Application Note

Secretion-based Single Cell Sorting with Nanovials

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Suspendable Hydrogel Nanovials for Massively Parallel Single-Cell Functional Analysis and Sorting
ACS Nano 2022, 16, 5, 7242–7257, March 24, 2022
https://doi.org/10.1021/acsnano.1c11420

Introduction

Over the last half-century, single-cell sorting technologies that utilize surface markers have evolved, leading to numerous fundamental discoveries in cell biology. However, there are still limitations in secretion-based sorting, despite its potential to accelerate our understanding of cell biology and the development of biologics. To overcome this limitation, Dr. Joseph de Rutte, Dino Di Carlo, a professor at UCLA, and his group have invented a novel microparticle that can capture a single cell and its secretion. The "Nanovials" are cavity-containing hydrogel microparticles that can be decorated with various carriers to hold the cell and its secretion in a target-specific manner. In addition, the Nanovials are easy-to-use because the fluid in the cavity can be easily replaced, and they can be sealed with biocompatible oil to prevent diffuse secretions and crosstalk between samples. These features allow the Nanovials to behave as "sortable microwells" that can perform bioassays such as ELISA at the single-cell level within the cavity, at speeds of 1,000 events/sec. or faster on a typical FACS instrument. This Application Note shows how Nanovials are a powerful tool for the development of biologics, such as cell therapy and antibody drugs, using high-throughput screening of highly antibody-producing cells and antigen-specific B cells from immunized mice as examples.

