be easily extended to other functional surfaces, such as microstructure-dependent superhydrophobic chips.^[6]

Limitation to the present approach is that all the microdroplet operations (i.e. drug screening) are performed manually, which typically require frequent practices and are time-consuming. To further confirm the reproducibility and reliability of this approach, an automatic robot system can be employed and integrated with our current method, which may meet the merit of high-throughput and high-content drug screening.

In summary, we have presented a novel ParaStamp technique that can enable deterministic cell patterning with precise control over individual shapes, and it can be applicable for synergy drug screening and microdroplet-based rewritable functional device as well. The ParaStamp includes a PDMS master and a liquid-phased paraffin oil at high temperature, which can transfer the patterned paramembrane onto varied material surfaces. This technique not only provides a simple and cost-effective solution to meet the high-throughput requirement for industries, but also it could ignite a new direction for coating such the transparent and hydrophobic material onto varied surfaces, such as flexible electric substrate for water repelling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Characteristics of ParaStamp. a) Schematic of hydrophobic paramembrane patterning *via* ParaStamp. b) Measurements of water-contact angle (WCA) for bare and parafilm-stamped glasses, respectively. c) Measurements of transmittance for bare glass, stamped glass, and commercial parafilm. d) shows the acquired self-healing ability of paramembrane furrowed on PDMS surface at 120 °C for 1 min rather than on glass. Scale bar, 500 µm. e) 5*5 or 5*3 array, with a diameter of 1.5 mm, is patterned on varied substrates. f) Shape-varied micropatterns *via* the ParaStamp. The detected fluorescence is from CellTiter-Blue dye. "Para" represents the paramembrane that is coated on the glass. Scale bar, 100 µm.

Figure 2.

Cell patterning *via* ParaStamp. a) Schematic showing the cell-patterning procedure. b) Cross-sectional view of the stamped paramembrane onto a cover glass. Scale bar, 50 μm . c–e) Characteristics of cell patterning by the ParaStamp, including patterning of single and multiple MCF7 cells in two circular shapes, numbers of “9” and “0”, and symbols of “+” and “n”. Cells were stained with calcein AM. Scale bars, 50 μm in c (left panel by 4 $\times$ objective lens and right panel by 10 $\times$ objective lens), 100 μm in d (by 10 $\times$ objective lens), 200 μm in e (by 10 $\times$ objective lens). f) Comparison of two F-actin expressions in MCF7 cells cultured on glasses, with and without paramembrane patterning. F-actin and nuclei

were stained with phalloidin (red) and Hoechst (blue), respectively. a.u., arbitrary unit. Data represents the mean $\pm$ SD from n = 40 (with pattern) and n = 82 (without pattern), respectively (**p < 0.01, ***p < 0.001). Scale bar, 10 μ m (by 63 $\times$ objective lens).

Figure 3.

Drug synergy screening *via* ParaStamp. a) Schematic showing a 2-drug combinational screening by the microwell array device made from ParaStamp. b) Fluorescent detection of live cells by CellTiter-Blue assay, under varied concentrations of cisplatin and paclitaxel in the cell array device. Scale bar, 1 mm (by 4 $\times$ objective lens). c) Toxicity profile of 48-h combinational cisplatin and paclitaxel treatment. Each data is from 3 independent experiments. d) Comparison of relative doses used for 81% cellular inhibition by single-used cisplatin or paclitaxel and optimized drug combination from c, which is highlighted by a red arrow (i.e. 25 $\mu\text{g/ml}$ cisplatin and 25 $\mu\text{g/ml}$ paclitaxel).

Figure 4.

Rewritable device for droplet storage *via* ParaStamp. a) Schematic showing the procedure of reconfigurable paramembrane patterning. b) Reliable test of the rewritable device from the measurements of contact angles (mean $\pm$ SD, $n = 2 \sim 3$ for each contact angle). Red and purple lines represent the average contact angles from all the coatings and removals of paramembranes, respectively. The time for each coating and removal are 30 seconds and 1 minute, respectively. *** $p < 0.001$. c) and d) Demonstrations of rewritable patterning on the identical glass substrate during the seven-time patterning and removal of paramembranes (“NTU” in c and “UTSW” in d, respectively). The diameter of pattern is 1.5 mm. The droplets utilized are CellTiter Blue dyes. e) One-step liquid loading and simultaneous droplet pinned in the patterned device after a 10-cycled operations. The liquid utilized is DI water, and the paramembrane pattern is a reverse “Z” symbol. See also the Movie S1, Supporting Information.