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High Throughput Secretion Based Single-Cell Screening for Antibody Discovery Using SIEVEWELL Arraying Device

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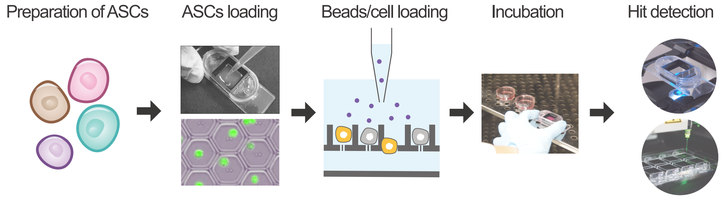
Abstract

We developed a high-throughput method for secretion-based single-cell screening in antibody discovery using the SIEVEWELL single-cell arraying device. SIEVEWELL features a microwell array with precisely fabricated through-holes at the bottom of each well. Cells are loaded by liquid flow, and once a single cell occupies a microwell, its through-holes are blocked, reducing local flow and directing remaining cells toward empty wells. This mechanism enables dense single-cell arraying (ca. 45,000 cells in a 17 × 17 mm area) beyond the limitations of Poisson distribution, achieving >95% single-cell occupancy at 50% microwell utilization. We applied SIEVEWELL to secretion-based screening of antibody-secreting cells. Antibody secretion was detected by co-loading antigen-conjugated beads into the same microwell and incubating with dye-conjugated anti-human IgG. Positive single cells were successfully retrieved using a glass capillary-based single-cell pick-up system (CellCelector, Sartorius). For functional screening, 293FT cells secreting anti-PD-1 antibodies (with or without PD-L1 blocking activity) and CHO cells expressing PD-1 were co-loaded into microwells, followed by incubation with Avi-tagged PD-L1. After staining with streptavidin-AF647, only antibodies that did not block PD-L1 binding allowed PD-L1 to bind PD-1 on CHO cells, resulting in AF647 fluorescence. This demonstrates the potential of SIEVEWELL for functional antibody screening. To scale throughput, we are developing an SBS/ANSI-compatible plate with eight independent chambers and a total of 720,000 microwells, enabling large-scale antibody discovery.

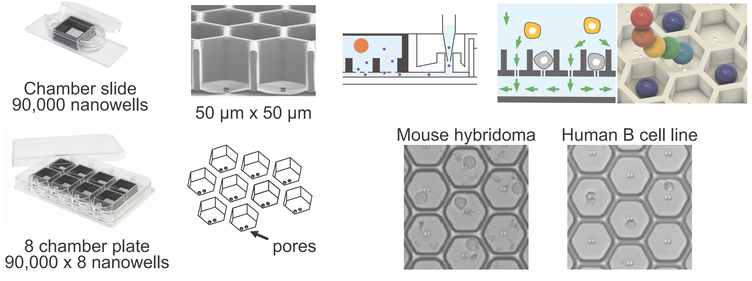
Materials and Methods

Screening Workflow



SIEVEWELL High Density Cell Arraying Device

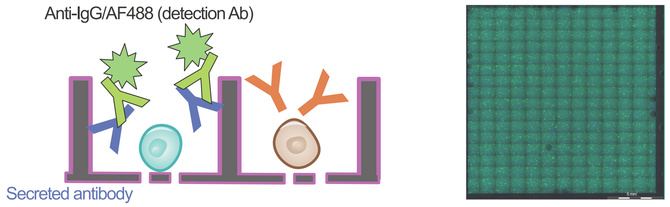
SIEVEWELL improves single-cell capture using nanowells with two basal pores that create a directed flow to pull cells into empty wells. When a cell enters and partially blocks the pores, the flow decreases. This causes untrapped cells to be guided toward other available wells. This active flow strategy surpasses the efficiencies of passive, Poisson-limited sedimentation.

