PHOTO-ASSISTED MICRO-GLUING FOR ASSEMBLING THREE-DIMENSIONAL MICROSTRUCTURES WITH LIVING CELLS

Shotaro Yoshida[1*], Koji Sato[1,2], Kaori Kuribayashi-Shigetomi[1], Tetsuhiko Teshima[1], and Shoji Takeuchi[1,2]

1. Institute of Industrial Science, The University of Tokyo, Japan
2. Takeuchi Biohybrid Innovation Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology (JST), Japan

ABSTRACT

This paper describes a method to assemble three-dimensional (3D) microstructures by gluing separated two-dimensional (2D) structures attaching with living cells at micron-scales using hydrogels. We utilized photo-curable, biocompatible poly(ethylene)-glycol-diacrylate (PEGDA) as a glue, and assembled 2D cell-laden microplates into 3D structures by photo-assisted local polymerizations. We demonstrated an assembly of 3D half-cubic microstructures with genetically-labeled cells, which enabled observing intracellular microstructure in 3D cellular constructs. We believe our method is useful for assembling 3D structures with targeted cells and into arbitrary geometries.

KEYWORDS: 3D Microstructure, Assembly, Hydrogel, Gene Expression, Cell Handling

INTRODUCTION

Construction of 3D microstructures has been a challenging issue in the microfabrication field [1], especially for bioengineering applications [2]. One of the promising approaches is self-assembly, such as self-folding [1, 3], or directed assembly [2]. However, these automatic assemblies are insufficient for building specific cellular structures because living cells have various genetical or phenotypical characters that demand manual cell targeting. In this respect, a possible approach for a precise 3D cellular assembly is that constructing them manually from 2D assembly parts that targeted cells are located.

In this study, we propose a method to assemble targeted 2D cell-laden microplates into a 3D microstructure by micro-gluing (**Figure 1**). The required conditions for the micro-gluing are follows: (i) the glue itself must be biocompatible, (ii) the gluing must be capable of being conducted under aqueous solution, such as culture medium, (iii) glue joints must be controlled at a micron-resolution. One possible material that meets the requirement (i) is a hydrogel, widely used material in tissue engineering [2] or in surgical applications [4]. There are various types of hydrogels that can be hardened by heat, ions, or pH, however these do not meet the requirement (ii)/(iii) because of their low gelation speed or need for mixing two-component solutions. We utilized a photo-curable hydrogel, PEGDA, which could be polymerized rapidly by local UV exposure. PEGDA can be polymerized into a micro-sized gel by UV without damaging cells [2]. Here, we demonstrated an assembly of 3D half-cubic structure with living cell by bonding 2D microplates with genetically-labeled cells by PEGDA micro-gluing.

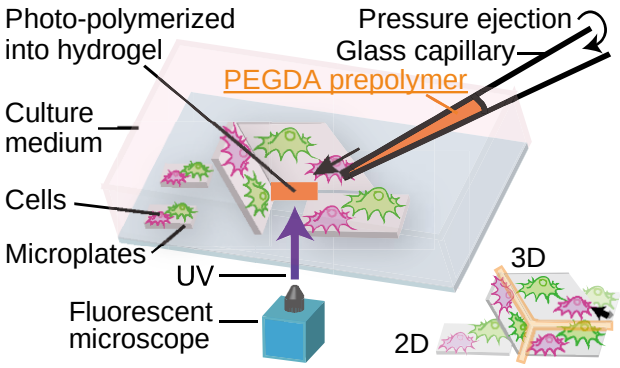

Figure 1. *Conceptual illustration of photo-assisted micro-gluing. Biocompatible, and photocurable PEGDA hydrogel was used for gluing 2D cell-laden microplates underwater. The position of each 2D microplate is arranged by micromanipulator driven glass capillaries. PEGDA prepolymer is applied on the edge of microplate, and UV exposed for gluing by polymerized PEGDA hydrogels. The connected microplates were further assembled into 3D microstructure by glass capillaries and the micro-gluing.*

