bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Massively parallel encapsulation of single cells with structured microparticles and secretion-based flow sorting

Joseph de Rutte[1], Robert Dimatteo[2], Mark van Zee[1], Robert Damoiseaux[4,5,6] and Dino Di Carlo*[1,3,4,5]

[1]Department of Bioengineering, University of California, Los Angeles
[2]Department of Chemical and Biomolecular Engineering, University of California, Los Angeles
[3]Department of Mechanical and Aerospace Engineering, University of California, Los Angeles
[4]California NanoSystems Institute, University of California, Los Angeles
[5]Jonsson Comprehensive Cancer Center, University of California, Los Angeles
[6]Department of Molecular and Medical Pharmacology, University of California, Los Angeles

*Correspondence to: dicarlo@ucla.edu

Abstract

Techniques to analyze and sort single cells based on secreted products have the potential to transform our understanding of cellular biology as well as accelerate the development of next generation cell and antibody therapies. However, secretions are rapidly transported away from cells, such that specialized equipment and expertise has been required to compartmentalize cells and capture their secretions. Herein we demonstrate the use of cavity-containing hydrogel microparticles to perform functional single-cell secretion analysis and sorting using only commonly accessible lab infrastructure. These microparticles act as a solid support which facilitates cell attachment, templates formation of uniform aqueous compartments which prevent cross-talk between cells, and captures secreted proteins. Using this platform we demonstrate high-throughput analysis and sorting of Chinese Hamster Ovary cells based on their relative production of human IgG using commercially available flow sorters. Microparticles are easily distributed and used, democratizing access to high-throughput functional cell screening.

Introduction

The ability to analyze and sort cells based on secretions (antibodies, cytokines, proteases, other enzymes, or even exosomes/microvesicles) is fundamental to our understanding of functional cellular heterogeneity underlying biology and to our ability to create new biotechnology products, such as biologics and cell therapies. However, consistent, easily accessible and automatable solutions for this basic biological problem do not exist.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Moreover, within the populations of secreting cells are subsets of rare cells with secretion profiles of outsized importance, with a quintessential example being B cells. The ability to sort rare B cells or B cell fusions, such as hybridomas, directly based on their production levels and activity of high affinity antibodies would allow for the acquisition of gene sequences that can be used to make new antibody drugs.[1–6] Further, sorting of stable, production-grade cells, such as Chinese hamster ovary (CHO) cells, based on their production rate of humanized IgG protein would allow for the selection of specific clones useful for antibody drug production environments.[7] Finally, the ability to sort rare populations of cells for outlier clones with ultra-high-performing secretion profiles promises to enable cycles of selection and mutagenesis important for directed cellular evolution of the next generation of cell therapies and also can enable performance surveillance of the clones themselves over time.[8]

Although secretion of proteins or other biological products is a key function of many cells, approaches to sort cells at high rates based on this function have significant tradeoffs and are not widespread. Intracellular staining of secreted proteins prior to secretion by cells can be performed following fixation and permeabilization. Fluorescence activated cell sorting (FACS) can then be used to isolate sub-populations based on levels of these retained intracellular proteins.[9,10] However, cells are non-viable after this process and intracellular stores of proteins may not directly reflect the secreted amount or functional state of secreted proteins. An alternative approach involves binding capture antibodies directly to the cell surface to capture secreted proteins. This has been done by directly biotinylating surface proteins or by using bi-functional antibody conjugates.[11–13] Captured secretions can then be labeled with fluorescently-conjugated antibodies, and cells can be sorted using standard FACS. Unfortunately, these strategies can affect cell viability or require the presence of specific surface proteins on the cell surface (e.g. CD45). Further, this general approach suffers from significant cross-talk for high secretors due to transport of secreted products away from the secreting cells. More recent approaches place single cells into nanoliter scale compartments to prevent cross-talk of secretions.[14,15]

Screening of live cells based on single cell secretions relies on the ability to isolate single cells into uniform compartments to reduce crosstalk, capture secretions onto a solid substrate, and readout/select cells based on an associated signal.[16] Droplet microfluidics can be used to compartmentalize cells with capture beads or within capture gel matrices and sort droplets containing cells of interest with custom droplet sorters or flow

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

cytometers.[17–20] Cells can also be seeded into microwells with surfaces modified to capture secretions and following secretion, target cells can be sorted one-by-one with a pipette.[21,22] Optofluidic approaches, e.g. from Berkeley Lights, transport and compartmentalize cells using optically induced dielectrophoretic manipulation, which allows loading and picking cells from specific wells embedded on a chip.[23,24] Despite the utility of these existing platforms, they each require users to acquire specialized, often extremely expensive equipment to perform the assays, which hinders widespread adoption.

We introduce a new approach to collect and quantify single-cell secretions without crosstalk in monodisperse droplets formed by precisely structured microparticles, enabling high-throughput screening based on secretions (Fig. 1). In our "dropicle" approach, uniform **droplets** are templated by **particles** through simple mechanical agitation induced by pipetting in bulk solution. Particles are structured with an open cavity enabling collection and protection of cells. Spherical gel particles have been used to template emulsions,[25,26] however, the lack of a cavity precluded use with mammalian cells, and no secretion assays were performed. In our system, cells are loaded into cavity-containing microparticles and adhere via integrin binding sites on the particle surfaces. Oil and surfactant are added and the suspension is agitated by pipetting to create incrementally smaller water-in-oil droplets. These resulting dropicles retain a fluid volume which is uniform and defined by the particle geometry. Secretions from encapsulated cells are captured on the associated particles, and particles and associated cells and secretions are transferred back to an aqueous phase by breaking the emulsion, enabling downstream analysis and sorting that is compatible with standard flow cytometers. As a result, the end user can form uniform droplets without the need for microfluidics, eliminating the need for new expensive equipment or specialized skills that would otherwise need to be acquired to perform these assays. Standard flow cytometers have been widely available to most research labs and companies over the past several decades making sorting methods for dropicles easily accessible.[27] This dropicle platform has the potential to dramatically increase the accessibility of single-cell screening tools to researchers which can add functional dimensions to our understanding of cell biology as well as accelerate the development of antibody drugs, affinity-based diagnostics, and emerging cellular therapies.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