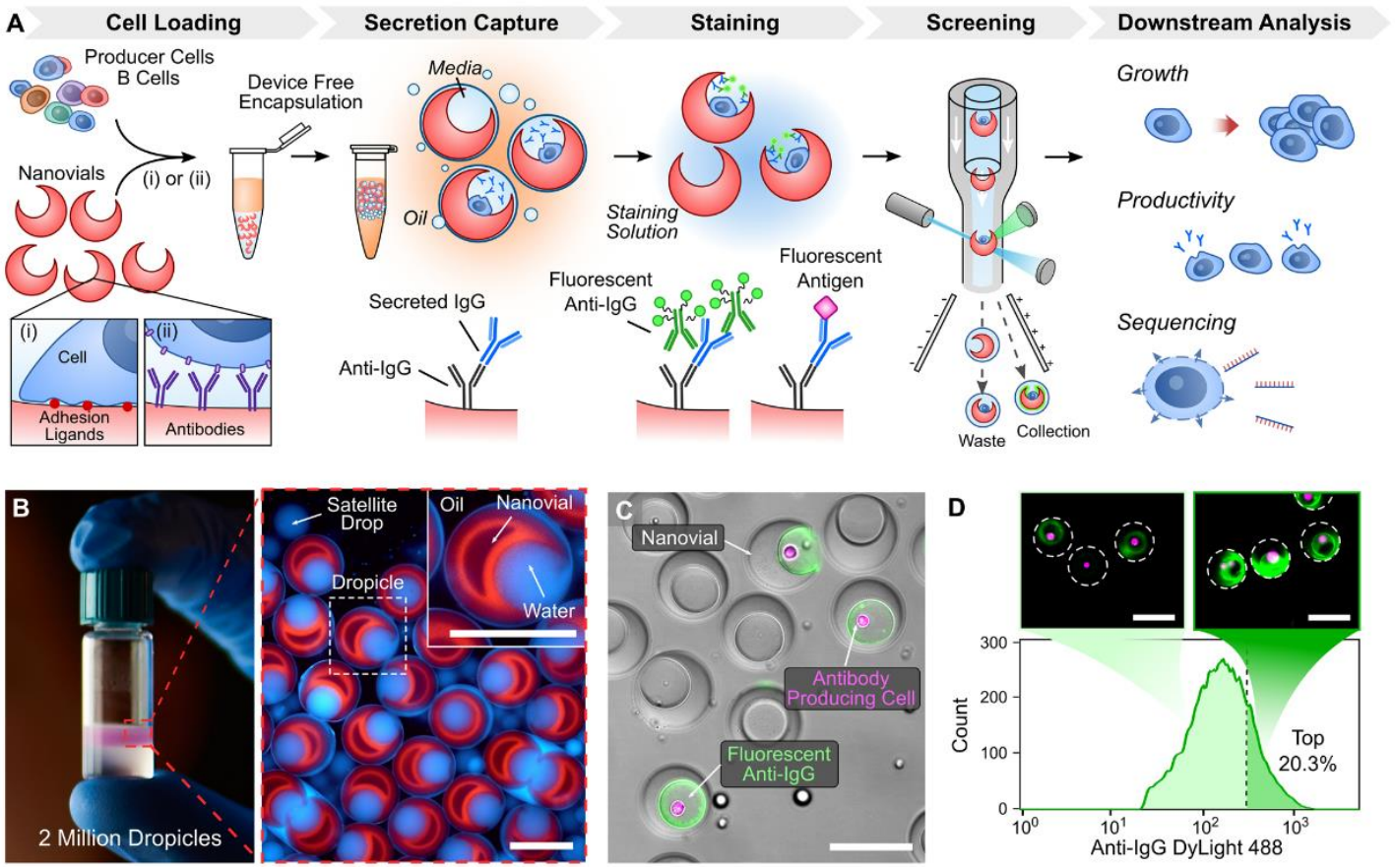

Fig. 1 Nanovials and secretion-based high throughput screening
(A) Workflow of secretion-based screening with Nanovials **(B)** Magnified image of Nanovials-contained water-in-oil emulsions. **(C)** Fluorescent image of captured cell that producing antibody in cavity of Nanovials. **(D)** Histogram data and fluorescent image of Nanovials after sorting each population.

Results

Nanovials as modular single-cell carriers

Nanovials suspended in the culture medium settle with their cavities facing upward, owing to the eccentricity of their center of gravity. As a result, a monolayer of Nanovials with the lumen facing upwards was formed on the culture plate. By adding the cell suspension from above, the cells settled toward the cavity and bound tightly to the Nanovial through the carrier (Fig. 2A). For example, RGD motifs, a well-known adhesive peptide motif present in fibronectin, tightly captured the adhesion cell line CHO DP-12 cell within cavities. They also showed that the surface proteins of HEK293 cells could be biotinylated and adhered tightly through biotin-streptavidin interactions. On the other hand, RGD coating alone was not sufficient to capture suspension-adapted cell lines, such as ExpiCHO. However, increased adhesion within Nanovial cavities was achieved by the combination with Poly-L-Lysine. Furthermore, coating Nanovials with antibodies against well-known surface markers such as CD45 and CD19 can be used to capture primary suspension cells. Indeed, antibodies against CD45, CD19, or both yield high levels of B cell adhesion in Nanovials, which was observed even after washing and sorting.
Cells were taken up into the Nanovial cavities along the Poisson distribution (Figure 2D). Therefore, by appropriately adjusting the cell concentration so that $\lambda \fallingdotseq 0.1$, one cell can be captured per one Nanovial particle, which is necessary for single-cell analysis. These results demonstrate that Nanovial is an exceptional single-cell analysis platform that is adaptable to various cell types.