FABRICATION OF TRANSPARENT 2D MICROPLATES AND CELL CULTURE

Biocompatible, transparent 2D microplates were fabricated as we reported previously (**Figure 2**) [5]. Briefly, on a glass substrate, we spin-coated thin-layers of gelatin, poly-monochloro-paraxylylene (parylene), aluminum, and patterned them into micro-sized plates by standard photolithography. Then, we coated 2-methacryloyloxyethyl-phosphorylcholine (MPC) polymers, which inhibit the cell adhesion and protein absorption on the surface of the glass substrate (**Figure 2a**). After the chemical etching of Al to remove MPC polymers on the parylene, collagen was coated on them (**Figure 2b**). Throughout the procedure, we achieved selective adhesion of cells only onto microplates with simply seeding them on the fabricated devices (**Figure 2c**).

We cultured PC12 cells on the microplates in serum-free DMEM containing 10 ng/ml Nerve Growth Factor (NGF) for 2 days. HEK293T cells were also cultured in normal culture dish with DMEM containing 10% FBS. pAcGFP-actin vector encoding N-terminal GFP-fused actin or pcDNA3.1-RFP vector was transiently transfected into the cells with FuGENE HD regent (**Figure 3**). Transgene labeled HEK293T cells were trypsinized, and seeded on microplates.

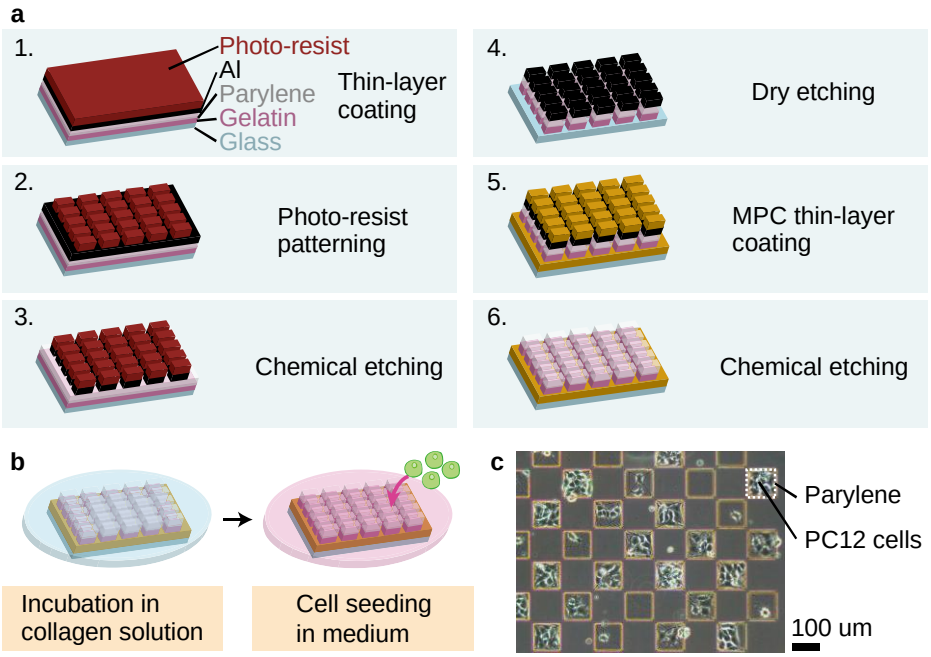

Figure 2. *Fabriction process of transparent 2D microplates with living cells. (a) Parylene microplates were fabricated by standard photolithography techniques.(b) Cell seeding and culturing on the microplates. (c) PC12 neural cells on microplates.*

EXPERIMENTAL SETUP FOR THE MICRO-GLUING

Microplates were observed by an inverted microscope, and manually moved for gluing by using the tip of a glass capillary combined with a hydraulic micromanipulator. PEGDA prepolymer solutions were loaded into another glass capillary and a pressure ejection system (**Figure 4**). PEGDA prepolymer solutions were projected to the edge of the microplate, and the duration of the projection was determined by a PIC-controlled solenoid valve. UV light was emitted through the objective lens, and its duration was regulated manually.