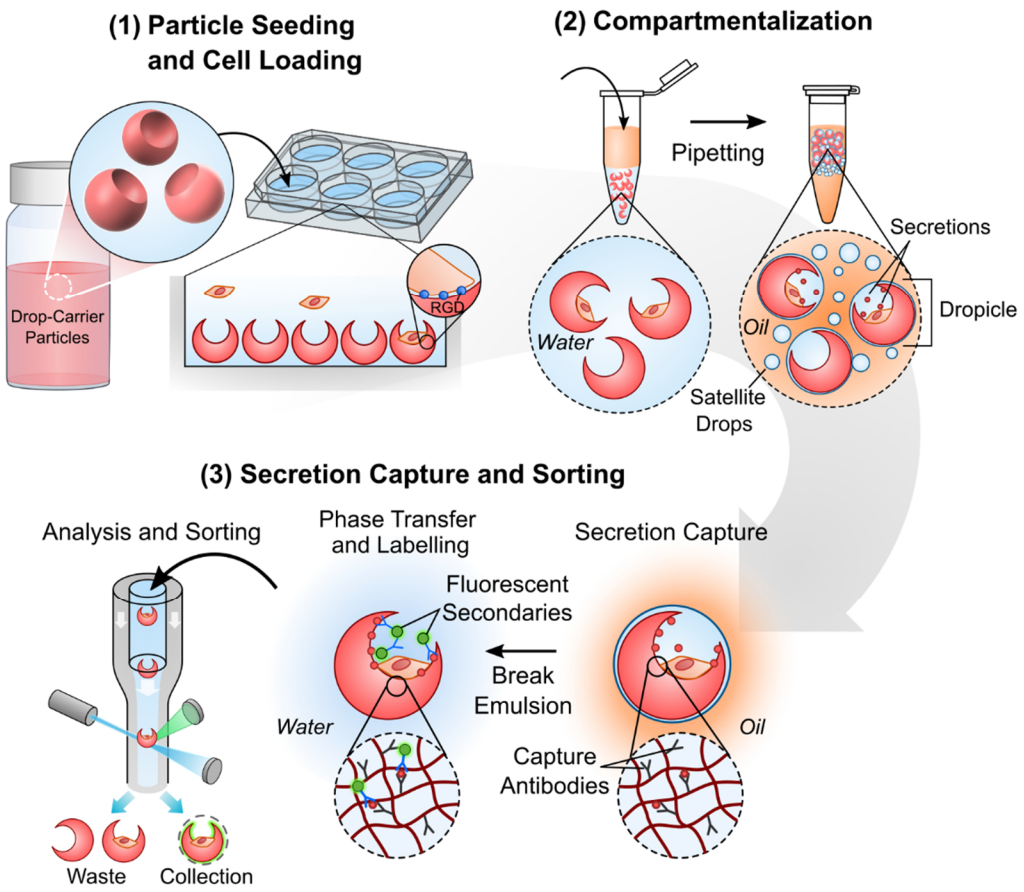

Figure 1. Overview of dropicle-based single cell secretion screening platform. (1) Prefabricated cavity-containing microparticles are seeded into a well plate and settle with their cavities remaining upright. Cells are seeded and adhere to the particle matrix via integrin binding sites (RGD). (2) Particles and associated cells are transferred into a tube and agitated by pipetting with biocompatible oil and surfactant to generate monodisperse compartments dictated by particle size (Dropicles). (3) Cells are incubated to accumulate secretions on associated particles and transferred back to an aqueous phase for labelling. Particle/cells/secretions can then be analyzed and sorted using high-throughput commercial flow cytometers.

Results

Device free encapsulation of single cells into monodisperse droplets

Our approach to encapsulate single cells in monodisperse drops requires structured microparticles with exposed cavities, biocompatible oils and surfactants, and simple pipetting steps. Structured hydrogel microparticles with a crescent-shaped cross-section are fabricated at high-throughput (~1000 s^{-1}) utilizing an aqueous two-phase system combined with droplet microfluidics as adapted from previous work (Figure S1-3).[28] By tuning the fabrication parameters, we achieve highly monodisperse drop-carrier particles (outer diameter CV

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

of 1.5%, cavity opening diameter CV of 2.1%) that are further functionalized with arginine-glycine-aspartate (RGD) peptide for cell adhesion and biotin as a versatile handle for affinity capture. Apart from this initial fabrication step that used a specialized microfluidic device, all further experiments are performed utilizing only

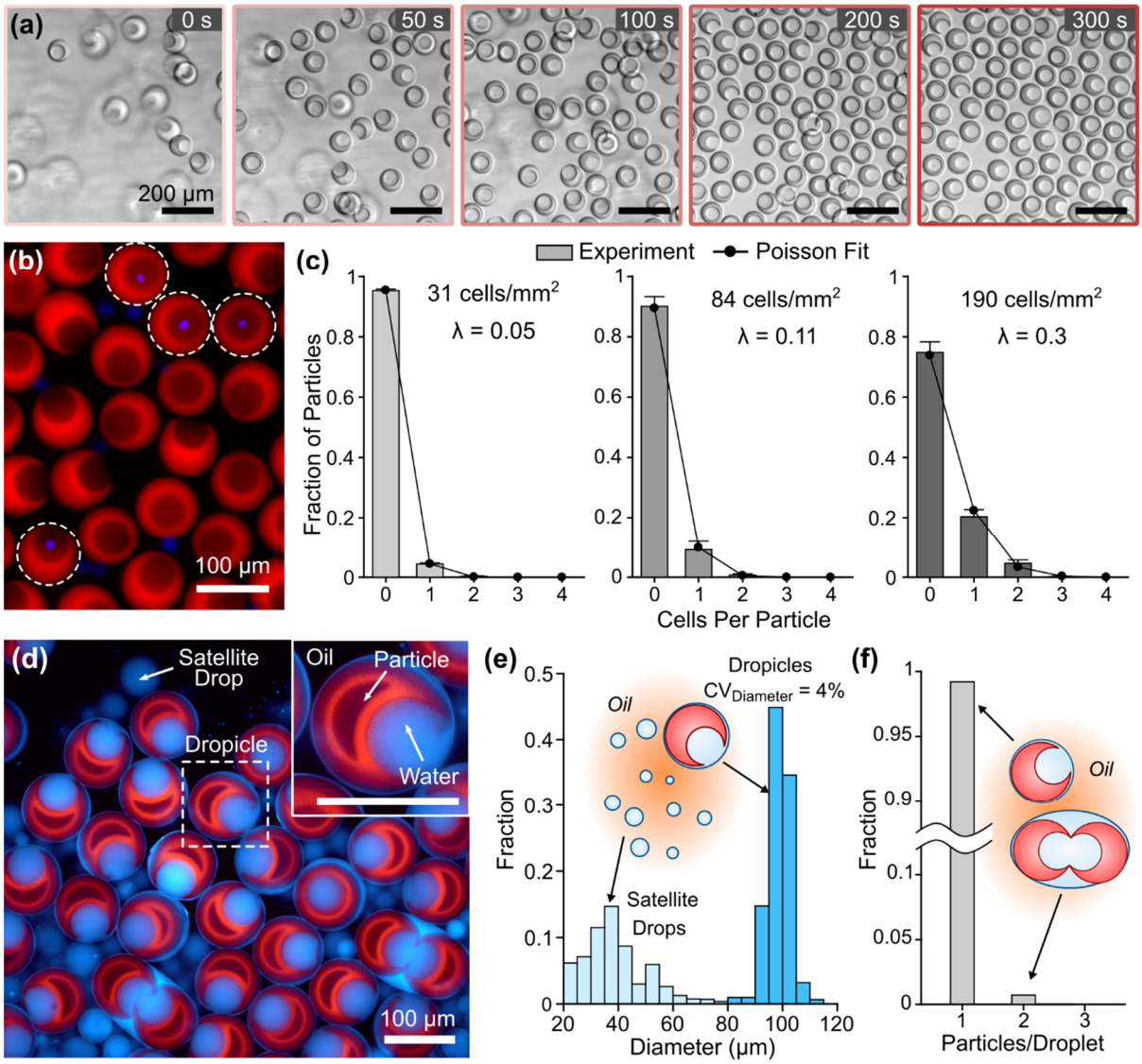

Figure 2. Characterization of cell loading and dropicle formation. (a) Particles are loaded into wells and settle with their cavities mostly upright due to their asymmetric morphology. (b) Cells are then seeded into the open cavities and adhere via integrin binding sites (RGD peptide) linked to the particle matrix. Fluorescence microscopy image of cells loaded into cavities where particles are labeled red and cell nuclei are labeled blue. (c) Cell loading closely follows single-Poisson statistics where λ is the average number of cells per particle. Loading fraction can be controlled by adjusting the seeding density. (d) Fluorescent image of dropicles formed by pipetting suspended particles with oil and surfactant. To aid in visualization drop-carrier particles were labeled with a red fluorophore and a large molecular weight dye (blue) was included in the water phase. (e) Droplet size analysis after emulsification shows two distinct subpopulations: smaller heterogeneous satellite drops and monodisperse dropicles (n = 561). (f) Nearly all droplets formed have either 0 or 1 particle associated with them, with only a small fraction (<1%) containing 2 or more particles per droplet.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

standard lab infrastructure. This is a critical feature of our platform as it enables the more complex microfluidic/particle fabrication work to be centralized and performed in advance. We envision that the end user can then directly use the pre-fabricated particles to perform the full single cell secretion assay workflow.

