SIEVEWELL

Spatially Controlled Culture of Single-Cell-Derived Organoids Using SIEVEWELL Single Cell Arraying Device

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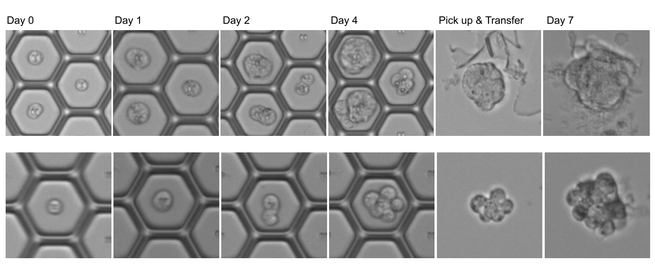
Abstract

Organoids are three-dimensional structures formed from stem cells or primary cells, widely used as physiologically relevant models of human tissue in drug discovery and disease research. Organoids derived from single cells, in particular, often exhibit significant heterogeneity in size and morphology due to differences in differentiation potential and cellular state. This results in challenges related to reproducibility and quantitative assessment of drug responses. SIEVEWELL is a device featuring regularly spaced microwells designed for single cells. By trapping one cell per well, it enables spatially controlled cell positioning and promotes organoid formation from individual cells. This technology contributes to standardizing organoid research and enhances the reliability of drug screening, making it a promising tool for disease modeling and personalized medicine applications.

Introduction

Cells used in *in vitro* experimental systems are often assumed to maintain uniform properties. However, it is clear that cellular diversity exists in normal tissues and organs, just as it is known that differences exist not only between cancer patients but also within tumor tissues from the same patient. SIEVEWELL is a high-density cell-arraying device designed to capture and arrange single cells with high efficiency. Through culturing from single cells, it has revealed that even common cell lines contain a mixture of cells with varying proliferative capacities and spheroid-forming abilities.

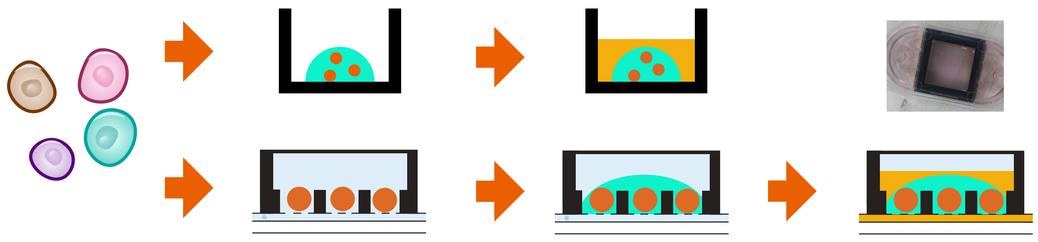


HepG2 spheroids formed from single cells using SIEVEWELL exhibited two distinct morphologies: one with indistinct cell-cell boundaries and another where individual cell shapes remained clearly visible.

The heterogeneity of the starting single cells is expected to significantly impact the quality and reproducibility of the resulting 3D cellular structures. However, with conventional 3D culture methods, it has been difficult to track and analyze the properties of individual cells, even though culturing from single cells is a common practice. While various methods exist for forming 3D cellular structures—particularly in organoid culture—the "gel dome method," which involves embedding dissociated single cells in Matrigel, is most widely used. In this study, we attempted to culture single cells within Matrigel using two types of SIEVEWELL with different well sizes.