RESULTS AND DISCUSSION

We assessed biocompatibility and cell adhesion property of PEGDA hydrogels by culturing HEK293T cells on them (**Figure 5**). Hydrogel precursor solutions were made of PEGDA and photo-initiator with volume ratio 95:5, and then mixed with aqueous solutions: water or type I-C collagen solution. The cells were cultured 2 days and stained with calcein-AM (live) / EthD-1 (dead) for viability assays. As a result, HEK293T cells adhered on PEGDA containing collagen (**Figure 5a, b**), and showed high viability rate (~98%) (**Figure 5c**), which ensured the biocompatibility of the PEGDA glue.

Using the micro-gluing system, PEGDA prepolymer solutions was focally projected on the edge of microplate. The polymerized into hydrogels by UV even under water, and the gelation resulted in gluing two targeted microplates.

Finally we glued microplates together in the system, and successfully assembled 3D microstructure from 2D microplates (**Figure 6**). We gathered target three 2D microplates with GFP-Actin/RFP expressing HEK293T cells, glued one by one, and succeeded in constructing a 3D half-cube structure (**Figure 6a, b**). Since the 3D object was constructed by gluing tareted parts, it may be possible to assemble it with heterogeneous cells, or microplates with different shape and materials.

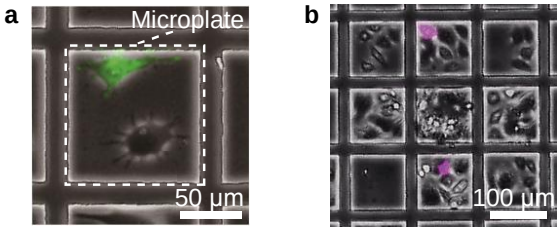

Figure 3. *Transgene expression in PC12 neural cells cultured on the microplate. (a) Green : N-terminus GFP-fused actin. (b) Magenta: RFP.*

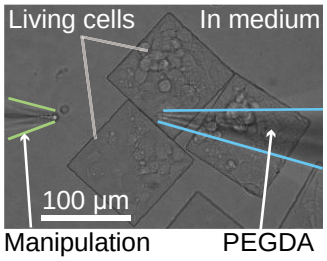

Figure 4. *Experimental setup for micro-gluing. Microplates were manipulated by a left glass pipette (green), and PEGDA was ejected by a right pipette (blue). UV light was exposed through an objective lens.*

Moreover, transparency of the parylene microplates enabled visualizing intracellular microstructures such as actin cytoskeletons with observation of GFP-fused actin proteins (**Figure 6c**). By integrating our micro-gluing technique, genetic engineering, and live cell imaging, it could be possible to study the basic mechanisms of cells and their internal molecules in 3D cellular environment, in chronological order. Also, the procedure could be fastened by using focal laser photo-activation in future.

CONCLUSION

We developed a photo-assisted micro-gluing technique applicable to 3D assembly of bio-samples. We successfully assembled 2D cell-laden microplates into a 3D half-cubic structure. We believe this method is useful in constructing 3D cellular structures only with targeted 2D parts.

ACKNOWLEDGEMENT

This work was partly supported by Grant-in-Aid for Scientific Research on Innovative Areas "Bio Assembler"(23106005) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

[1] T. G. Leong, et al., Three-Dimensional Fabrication at Small Size Scales, ***Small***, **6**, 792, (2010)

[2] P. Zorlutuna, et al., Microfabricated Biomaterials for Engineering 3D Tissues, ***Adv. Mater***, **24**(14) 1782, (2012)

[3] K. Kuribayashi-Shigetomi, et al., Assembly of 3D Microstructures Powered by Cells, ***Proc. in MicroTAS2009***, 1321, (2009)

[4] A. Lauto, et al., Adhesive biomaterials for tissue reconstruction, ***J. Chem. Technol. Biotechnol***, **83**, 464, (2008)