Cell seeding onto the particles uses standard pipetting steps followed by incubation. To seed cells within the particle cavities, a monolayer of drop-carrier particles is first pipetted onto the bottom of a well plate. Due to the unique morphology of the particles, they settle with their cavities mostly upright (Figure 2a, Video S1).[29] Cells are then seeded over the particles and allowed to settle with a sizeable fraction coming to rest in the particle cavities (>15%) (Figure 2b). It was noted that this seeding approach led to cell occupancies that closely followed Poisson statistics (Figure 2c), as is expected for loading of single cells into microfluidically-generated droplets.[30,31] Therefore, by controlling the cell seeding density, we can control occupancy such that most particles have either 0 or 1 cells associated with them.

By exploiting the structure of the microparticles, uniform droplets are formed via simple agitation steps with oil and surfactant. An aqueous solution of drop-carrier particles is first concentrated in a conical or microcentrifuge tube by centrifuging and aspirating the supernatant. A layer of biocompatible oil with surfactant is added and the suspension is then pipetted vigorously for 30 seconds to create smaller and smaller water-in-oil droplets. Eventually the droplet size is maintained by the outer periphery of the microparticle (Figure 2d). We find that, in agreement with minimal energy considerations[32], fluid from the cavity remained stably trapped by the hydrophilic drop-carrier particle. Any excess fluid in the suspension is partitioned into much smaller satellite droplets. The resulting emulsion shows two unique distributions; (1) small non-uniform satellite droplets and (2) monodisperse droplets each templated by single microparticles (Dropicles) (Figure 2e). Importantly it was observed that only a small fraction of droplets contained multiple particles (<1%, Figure 2f).

For functional cell assays it is critical that cells remain viable during the dropicle formation and emulsion breaking process. Using calcein staining we demonstrate that the cell membranes remain intact during the emulsification steps (Figure S5a). Live/dead analysis of CHO cells following encapsulation and subsequent emulsion breaking shows high viability over a 24-hour period (>80%) indicating that the workflow is biocompatible (Figure S5b). Further, we compared cell growth after dropicle release with cells seeded into a well

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

plate. Growth was determined to be not significantly affected by the process with comparable growth rates (Figure S6).

Single cell secretion analysis using dropicles

Using the dropicle platform we demonstrate a device free workflow to perform single-cell secretion assays with minimal crosstalk (Figure 3a). Given the importance of selecting high antibody titer cell lines for therapeutic production,[33] we chose a Chinese hamster ovary (CHO) cell line that produces human IgGs targeting interleukin 8 (IL-8) and particles modified to capture human IgG as our model system. Cells are first loaded into the microparticle cavities and adhere via integrin binding sites as previously described. After initial cell seeding, particles and associated cells are collected and washed to remove background secretions. Particles are then coated with anti-human IgG Fc antibodies by binding to biotin groups linked to the particle matrix. Following this step, the particles and associated cells are rapidly compartmentalized via dropicle formation. The compartmentalized CHO cells are then incubated, and the secreted antibodies are captured onto the associated particle matrix, which was confirmed via imaging in dropicles (Figure S7). After incubation, the emulsions are broken and the particles with attached cells and secretions are collected and washed. The particles with the associated cells and secretions are then labeled with secondary fluorescent antibodies targeting the secreted anti-IL-8 antibodies. Using fluorescence microscopy, we confirmed retention of cells and associated secretion signal on the particle surface after recovering out of oil into an aqueous phase (Figure 3b).

We incubated cells from 0 to 8 hours in dropicles and observed an increase in mean accumulated fluorescence signal on the particles that trends, as expected, with increasing incubation time and corresponding antibody production (Figure 3c,d). Significant signal over background (3 standard deviations) was observed after 1 hour of incubation (Figure 3e). Interestingly, we noted across all incubation times that there was a sizeable population of cells (~25%) that had no measurable secretion signal (Figure 3c,e). Further analysis showed that this was not due to cell viability (Figure S9), but instead likely due to a fraction of the cell population no longer expressing the antibody production gene or secreting the produced antibodies. Further we demonstrate that this assay workflow is compatible with the CHO cells after adapting them to suspension culture (Figure S8). With suspension-adapted

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

CHO cells we also saw distinct punctate signals on particles containing cells that was observable after as little as 1 hour.

Because of the heterogeneity across cell populations, preventing secretion cross-talk between particles is critical to enable quantitative analysis and sorting. Dropicle formation is key to preventing cross-talk of secretions as demonstrated by a side by side comparison of the secretion assay with and without the dropicle formation step. When secretions are captured on particles in bulk (without a surrounding oil), particles without associated cells show high secretion signals indicating significant cross-talk (8.2% of non-cell containing particles have signal above threshold) (Figure 3d). Conversely, with the dropicle formation step there are two visibly distinguishable populations, particles with and without secreting cells, and only 1% of particles without cells have signal above the cut off threshold. Minimizing the fraction of false positives is particularly critical when secretions can spill over into neighboring particles containing non-secreting cells that would contaminate downstream cultures or sequencing assays.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