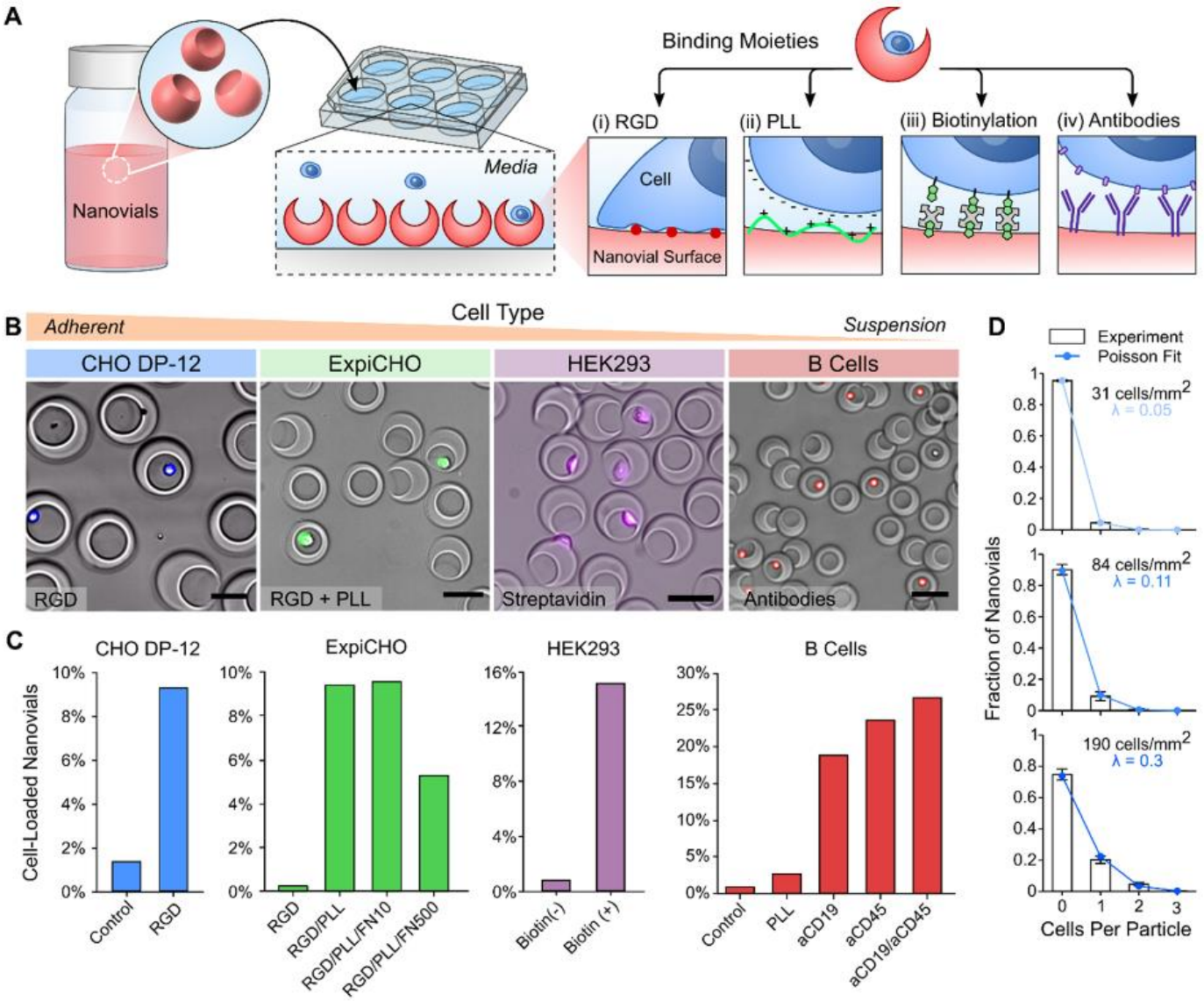

Fig 2. Loading and binding of single cells into Nanovials

(A) Workflow of loading and binding single cells into Nanovials **(B)** Fluorescent microscope image of captured cells within Nanovial cavities. **(C)** Comparison of cell binding efficiency to Nanovials for different cell type and moieties. **(D)** Relationship between number of loading cells into Nanovials and Poisson statistics.

Nanovial emulsification for secretion analysis and sorting at the single cell level.

