Hierarchical shape-by-shape assembly of microparticles for micrometer-scale viral delivery of two different genes

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(Received 23 March 2018; accepted 24 April 2018; published online 4 May 2018)

Understanding tissue engineering using a bottom-up approach has been hindered by technical limitations because no platform can demonstrate the controlled formation of a heterogeneous population of cells in microscale. Here, we demonstrate hierarchical shape-by-shape assembly of virus-laden particles into larger ones to transfect two different genes on the seeded cells. We show that smaller daughter particles with different sizes and shapes can be assembled into the matching indentations of larger parent particles with different sizes and shapes. Then, we transfected a population of cells with two different gene-transfecting viruses, each of which was laden on the parent or daughter particles. *Published by AIP Publishing.*
<https://doi.org/10.1063/1.5030597>

I. INTRODUCTION

Mimicking physiological conditions *in vitro*, which is essential for studying cellular communication and disease mechanisms, requires a heterogeneously populated environment in microscale. Populating different types of cells *in vitro* in a controlled manner is crucial to understand or model tumorigenesis, morphogenesis, stem cell differentiation, and wound healing.^{1,2} In particular, embryonic, adult, and induced pluripotent stem cells differentiate depending on the composition of materials and neighboring cell niche, which is heavily formed by heterogeneous population.^{3,4} Therefore, in order to mirror *in vivo* conditions effectively for different *in vitro* modeling, a platform that can provide and precisely control multiplex and adaptable environments in microscale is needed to study cellular pathways.

Powerful approaches, such as mechanical cell contacting methods,⁵ patterning different materials for different types of cells,⁶ and selective transfection,⁷ have been developed to address this issue. The systems that use macroscale systems, however, are not only limited in their flexibility in design but also restricted to millimeter scale, which are far from genuine *in vivo* systems. Microfluidic systems have emerged to pattern different types of cells to mimic

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cellular communities in microscale, but these require bulky instruments to run experiments^{6,8} Also, no microfluidic platform has demonstrated controlled patterning of heterogeneous population of cells in direct contact and multiplex.

Microscale systems using microparticles can address the above-mentioned issues, providing much higher complexity and surface area per volume.^{9–13} However, particulate materials face challenges in tissue engineering because the intricate control of individual particles and imaging are difficult.¹⁴ While retaining the advantages of microparticles, multicompartmental particles that can incorporate multiple materials or cells can complement controllability by situating the cells in a specific compartment.^{15,16} Therefore, multicompartmental particles, such as alginate-based particles, electrohydrodynamic co-jetting-based core-shell particles, cylinder-shaped microparticles, and hydrogel particle assembly, have been developed.^{17–20} Although these platforms have demonstrated multiple compartments of cells and other materials, none of them had controllability over direct or indirect contact of heterogeneous cell populations in microscale. Moreover, since most multicompartmental particles are dissociated in solution phase, imaging for tissue engineering assay remains difficult, limiting plexity in assays.

Here, we describe the shape-by-shape assembly of multiple virus-laden compartments inside microscale virus-laden particles to form a heterogeneous population of cells using microscale transfection, which builds on our previous report on viral micropatches.⁷ By assembling daughter microparticles with different shapes and sizes to a parent particle, which has indentations that match the size and shape of the daughter particles [Figs. 1(a)], we were able to demonstrate two different populations of cells in direct contact on a single particle. Because these particles are assembled in 2D plane in a well-controlled manner, multiplex imaging is convenient for any tissue engineering applications.

FIG. 1. The particles in the particle system, by which the daughter particles are assembled into the parent particles according to shape codes, is applied to transfect cells with two different genes. (a) Concept of the particles in particle platform. Larger parent particles are assembled into the PDMS microwell plate according to their shapes. Then, the smaller daughter particles are assembled into the indentations of the parent particles of corresponding shape. Scale bar, 200 μm. (b) Fabrication of the daughter and parent particles using OFML system. UV light is exposed to the dynamic photomask and then reflected to the substrate with photocurable polymers on automated stage. (c) Different viruses were laden inside the different particles whose shape will encode the virus type. Cells are then seeded on top of the assembled particles and incubated. The cells are transfected locally by gene delivery from viral particles. Scale bar, 200 μm.