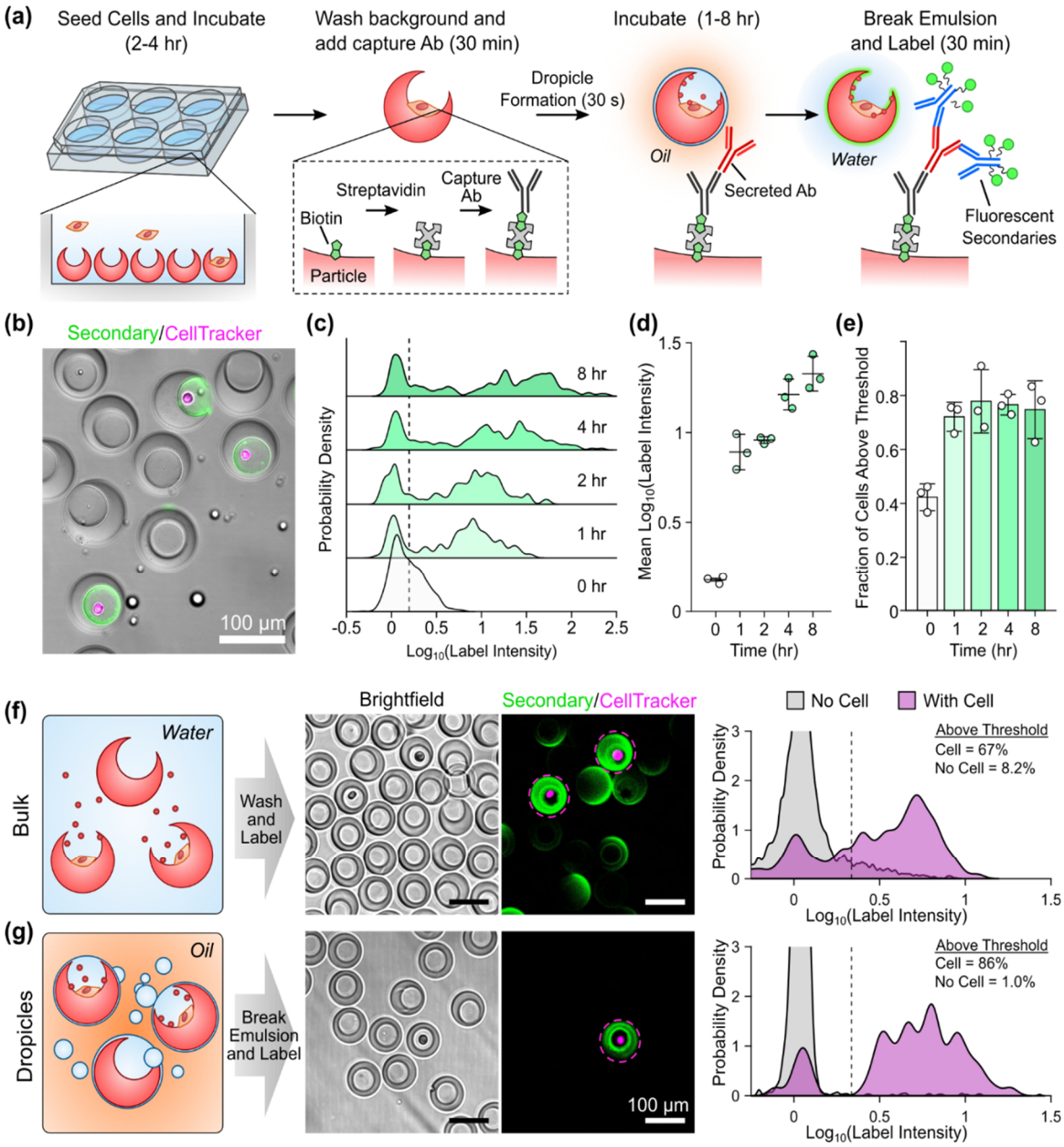

Figure 3. Analysis of single-cell secretions using dropicles. (a) Single cell secretion assay workflow. Cells are first seeded onto particles and washed. Biotinylated anti-human IgG Fc capture antibody is linked to the particle via biotin-streptavidin interactions. Dropicles are formed and cells are incubated inside. After secretion accumulation, emulsions are broken. Particles and associated cells are then washed and stained using fluorescent secondary anti-human antibodies. (b) Example image of particles with associated cells and secretion signal after performing full secretion assay. Fluorescent images are overlaid onto a brightfield image. (c-d) Sweep of incubation times shows increased accumulation of secretions over time as expected, providing further validation of the assay. Signal intensity of only particles with cells is shown. (d) Mean intensity is consistent across triplicate samples. (e) Fraction of cell population that is secreting human antibodies. Secreting criteria defined as 3 standard deviations above empty particle signal (dashed line shown in (c)). Significant signal over background is observed after as little as 1 hour of incubation. (f) Cross-talk is observed when the secretion assay is performed on particles without the dropicle formation step. (g) Minimal cross-talk is observed when dropicle formation compartmentalizes cell secretions during the incubation step. Threshold in both (f) and (g) is set at 3 standard deviations above empty particle signal for the dropicle condition (g).

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Isolation of secreting subpopulation using FACS

Following emulsion breaking, particles with attached viable cells are sorted based on secretion quantity using a commercial flow sorter. We performed secretion-based sorting with a mixed population of the anti-IL-8 producing CHO cells and non-producing CHO cells each labeled with a separate Cell Tracker™ dye (Figure 4a). After the secretion assay and prior to sorting, fluorescence imaging of the stained particles showed 100-fold higher signal on particles containing the antibody-secreting cells of interest compared to those containing non-secreting cells (Figure 4b). This further demonstrates the lack of cross-talk in our system as well as the specificity of the labels only to the secretions of interest. The particles with associated cells and secretions were then sorted based on the labeled secretion intensity (Figure 4c). Downstream analysis showed effective isolation of the sub-

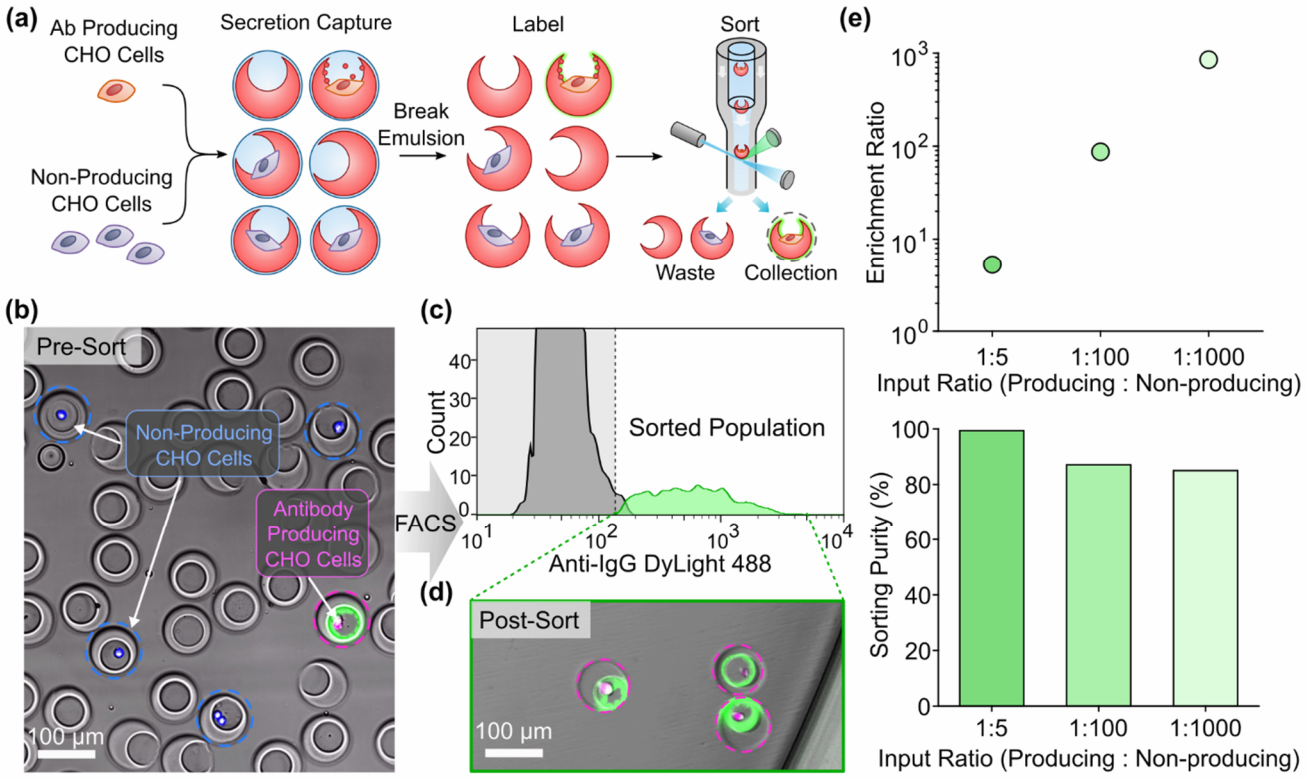

Figure 4. Sorting of cells based on secretions using commercial FACS. (a) Selection of secreting cells of interest using FACS. Antibody (Ab) producing cells are mixed with non-producing cells (1:4 ratio), each stained with different colored Cell Tracker dyes and seeded on the particles. A secretion capture assay is performed as previously described and samples are then collected, stained for secretions, and sorted using a commercial flow cytometer. (b) Microscopy image of samples after staining shows particles with Ab producing cells (magenta) yield clear secretion signals (green) which are not observed for non-producing cells (blue). (c) Samples are sorted by gating on the peak fluorescence intensity of the secretion label channel. (d) Collected samples are then imaged and analyzed. (e) Spiked target cells were isolated across a range of initial fractions in a background of non-secreting cells (1:5 – 1:1000) and imaged to determine enrichment ratio and purity.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

population of interest with high-purity (85-99%) (Figure 4d-e) over a range of target cell dilutions. In the most dilute case (1:1000) an enrichment ratio of 850X was achieved indicating the capability to isolate rare target cell events. Cells isolated using this approach could be expanded directly from the particle matrix enabling a streamlined workflow with minimization of trypsinization steps (Figure S6).