Materials and Methods

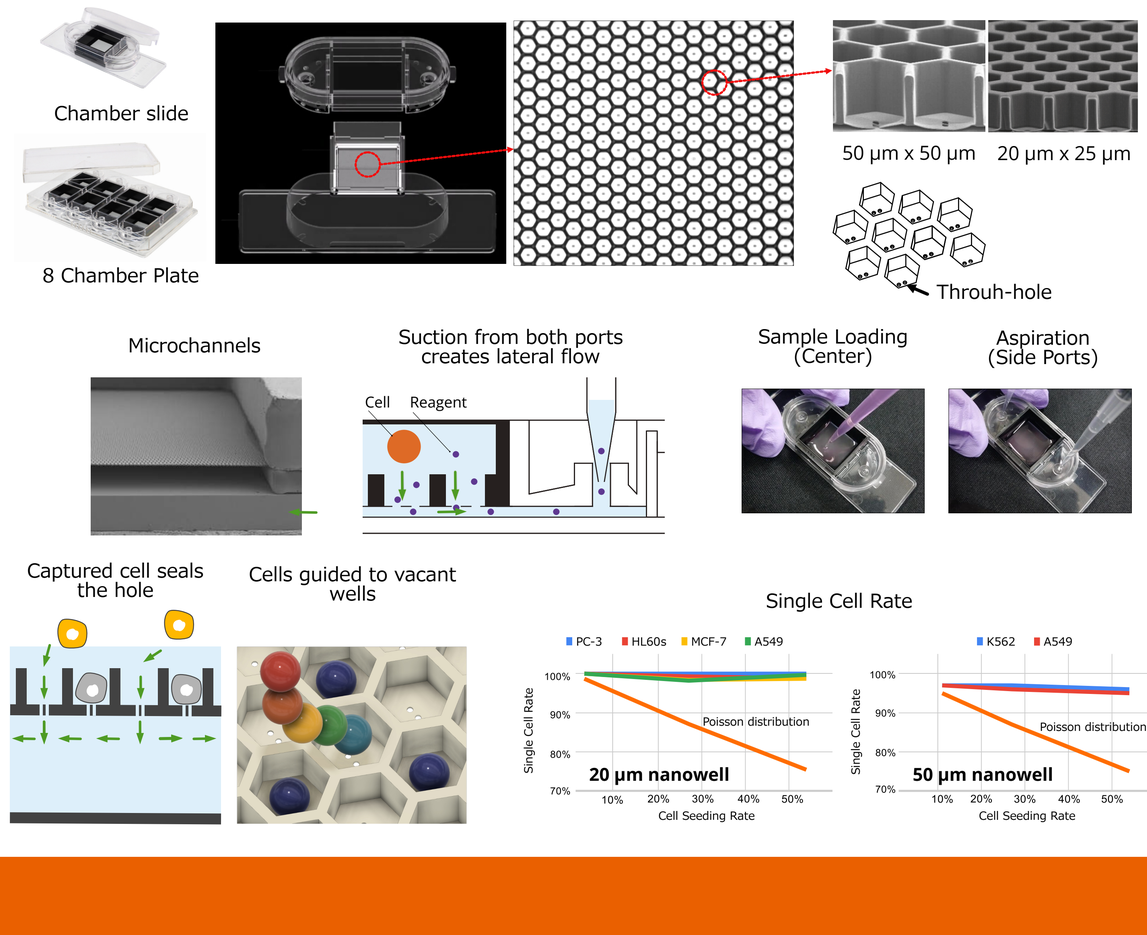
- Conventional Gel Dome Method: 5,000 dissociated single cells were mixed with Matrigel and seeded onto a 24-well plate to form gel domes. After gelation, culture medium was added to initiate the culture.
- Gel Dome Method using SIEVEWELL: 5,000 dissociated single cells were seeded onto the device to ensure they were captured into individual nanowells. Matrigel was then overlaid and allowed to gel, followed by the addition of culture medium for subsequent culture.



SIEVEWELL High Density Cell Arraying Device

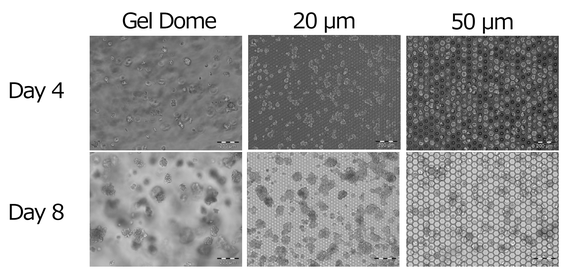
Conventional microcavity arrays used for single-cell capture often rely on sedimentation to trap cells, which results in a cell distribution governed by Poisson distribution. In such systems, the single-cell occupancy rate inevitably decreases as the cell loading density increases.

In contrast, SIEVEWELL features a through-hole at the bottom of each nanowell. During loading, a fluid flow is generated from the chamber toward the side ports, which actively guides cells into the nanowells. Once a cell enters a well, it partially occludes the pore, thereby reducing the flow rate in that specific well and directing subsequent cells toward unoccupied nanowells. This mechanism enables SIEVEWELL to achieve a higher single-cell capture rate than what is theoretically possible under Poisson distribution.



Results

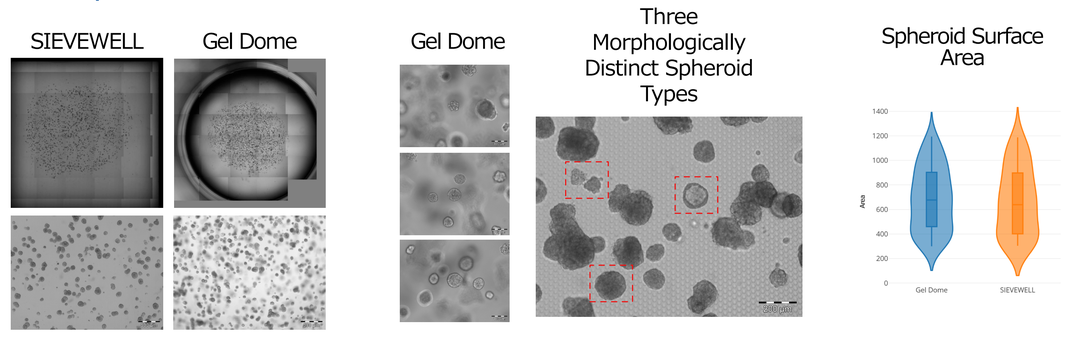
HepG2 Spheroid Formation



When using 50 µm wells, the formed spheroids began to contact the well walls by day 4 of culture. Likely due to contact inhibition, some spheroids showed no further increase in size even at day 8.