Antibody coating allows the different types of cells to be captured in the Nanovials cavity, but can also trap secretions from the cells. However, secretions from captured cells can quickly diffuse outside the Nanovials and cause cross-talk between samples. To prevent this, Nanovials can be emulsified by adding oil and surfactant and a simple pipetting operation to obtain an emulsion with cells and medium sealed in their cavity (Fig. 3A). In addition, the Nanovials can be easily recovered from the oil by using a biocompatible destabilizing agent and can proceed to immunostaining for secretion assay (Fig. 3B).

These features of the Nanovials enable precise and quantitative analysis and sorting based on secretion differences at the single-cell level. Indeed, by using the FACS instrument with the mixed population of the IgG-producing CHO cells and non-producing CHO cells, they clearly demonstrated that the Nanovials platform can label to the secretion of interest and sort with high-purity (85-99%) and high-enrichment ratio (850-fold) (Fig. 3C). Furthermore, sorted cells were able to expand out of the Nanovials and retained the ability to produce antibodies at levels that correlated with the mean intensity of the single-cell secretion signal detected at the sorting steps (Fig. 3D-F). These data indicate the versatility of the Nanovials technology for identifying and recovering rare populations of high-yielding producer cells using standard flow cytometry workflows.

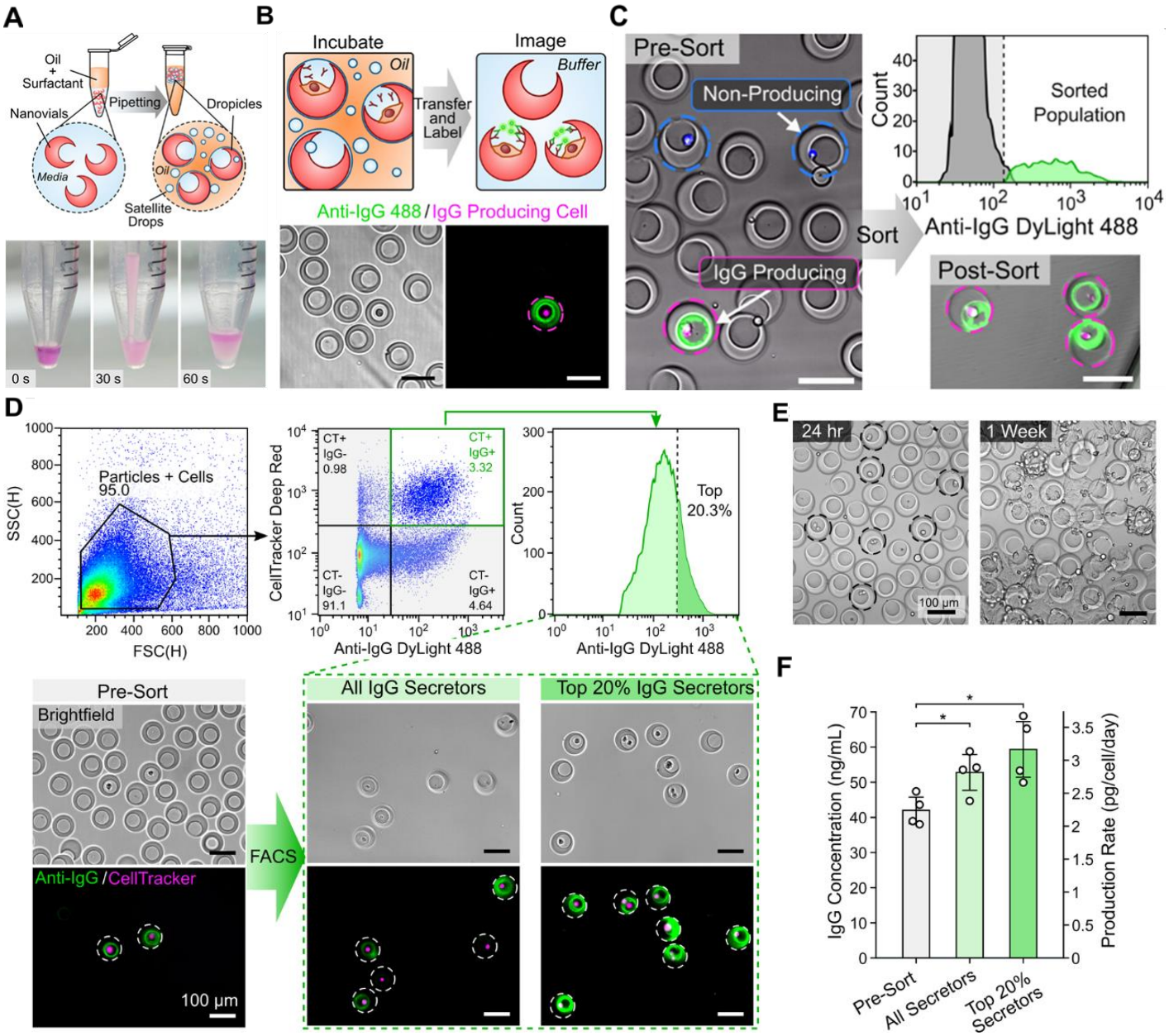

Figure 3. single-cell secretion analysis and sorting by emulsification of Nanovial.

(A) Emulsification of Nanovials by pipetting **(B)** Cell culture within the emulsion and adsorption of secretions into the lumen, staining with secondary antibodies **(C)** Sorting of antibody-producing cells using Nanovial and On-chip Sort **(D)** Analysis of antibody-producing cells and screening of high-producing cells using Nanovial and On-chip Sort **(E)** Microscopic images of post-sort samples **(F)** Differences in the culture supernatant between the pre- and post-sort samples **(F)** Differences in antibody production in the culture supernatant between the pre- and post-sort samples