Viable sorting of high antibody producing cells

Sorted cells maintain the ability to produce antibodies at levels correlating with the mean intensity of initial secreted signal. In a separate set of experiments, we performed a secretion assay on human anti-IL8 producing CHO cells and selected out sub-populations based on level of secretion signal using FACS (Figure 5). Cells secreting antibodies were sorted in high-throughput (>200 events/s) by gating off both the fluorescently labeled secretion channel as well as CellTracker^{TM} dye (Figure 5a). For each separate passage of cells analyzed (n = 4) we sorted two sub-populations: (1) all particles with cells and detectable secretion signal above background and (2) particles with cells and the top 20% of antibody secretion signal. Microscopy images of the sorted particles and associated cells showed successful isolation of secreting cells with signal proportional to their respective FACS gating (Figure 5b). The sorted sub-populations were seeded into a 96-well plate and expanded out of the particles over the course of ~10 days. Samples were then plated at the same cell density and bulk antibody production of the different subpopulations was measured by ELISA and compared with non-sorted control samples. A 26% increase in total IgG production was observed for sorted sub-population 1 (all secreting cells) in comparison to the pre-sort control population (Figure 5c). This increase is most likely due to the removal of cells that are no longer secreting IgG following the sort, which was measured to be ~25% based on microscopy analysis (Figure 3c) as well as flow analysis (Figure 5a). For sorted sub-population 2 (the top 20% of secretors) we measured an average increase of 41% in total IgG production (n =4) with a maximum increase among the samples of 58% (Figure S10), indicating the capability of the platform to select out functionally higher producing sub-populations that maintain the phenotype for at least 10 days.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

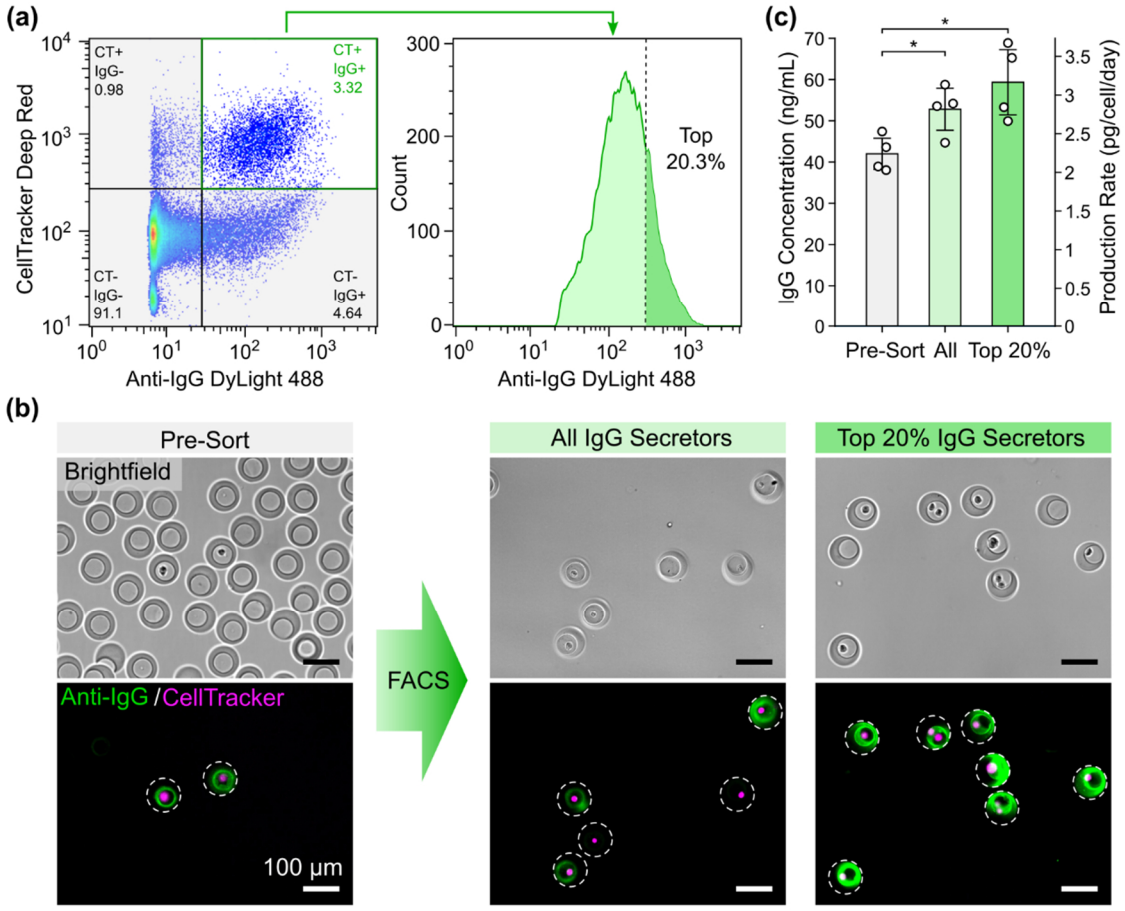

Figure 5. Selection of high secretion cell sub-populations using FACS. (a) The secreting population of cells were gated and sorted based on IgG secretion signal and CellTracker (CT) labelling. Both cells with any secretion signal disparate from background as well as the top 20% of secretors were separately sorted. (b) Microscopy images before and after sorting show enrichment of secreting cells with fluorescent intensity proportional to the selection criteria. (c) Isolated cells were expanded and bulk ELISA was performed to determine the production rate for the different sub-populations. Full experiment was performed with separate cell passages (n = 4). Statistical significance based on standard two-tailed *t*-test (*p<0.05).

Discussion

We show that two key features of the dropicle platform allow for widely accessible analysis and sorting of cells based on secretions: (i) the ability to form uniform compartments containing single cells in small volumes with minimal crosstalk using simple pipetting and no devices, and (ii) the compatibility of the drop-carrier particles with commercially available flow sorters such that viable cells can be sorted based on their associated secretions. Particles allow for seeding of cells into their open cavities and emulsification to form hundreds of

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

thousands to millions of dropicles all in parallel using simple pipetting operations. Parallel encapsulation in < 1 min is an important differentiator from microfluidic techniques which often require tens of minutes to several hours to sequentially form droplets while cells remain mixed and secreting in the input sample volume.[34] Rapid emulsification minimizes cross-talk from cells secreting into the mixed input sample volume during the time over which secreting cells are encapsulated which is critical for enabling screening of large cell populations. Dropicle emulsification and analysis steps can also be more easily performed in a sterile environment (e.g. biosafety hood) using standard sterile plasticware (e.g. well plates, pipette tips), as opposed to bulkier equipment. Although microfluidics is used to manufacture the drop-carrier particles, the particles can be easily produced in batch (>10 million drop-carrier particles per batch, Figure S1e) and provided to other labs who do not have expertise in microfluidics. Shipping of particles is significantly more cost-effective and rapid than replicating microfluidic setups for generation and sorting of droplets. Further, the amount of additional expertise required to use the dropicle system is substantially lower than microfluidic based approaches.

The dropicle system is also compatible with commercial flow cytometers which enabled us to screen over 100,000 cells across the experiments described in this report at rates of ~100 events/second. This throughput is comparable to state-of-the-art microfluidic approaches and by further optimizing the loading of cells into the particles as well using higher-throughput FACS machines this throughput could exceed 10 million cells per day. Besides increasing availability to labs without expertise in microfluidics or specialized commercial instruments, there are some unique advantages of this new approach for encapsulation of mammalian cells.