In contrast, in 20 µm wells, the spheroids grew to sizes exceeding 200 µm. This is likely because the spheroids were pushed out of the wells during the early stages of formation, thereby avoiding contact inhibition from the well walls.

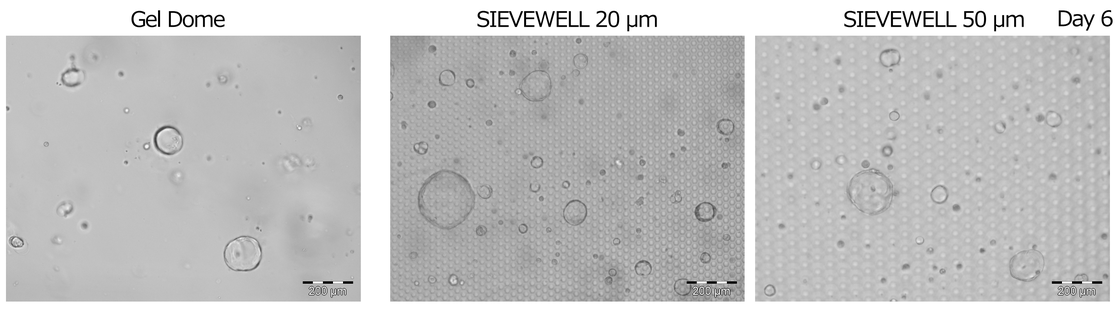
A549 Spheroid Formation



Three distinct types of spheroid morphologies were observed during the culture of A549 cells in Matrigel. Since these diverse morphologies were also confirmed in the conventional gel dome method, this morphological diversity likely stems from the intrinsic heterogeneity of the cell line, rather than being an artifact of the culture technique. Furthermore, a comparison of the spheroid surface areas revealed no significant difference between the SIEVEWELL and gel dome groups, demonstrating that the growth performance in SIEVEWELL is equivalent to traditional methods.

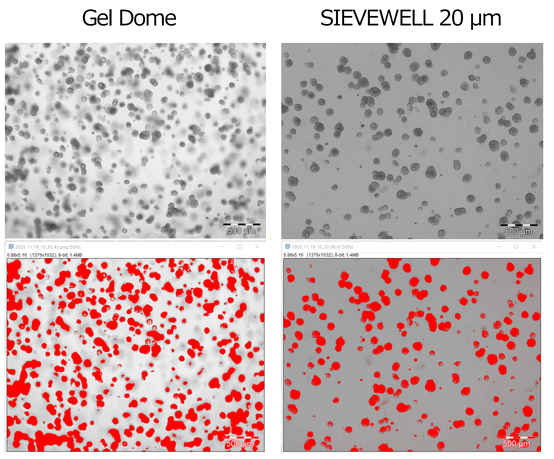
Liver Organoid Culture

- PXB-cells RF: Human hepatocytes isolated from PXB mice (human-liver chimeric mice).
- Method: Liver organoids were initially formed using the gel dome method according to the Human HepatiCult Organoid Kit protocol. The resulting organoids were then dissociated and subjected to secondary culture using both the conventional gel dome method and SIEVEWELL.



When applied to liver organoid culture, SIEVEWELL successfully supported the formation of numerous single-cell-derived organoids, with efficiency comparable to the conventional gel dome method.

Imaging Challenges



In the conventional gel dome method, cell aggregates form randomly in a three-dimensional space and frequently overlap, which can make quantitative analysis challenging.

In contrast, the SIEVEWELL method aligns spheroids on a single focal plane across the device, which is expected to facilitate easier and more accurate quantitative analysis. However, it has been suggested that the micro-well structures of the SIEVEWELL device itself could potentially act as optical noise during image processing and analysis.

Applied ImageJ Auto Threshold to the images.

Conclusion and Future Perspectives

The SIEVEWELL device maintains a formation efficiency for single-cell-derived 3D cultures of cell lines and organoids that is equivalent to the conventional gel dome method. Furthermore, because it ensures that cell aggregates form on a single focal plane, it has been demonstrated to be a powerful platform for tracking the diversity of individual cells. Moving forward, the key challenge will be to maximize the quantitative analysis capabilities of this system.