Screening of cells producing antigen-specific antibodies using Nanovial.

Nanovials can also be used to screen for antigen-specific antibodies and their producing cells. The HyHEL-5 strain, a hybridoma that produces egg white lysozyme, and the 9E10 strain, which produces anti-Myc tag antibodies, are mixed and captured on Nanovial cavity modified with biotin and anti-CD45 antibodies. Secreted antibodies during culture were captured using anti-mouse H&L chain antibodies. Following incubation, the oil was removed and the particles were labelled with fluorescence-conjugated lysozyme to screen for antigen-specific antibody secretion from HyHEL-5 (Fig. 4A-B). Subsequent microscopic analysis revealed that antigen-positive/live cell-loaded Nanovials were highly enriched (>90% purity) in the post-sort population, despite being present in only 0.18% of the one million pre-sort population (Fig 4C). In addition to the bulk recovery of enriched cell samples producing antigen-specific antibodies, it was confirmed that a single hybridoma could be loaded into the individual wells of a 96-well plate for downstream sequence recovery and regrowth (Fig. 4D).

It is also possible to screen B cells that produce antibodies of interest in immunized mice. When B cells from mice immunized with OVA were analyzed and sorted using a combination of Nanovial and fluorescent OVA, the top 2% of the population with the highest fluorescence intensity contained a variety of positive Nanovials, including those with a strong signal on the cell surface and those without a signal on the cell surface but with a strong signal from the Nanovial. These data strongly suggest that more differentiated plasma cells, which could not be detected and isolated by existing antigen-specific B-cell screening using BCR antigen binding, could also be detected (Fig. 4E).