The structure and surface of the drop-carrier particles provide unique capabilities for tuning the number and type of captured cells, analysis of adherent cell secretions, and analysis and sorting of clonal colonies. The size and opening diameter of the cavity within a drop-carrier particle can be tuned (Figure S2), potentially enabling deterministic loading of single cells based on size exclusion effects. This could enable more rapid screening because cell loading rates may not be limited by Poisson statistics. Besides structural changes to the particles, the surface of particles can also be functionalized with affinity agents that specifically enrich certain populations of cells, such as antibodies to CD3 that enrich T cells from a mixed population. The surface of the particle also enables the attachment and growth of adherent cell populations, such as the CHO cells used herein. Analysis of

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

secretions from adherent cells can be challenging with other microfluidic techniques which require cells to be in suspension to flow into devices and sort afterwards. Adherent cells begin to undergo apoptosis when remaining in suspension and it is expected that secretion rates of biomolecules would change in this condition.[35] The inability to perform these assays has led to a dearth of information on the secretion phenotypes and heterogeneity of adherent cells with important secretion products in health and disease, such as mesenchymal stem cells, glandular epithelial cells, endothelial cells, glial cells, and even adherent bacterial biofilms. Further, in the dropicle system, when single cells are initially seeded onto particles they adhere and grow without nutrient limitations (Figure S6). The clonal colonies can then be encapsulated to form dropicles, enabling high-throughput screening based on the combination of growth and per cell secretion (i.e. overall biomass produced per unit time), potentially overcoming previous challenges in cell line selection in which growth and biologic production can be in a trade-off relation.[13]

Sorting based on secretions extends beyond selection of high antibody-titer cell lines to many applications of importance in life sciences and biotechnology. Discovering high affinity therapeutic antibodies relies on the selection of B cells producing antibodies with high affinity amongst a large background.[6] The activity of immune cells is largely connected to their secretion profiles, which direct communication and effector functions and can be better studied by sorting out specific sub-populations for further functional testing in vivo or in vitro. The effectiveness of chimeric antigen receptor-T cell batches also appears to depend on a multifunctional secretion of cytokines, such that sorting populations based on secretion profiles may enhance therapeutic activity.[36,37] Finally, processes of directed evolution of cell products and cells themselves can benefit from larger numbers of clones being screened, mutagenized, and expanded across multiple cycles using an efficient process relying on standard equipment and expertise.[8]

More broadly, our new approach to encapsulate single entities into uniform compartments with a solid phase can enable a centralized “lab on a particle” platform for a number of single-cell and single-molecule assays. Encapsulation is a key component for single-cell nucleic acid sequencing. Clonal colonies of bacteria, yeast, or algae producing engineered proteins (e.g. fluorescent proteins) can also be maintained in dropicles and sorted based on desirable features (e.g. intensity at particular excitation/emission wavelengths). Moving beyond cells, the compartments formed can enable digital nucleic acid amplification assays and immunoassays, where the solid

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

phase provides potential for barcoding and capturing of amplified assay signals. Given the ability to rapidly deploy our approach with established lab infrastructure we anticipate widespread applications of lab on a particle technology across a range of these single cell and single molecule assays in the near future.

References

1. Tiller, T. *et al.* Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J. Immunol. Methods* **329**, 112–124 (2008).
2. Tiller, T., Busse, C. E. & Wardemann, H. Cloning and expression of murine Ig genes from single B cells. *J. Immunol. Methods* **350**, 183–193 (2009).
3. Dekosky, B. J. *et al.* High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. *Nat. Biotechnol.* **31**, 166–169 (2013).
4. DeKosky, B. J. *et al.* In-depth determination and analysis of the human paired heavy- and light-chain antibody repertoire. *Nat. Med.* **21**, 86–91 (2015).
5. Mcdaniel, J. R., DeKosky, B. J., Tanno, H., Ellington, A. D. & Georgiou, G. Ultra-high-throughput sequencing of the immune receptor repertoire from millions of lymphocytes. *Nat. Protoc.* **11**, 429–442 (2016).
6. Parola, C., Neumeier, D. & Reddy, S. T. Integrating high-throughput screening and sequencing for monoclonal antibody discovery and engineering. *Immunology* vol. 153 31–41 (2018).
7. Wurm, F. M. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nature Biotechnology* vol. 22 1393–1398 (2004).
8. Di Carlo, D. Technologies for the Directed Evolution of Cell Therapies. *SLAS Technol. Transl. Life Sci. Innov.* **24**, 359–372 (2019).
9. Sander, B., Andersson, J. & Andersson, U. Assessment of Cytokines by Immunofluorescence and the Paraformaldehyde-Saponin Procedure. *Immunol. Rev.* **119**, 65–93 (1991).
10. Freer, G. & Rindi, L. Intracellular cytokine detection by fluorescence-activated flow cytometry: Basic

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

principles and recent advances. *Methods* **61**, 30–38 (2013).

11. Manz, R., Assenmacher, M., Pflüger, E., Miltenyi, S. & Radbruch, A. Analysis and sorting of live cells according to secreted molecules, relocated to a cell-surface affinity matrix. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1921–1925 (1995).

12. Assenmacher, M., Löhning, M. & Radbruch, A. Detection and Isolation of Cytokine Secreting Cells Using the Cytometric Cytokine Secretion Assay. *Curr. Protoc. Immunol.* **46**, (2001).

13. Borth, N., Zeyda, M. & Katinger, H. Efficient selection of high-producing subclones during gene amplification of recombinant Chinese hamster ovary cells by flow cytometry and cell sorting. *Biotechnol. Bioeng.* **71**, 266–273 (2000).

14. Wimmers, F. *et al.* Single-cell analysis reveals that stochasticity and paracrine signaling control interferon-alpha production by plasmacytoid dendritic cells. *Nat. Commun.* **9**, (2018).

15. Dimatteo, R. & Di Carlo, D. Droplet-Enhanced On-Cell Encoding of Single Cell Secretory Function. in *23rd International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2019* (2019).

16. Chen, Z., Chen, J. J. & Fan, R. Single-Cell Protein Secretion Detection and Profiling. *Annu. Rev. Anal. Chem.* **12**, 431–449 (2019).

17. Mazutis, L. *et al.* Single-cell analysis and sorting using droplet-based microfluidics. *Nat. Protoc.* **8**, 870–891 (2013).

18. Ahn, K. *et al.* Dielectrophoretic manipulation of drops for high-speed microfluidic sorting devices. *Appl. Phys. Lett.* **88**, 1–3 (2006).

19. Baret, J. C. *et al.* Fluorescence-activated droplet sorting (FADS): Efficient microfluidic cell sorting based on enzymatic activity. *Lab Chip* **9**, 1850–1858 (2009).

20. Li, M. *et al.* A Gelatin Microdroplet Platform for High-Throughput Sorting of Hyperproducing Single-Cell-Derived Microalgal Clones. *Small* **14**, 1803315 (2018).

21. Love, J. C., Ronan, J. L., Grotenbreg, G. M., Van Der Veen, A. G. & Ploegh, H. L. A microengraving method for rapid selection of single cells producing antigen-specific antibodies. *Nat. Biotechnol.* **24**, 703–

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

707 (2006).

22. Park, S., Han, J., Kim, W., Lee, G. M. & Kim, H. S. Rapid selection of single cells with high antibody production rates by microwell array. *J. Biotechnol.* **156**, 197–202 (2011).

23. Chiou, P. Y., Ohta, A. T. & Wu, M. C. Massively parallel manipulation of single cells and microparticles using optical images. *Nature* **436**, 370–372 (2005).

24. Le, K. *et al.* A novel mammalian cell line development platform utilizing nanofluidics and optoelectro positioning technology. *Biotechnol. Prog.* **34**, 1438–1446 (2018).