[5] S. Yoshida, et al., Single neural cells on mobile microplates for precise neural network assembly, ***Proc. in MicroTAS2011***, 1749, (2011)

CONTACT

*Shotaro Yoshida, Institute of Industrial Science, The University of Tokyo, 4-6-1, Komaba Meguro-ku, Tokyo, JAPAN,
tel: +81-3-5452-6650; Fax: +81-3-5452-6649, Email: syoshida@iis.u-tokyo.ac.jp

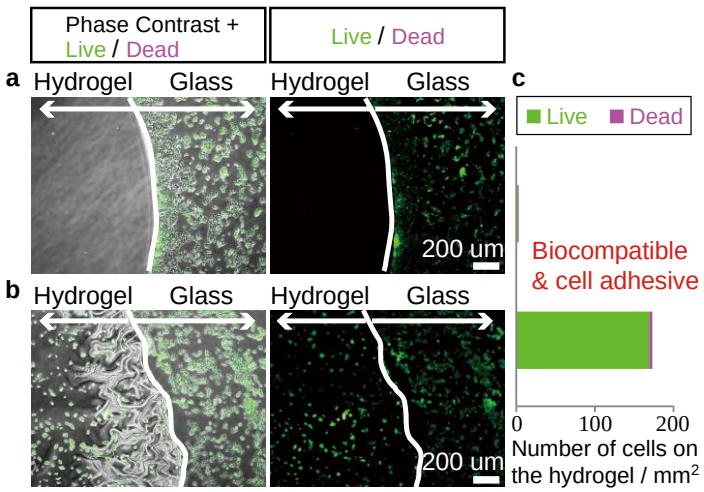

Figure 5. *Biocompatibility and cell adhesive property of PEGDA hydrogel. (a, b) fluorescence image of HEK293T cells on the 9.5% PEGDA (a), or 9.5% PEGDA including 1.8% typeI-C collagen (b). Green: calcein, Magenta:EthD-1. (c) the summary of cell adhesion and viability on the PEGDA hydrogel.*

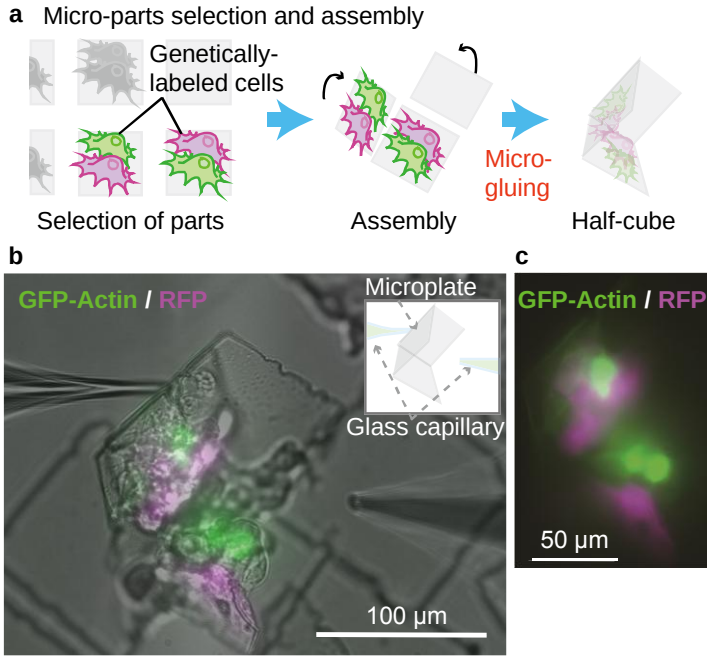

Figure 6. *3D assembly by micro-gluing. (a) microplates with transgene expressing cells were selected, assembled, and glued into a 3D half-cube object. (b) a picture of a micro-glued 3D half-cube structure attached with HEK293T cells expressing GFP-actin or RFP. (c) intracellular cytoskeletons (green) and cytosol (magenta) were visible through the half-cube.*