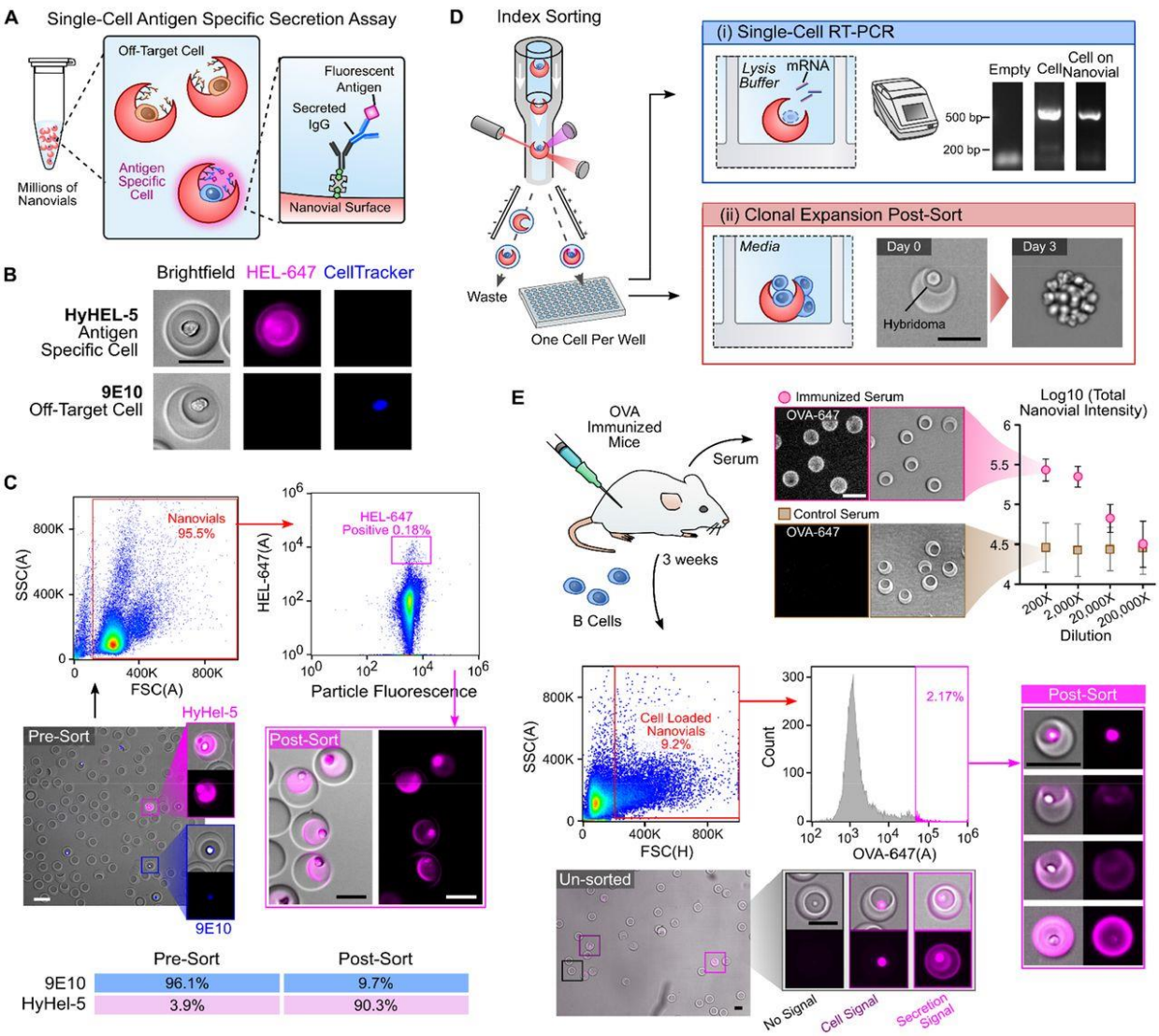

Figure 4. Screening of antigen-specific antibody-producing cells using Nanovial.

(A) Schematic diagram of antigen-specific antibody-producing cell screening using fluorescent antigen.
(B-D) Detection of target antibody-producing cells using HyHEL-5 and fluorescence-labelled antigen as a model **(C)** and analysis and sorting results and **(D)** examples of antibody gene amplification by RT-PCR and expanded culture. **(E)** Detection of anti-OVA antibodies in serum and screening of anti-OVA-producing B cells in OVA-immunized mice.

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