25. Novak, R. *et al.* Single-Cell Multiplex Gene Detection and Sequencing with Microfluidically Generated Agarose Emulsions. *Angew. Chemie Int. Ed.* **50**, 390–395 (2011).

26. Hatori, M. N., Kim, S. C. & Abate, A. R. Particle-Templated Emulsification for Microfluidics-Free Digital Biology. *Anal. Chem.* **90**, 9813–9820 (2018).

27. Herzenberg, L. A. *et al. The History and Future of the Fluorescence Activated Cell Sorter and Flow Cytometry: A View from Stanford*. (2002).

28. Ma, S. *et al.* Fabrication of Microgel Particles with Complex Shape via Selective Polymerization of Aqueous Two-Phase Systems. *Small* **8**, 2356–2360 (2012).

29. Liu, Q. *et al.* Self-Orienting Hydrogel Micro-Buckets as Novel Cell Carriers. *Angew. Chemie Int. Ed.* **58**, 547–551 (2019).

30. Collins, D. J., Neild, A., deMello, A., Liu, A. Q. & Ai, Y. The Poisson distribution and beyond: Methods for microfluidic droplet production and single cell encapsulation. *Lab on a Chip* vol. 15 3439–3459 (2015).

31. Basu, A. S. Digital Assays Part II: Digital Protein and Cell Assays. *SLAS Technol.* **22**, 387–405 (2017).

32. Ha, K., de Rutte, J., Di Carlo, D. & Bertozzi, A. Minimal surface configurations for axisymmetric microparticles. *Prep.*

33. Hong, J. K., Lakshmanan, M., Goudar, C. & Lee, D. Y. Towards next generation CHO cell line development and engineering by systems approaches. *Current Opinion in Chemical Engineering* vol. 22 1–10 (2018).

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

34. Eyer, K. *et al.* Single-cell deep phenotyping of IgG-secreting cells for high-resolution immune monitoring. *Nat. Biotechnol.* **35**, 977–982 (2017).

35. Zhong, X. & Rescorla, F. J. Cell surface adhesion molecules and adhesion-initiated signaling: Understanding of anoikis resistance mechanisms and therapeutic opportunities. *Cellular Signalling* vol. 24 393–401 (2012).

36. Fraietta, J. A. *et al.* Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat. Med.* **24**, 563–571 (2018).

37. Rossi, J. *et al.* Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood* **132**, 804–814 (2018).

Methods

Fabrication of drop-carrier particles. Particles were fabricated using a standard PDMS microfluidic flow focusing droplet generator. A PEG phase comprised of 17.5% w/w 4-arm PEG-Norbornene (Sigma), 2% w/w LAP (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate,sigma), and 0.5 mg/ml Biotin-PEG-thiol (5000 MW, Nanocs) in Phosphate Buffered Saline (PBS, pH7.2) was co-injected with a dextran phase comprised of 20% w/w 40 kDa dextran (Sigma), 1.3% w/w DTT (dithiothreitol, Sigma), and 5 mM RGD peptide (Ac-RGDSPGERCG-NH2, Genscript) in PBS at a rate of 8 µL/min and 2.67 µL/min, respectively using syringe pumps (Harvard Apparatus PHD 2000). An oil phase comprised of Novec 7500 (3M) and 0.25% w/w PicoSurf (Sphere Fluidics) was injected at a rate of 42 µL/min to partition the aqueous phases into monodisperse water and oil droplets. PEG and Dextran polymers phase separated on chip after approximately 5 seconds. The PEG phase was crosslinked with focused UV light through a DAPI filter set and 4X objective (Nikon, Eclipse Ti-S) at a power of 50 mW/cm^2 over an approximate duration of 1-3 seconds near the outlet region of the microfluidic device. Crosslinked particles were collected and oil and dextran were removed using a series of washing steps. Briefly, excess oil was removed by pipetting and a layer of PBS was added on top of the remaining emulsions. A solution of 20% v/v perfluorooctanol (PFO, Sigma) in Novec 7500 was then added to destabilize the emulsions and transfer particles to the PBS phase. Excess oil was removed and samples were washed 2X with Novec 7500 to remove remaining

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

surfactant. Novec 7500 was removed by pipetting and residual oil was removed by washing 2-3X with hexane (Sigma). Samples were then washed 3X with PBS to remove dextran from the system. For cell experiments particles were sterilized by incubating in 70% Ethanol overnight. Particles were then washed 5X with a storage solution comprised of PBS + 0.1% Pluronic F-127 (Sigma) +1% penicillin/streptomycin (Invitrogen) and stored in a conical tube at 4°C.

Particle seeding and cell seeding characterization. Stock drop-carrier particles were first concentrated in a conical tube by centrifugation and supernatant was aspirated. Particles were then fluorescently labeled by diluting at a 1:1 ratio with PBS containing 10 µg/ml of Alexa Fluor 568 streptavidin (Fisher Scientific) and incubating for 10 min. Particles were washed 3X with PBS and then dispersed in a well plate at a concentration of 7.5 µL of concentrated particles per cm of well surface area. Particles were then allowed to settle for 10 min. CHO cells pre-stained with Hoechst were then seeded at a range of concentrations (31-190 cells/mm^2) into the well by carefully pipetting evenly across the well area. Cells were allowed to settle for 15 min and then imaged using a fluorescent microscope. Loading statistics was calculated using custom image analysis algorithms in MATLAB. Particles were first identified using the particle fluorescence channel and cell number was calculated by counting nuclei number within each particle cavity.

Dropicle formation and characterization. Drop-carrier particles were suspended in DMEM base media (Invitrogen) and then concentrated by centrifuging at 300 g for 3 minutes and aspirating supernatant. An oil phase comprised of Novec 7500 and 2% w/w PicoSurf was added to the particle suspension at approximately 2X the remaining volume. The sample was then vigorously pipetted for 30 s (~50 pipettes) using a 200 µL micropipette (Eppendorf). The resulting water-in-oil emulsion was then carefully transferred into a PDMS reservoir by pipetting and imaged. Size distribution characterization was then performed using custom image analysis algorithms in MATLAB. For the fluorescent images shown in Figure 2D, particles were first labeled with Alexa Fluor 568 streptavidin and the DMEM was replaced with PBS containing 2 mg/ml fluorescein isothiocyanate-dextran (500k MW, Sigma).

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

CHO K1 and CHO DP-12 cell culture. All cells were cultured in incubators at 37°C and 5% CO_2. CHO K1 cells (ATCC CCL61) were cultured in F12 base media (Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin/streptomycin (Invitrogen). CHO DP-12 cells (ATCC CRL-12445) were maintained according to manufacturer's specifications. Cell culture media was comprised of DMEM (Invitrogen) supplemented with 10% FBS, 1% penicillin/streptomycin, 0.002 mg/ml recombinant human insulin (Sigma), 0.1% Trace Elements A (Fisher Scientific), 0.1% Trace Elements B (Fisher Scientific), and 200 nM Methotrexate (MTX, SIGMA). Suspension adapted CHO DP-12 cells were cultured in TC42 base media (Xell) supplemented with 1% penicillin/streptomycin, 0.002 mg/ml recombinant insulin, and 200 nM MTX.