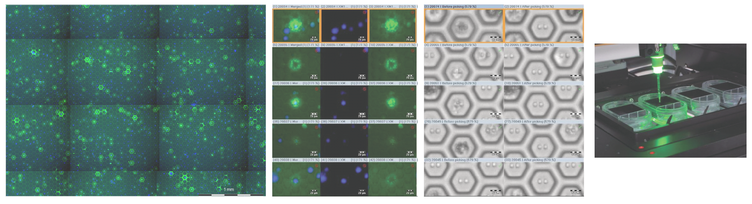


Results

Single Cell ELISA

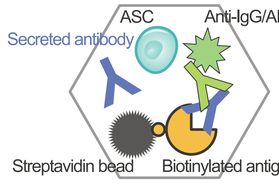
- The nanowell surfaces were coated with recombinant cytomegalovirus pp65 protein via physical adsorption
- EBV-transformed human B cell line XYFMGG was seeded into the SIEVEWELL device
- The cells were cultured in a medium containing AF488-labeled anti-human IgG

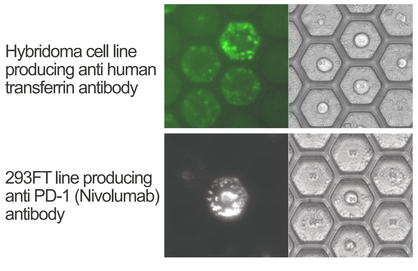




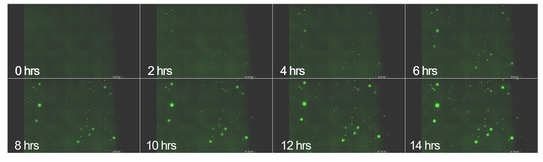
Beads-based Assay

- Biotinylated antigen was conjugated to streptavidin-labeled beads
- ASCs were seeded into the SIEVEWELL device
- The cells were cultured in a medium containing AF488-labeled anti-human IgG





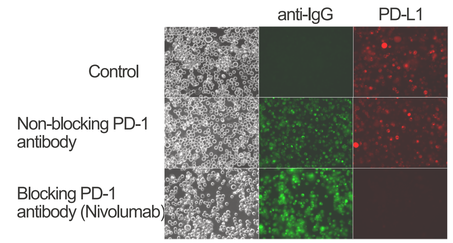
The time-course formation of AF488-labeled detection antibody and antigen-secreted antibody complexes was monitored using IncuCyte S3.

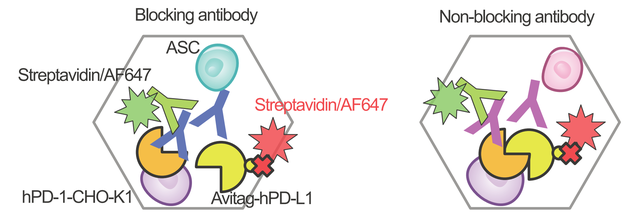


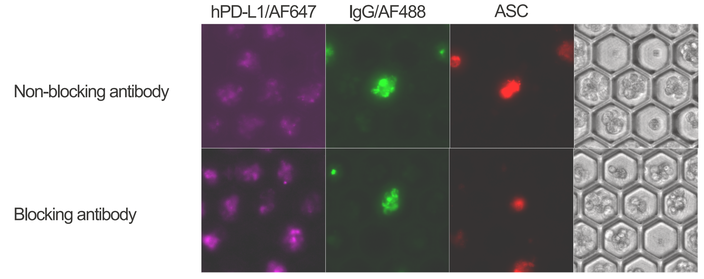
Cell-based Assay

- We established 293FT cell lines that secrete blocking and non-blocking anti-PD-1 antibodies
- Anti-PD-1 antibody producing 293FT cells were seeded into the SIEVEWELL device
- hPD1-expressing CHO-K1 cells were seeded into the SIEVEWELL device
- Cells were cultured in AF488-labeled anti-human IgG medium
- The cells were incubated with Avitag-hPD-L1 in PBS
- The cells were incubated with AF647-labeled streptavidin in PBS

hPD-L1/CHO-K1 cells were stained with individual antibodies, subsequently incubated with Avitag-hPD-L1, and then stained with streptavidin-conjugated AF647.







Conclusions

SIEVEWELL enables highly efficient, high-throughput single-cell antibody screening by achieving excellent single-cell occupancy and reliable functional readouts. It simplifies the identification and isolation of blocking and non-blocking antibodies, accelerating the development of next-generation therapeutic antibodies.

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