II. METHOD AND MATERIALS

All particles of the platform were fabricated using the homemade optofluidic maskless lithography (OFML) system²¹ [Fig. 1(b)]. The system comprised a UV source, dynamic photomask device, objective lens, and automated XY stages. The shape of the UV light from the source is modulated by the dynamic photomask device, from which the modulated light was focused through the objective lens. The photopolymer (ETPTA) resin on the exposed area of the automated XY stages was cured according to the shape and size of the modulated UV light. The particles with various shapes and indentations were fabricated to form two libraries of particles: a library with the parent particles and one with the daughter particles. Daughter particles are smaller particles that can be assembled into the indentations of the larger parent particles. For the hierarchical shape-by-shape assembly, the parent particles were assembled first into the PDMS microwells according to their shapes. When the square-shaped particles are dispensed on the PDMS well, approximately 24.9% of particles were self-assembled initially (Fig. S2). Likewise, differently shaped particles were assembled and the residual particles were manually picked and placed on PDMS substrate. Then, the daughter particles were assembled into the indentations of the parent particles to fit in, first by simple dispensing and second by picking and placing. To demonstrate the applicability of this platform, multi-virus-laden compartmental particles were formed to demonstrate gene transfection to the seeded cells [Fig. 1(c)]. The Ad-GFP virus was attached to the library of the daughter particles, while the Ad-RFP virus was attached to the library of parent particles (Fig. S3). The attachment of the virus to particles was done by silica coating and functionalization process.²² The photocured ETPTA particles were coated with silica through modified Stöber process. When (3-aminopropyl)triethoxysilane (APTES) is added onto the silica, the surface is modified to amine groups. The amine surface changes to the carboxyl surface when succinic anhydride is reacted thereto. The EDC/NHS is used as a linker to connect the carboxyl surface and the amine moieties of the virus surface proteins. After the assembly of the virus-laden particles, the seeded cells on the particles will be transfected by local delivery, forming a microscale and heterogeneous environment with two different types of cells.

III. RESULTS

The technological potential of this platform was demonstrated by assembling the daughter particles with different sizes, shapes, and numbers into the parent particles with different sizes and shapes [Figs. 2(a) and S1]. The multicompartmental particles were successfully formed and confirmed by fluorescently staining the library of daughter particles with one color and that of parent particles with another.

To further apply this platform, we demonstrated the two-gene transfection of cells forming a heterogeneous population of cells by microscale gene transfection [Figs. 2(b) and S4]. If a daughter particle is virus-laden with a virus that transfects gene 1, then the parent particle can be virus-laden with another virus that transfects gene 2. Using this concept, we used two adenoviruses: one that transfects green fluorescent protein (GFP) and another that transfects red fluorescent protein (RFP). After the two types of virus-laden particles are assembled to form one multicompartmental particle, local delivery of the genes enabled microscale patterning of two different cell populations in direct contact.

IV. CONCLUSION

In this paper, we demonstrated the transfection of two different genes on cells using hierarchical shape-by-shape assembly of virus-laden daughter particles into virus-laden parent particles. Although we only demonstrated two-gene transfection of the cells, this platform can form multicompartmental particles with more different substances. In a technical sense, because the M:N ratio of daughter particles to parent particles can be controlled, this platform enables high throughput and multiplex examination of cells according to combinations of $M \times N$. In a biological sense, the demonstrated transfections of cells in microscale will enable the investigation of cell-cell, cell-substance, and cell-matrix topological interactions in microscale. Also,

FIG. 2. The shapes of the daughter and parent particles are controlled, and thereby two gene transfections are demonstrated. (a) In the first assembly process, the parent particles with different shapes are assembled into the PDMS microwell. In the second assembly process, the daughter particles with different shapes are assembled into the parent particles according to their shapes. Scale bar, 400 μm . (b) Two genes Ad-RFP and Ad-GFP were successfully transfected in microscale and locally using the two viral-laden microparticles assembled together. Scale bar, 200 μm .

because OFML technology enables simple fabrication of nanocomposites, such as magnetic particles, drug-laden particles, encoded particles, and more, the particle-in-particle platform has potential applications in more complex studies. For future directions, even though we demonstrated simple shapes that can fit into wells and particles, we can form complex shapes that can mimic a natural system through the OFML system. If only the corresponding indentations can be formed in the microwell, the particles with different shapes will fit in like a puzzle. In terms of assembly efficiency, self-assembly methods can be incorporated for faster and more robust assembly of these particles. Finally, because this system is a well-based 2D system, we can apply advanced optic systems such as laser systems or scanning electron microscopy imaging to study tissue engineering according to the substances, shapes, sizes, and heterogeneity of the cells.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for photographs of the hierarchical shape-by-shape assembly of daughter particles with different sizes into the parent particles with different sizes (Fig. S1); assembly yield of the square-shaped parent particle (Fig. S2); schematic for chemical coating process for fabricating virus-laden microparticles (Fig. S3); and additional photographs of two-gene transfected cells using the hierarchical shape-by-shape assembly of the virus-laden particles (Fig. S4). See [supplementary material](#) for complete methods and experimental data.

ACKNOWLEDGMENTS

This research was supported by Global Research Development Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT) (2015K1A4A3047345), by the Nano-Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning (2012M3A7A9671610), by a grant from the Korean Health Technology R&D Project, Ministry of Health and Welfare (HI13C2162), and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning (NRF-2017R1A2B4005933). This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1A6A3A04010692), and by the Brain Korea 21 Plus Project in 2018.

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