Cell viability characterization. 30 µL of concentrated drop-carrier particles and 48k CHO DP-12 cells were suspended in media and sequentially seeded into individual wells of a 12-well plate. A separate well plate control sample containing no particles was prepared in parallel. Cells were allowed to adhere for 4 hours. Samples containing both the cells and particles were transferred into a 15 ml conical tube, exchanged with fresh media and then concentrated via centrifugation. 100 µL of Novec + 2% w/w PicoSurf was added to the concentrated sample and pipetted for 30 s to encapsulate the particles and associated cells into dropicles. 150 µL of light mineral oil was added on top of the samples to mitigate evaporation during incubation. Samples were then incubated for 2, 12, and 24 hours in an incubator at 37°C and 5% CO_2. To recover cells back into an aqueous phase excess oil was first removed via pipetting and several ml of media was added on top of the emulsions. To destabilize the droplets 50 µL of 20% v/v PFO in Novec 7500 was then pipetted on top of the emulsion layer and the sample was gently agitated. After 5 min most of the droplets were merged and particles and associated cells transferred into the bulk media phase. Optionally, samples can be centrifuged for 15-30 s at 200g to coalesce remaining droplets. The suspension of particles and cells were then transferred by pipetting into a separate conical tube. Samples were washed with PBS and then sequentially stained with calcein AM and propidium iodide. Particle samples were transferred back into a well plate, imaged, and then analyzed in MATLAB to determine cell viability statistics.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Cell secretion assay. To identify cells during downstream analysis CHO DP-12 cells were first stained with CellTracker™ Blue CMAC Dye (Thermo Fisher). Particles and cells were seeded into a 12-well plate as described above and then incubated at 37°C for 2 hours to allow cells to adhere to the particles. Particles were then recovered by tilting the well plate and transferring by pipetting. In order to remove unattached cells from the background, samples were strained using a 37 µm reversible cell strainer (STEMCELL Technologies) and then particles were recovered by flipping the cell strainer and washing with a washing buffer comprised of PBS (Ca^{2+},Mg^{2+}), 0.5% BSA (GeminiBio), 1% penicillin/streptomycin and 0.05% Pluronic F-127. To reduce sample loss all conical tubes and pipette tips were first precoated with washing buffer prior to handling particle containing solutions. After recovering from the well plate, samples were washed 2X by centrifuging particles and associated cells at 300 rpm for 3 min, aspirating, and then resuspending in washing buffer. Particles were then labeled with streptavidin (Thermo Fisher, 434302) by adding 0.415 ng per µL of concentrated particle solution and incubating for 10 min. Samples were then washed 3X with washing buffer and resuspended. Particles were then labeled with Biotin Anti-FC (Thermo Fisher, A18821) by adding 75 pg of the antibodies per µL of concentrated particle solution and incubating for 10 min. After incubation, samples were washed and resuspended in CHO DP-12 media. After re-concentrating, samples were compartmentalized by pipetting with oil and surfactant as described above to create dropicles. Samples were then incubated for a range of times 0, 1, 2, 4, and 8 hours to allow cells to secrete and to facilitate capture of secreted antibodies onto the associated particle matrix via Anti-FC sites. After the incubation period, particles and associated cells were transferred back into media by breaking the emulsions (see live dead section). Samples were then washed and captured secretions were stained with Goat anti-human IgG H&L (Dylight® 488, Abcam ab96911) at a final concentration of 30 pg per µL of initial concentrated particle solution. After 30 min of staining, samples were then washed 5X with a solution of PBS (Ca^{2+}, Mg^{2+}), 2% FBS, 1% penicillin/streptomycin and 0.05% Pluronic F-127 and optionally stained with propidium iodide. Samples were then imaged in both brightfield and fluorescence channels in a well plate. To characterize secretion amount per particle a custom MATLAB algorithm was used to identify particles in brightfield and then count the number of cells, check for the presence of dead stain, and integrate the total secretion label fluorescence intensity for each particle.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Secretion cross-talk assay. To analyze potential cross-talk in our single cell secretion system, samples were prepared as described in the cell secretion assay section with several modifications to the protocol. Two sets of samples were prepared, a control sample that was incubated in bulk solution and one incubated after dropicle formation. Prior to the incubation step a separate suspension of particles containing no cells and tagged with Alexa Fluor 647 streptavidin were mixed into the samples. This was done in order to ensure signal on empty particles that was measured did not arise from cells that may have detached from the particles during various steps of the assay. Samples were washed and capture sites were then added. The bulk samples were then left to incubate in media while the dropicle samples was emulsified in oil via pipetting. After incubating for 15 hours samples were recovered and washed with washing buffer, stained, and imaged as described in the cell secretion assay section. The amount of cross talk was determined by comparing secretion staining intensity on particles with cells with the intensity on the control particles.

Secretion based cell isolation using FACS. Samples were prepared as described in the cell secretion assay with the following modifications. CHO DP-12 cells and CHO K1 cells were prelabeled with CellTracker™ Deep Red and CellTracker™ Blue (Thermo Fisher). After labelling, cells were mixed together at various ratios (1:5, 1:100, 1:1000) and loaded into particles (Seeding density ~84/mm^2, lambda ~0.1. All remaining secretion assay steps were as previously described. After labelling secretions on samples with fluorescent secondary antibodies, samples were sorted using a FACS machine (BioSorter, Unionbio). Samples were excited using both 488 nm and 561 nm lasers. Events were triggered based on particle absorbance from the 561 nm laser. Single particle events were gated based on time of flight. Particles with secretion signal were then sorted by thresholding the peak intensity height collected through a 543/22 nm filter. Samples were sorted directly into a 96-well plate and imaged with a fluorescence microscope.

Quantification of high producers after sorting. Samples were prepared as described in the cell secretion assay section. After labelling secretions, a fraction of each sample was kept and imaged using fluorescence microscopy.

bioRxiv preprint doi: https://doi.org/10.1101/2020.03.09.984245; this version posted March 11, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Remaining samples were then sorted using a FACS machine (On-Chip Sort, On Chip Biotechnologies). Samples were excited with both a 488 nm and 637 nm laser. Particle events were screened based on the forward and side scatter. Particles positive for both cells and secretion signal were gated based on peak fluorescence height collected through a 676/37 nm emission filter and a 543/22 nm emission filter respectively. Two sub-populations were sorted for each sample: (1) particles with cells and positive secretion signal, (2) particles with cells and the top 20% of positive secretion signal. Collected samples were plated and expanded for >10 days. To quantify antibody production of the isolated sub-populations, 30k cells from the expanded sub-populations as well as un-sorted control samples were plated into a 48-well plate. After cells attached the samples were washed, replaced with 400 μL of fresh media, and incubated for 6 hours. Supernatant was then collected and total human IgG amount was measured using ELISA (IgG (Total) Human ELISA Kit, Invitrogen, BMS2091). Production rate was calculated based off the measured IgG concentration, incubation time, and initial number of cells seeded.

Acknowledgements

We acknowledge support from the National Institutes of Health Grant #R21GM126414 and the Simons Foundation Math+X Investigator Award #510776. Device mold fabrication was completed using equipment provided by the Integrated Systems Nanofabrication Cleanroom at the California NanoSystems Institute at the University of California, Los Angeles. On-Chip sorting experiments were performed in the UCLA Jonsson Comprehensive Cancer Center (JCCC) and Center for AIDS Research Flow Cytometry Core Facility that is supported by National Institutes of Health awards P30 CA016042 and 5P30 AI028697, and by the JCCC, the UCLA AIDS Institute, the David Geffen School of Medicine at UCLA, the UCLA Chancellor's Office, and the UCLA Vice Chancellor's Office of Research.

Author Contributions:

J.D. and D.D. conceived and designed initial dropicle system and protocols. J.D. designed the fabrication workflow for the drop-carrier particles, performed experiments, and analyzed data. J.D. and R.Di. fabricated drop-carrier particles. J.D, R.Di. and M.V. developed cell secretion protocols. J.D., R.Di., M.V., R.Da. and D.D. designed the flow sorting studies. J.D., R.Di and R.Da performed cell sorting experiments. R.Di performed secretion cross-talk experiments. M.V. performed suspension adapted CHO cell experiments. J.D. and D.D. wrote the manuscript with input from all authors. J.D. prepared figures. D.D, supervised the study.