

functions greatly increased both the convenience and usefulness of these devices, and these results confirmed that this approach was feasible.

[0211] For comparison, drops were also occasionally collected directly into a syringe where the piston had been removed to allow gas exchange. In these cases, the monolayer packing of the drops was no longer maintained, even when the syringe was placed almost horizontally to increase the surface area of the fluid. As a result, cell viability was degraded, and after only 3 hours the survival rate was already only 80% as shown in FIG. 8*b*. These results confirmed the importance of the monolayer packing in our microfluidic incubation device for these hybridoma cells.

[0212] A confined cell-culture volume without perfusion leads to a decrease in nutrient levels and an increase in waste levels, compromising cell proliferation and growth. Therefore, the survival rate as a function of drop size was also tested. Drops with volumes of 21 pL and 12 pL showed poor results, as shown in FIG. 8*b*. This is clearly a function of incubation time with the survival rate decreasing dramatically with increasing time as shown in FIG. 8*c*. Drops of approximately 33 pL were used in the microfluidic incubation device, ensuring a good rate of cell survival for at least 6 hours. This inverse relationship between drop size and survival time is consistent with studies using other mammalian cell lines (Jurkat and HEK293T), in which microfluidic systems were used to compartmentalize single cells in larger (660 pL) drops in Fluorinert FC40 fluorocarbon oil stabilized with a PFPE-dimorpholinophosphate surfactant. In these larger drops, the cells survived and proliferated for several days before viability started to decrease.

[0213] In addition to live-dead tests for cell viability, more rigorous experiments were performed to ensure that cell metabolism was not harmed by their encapsulation. This was accomplished by breaking the emulsion, recovering the cells, and recultivating them on microplates. Normal growth was observed; cells split directly from bulk were indistinguishable from those recultivated from the broken emulsion, as shown by the images, taken after 2 days growth, in FIGS. 9*a* and *b*. This set of experiments demonstrated the viability of cells encapsulated in drops and confirmed that new cell lines could, in principle, be established from encapsulated cells.

[0214] It was also ascertained that the production of antibodies was not hindered by the confinement of the hybridomas in the small volume of the drops. To prepare the hybridomas for this test, cells were provided at a density of about 2×10^6 cells/mL, and the cells were grown for 3 days, at which time the density had increased to about 8×10^6 cells/mL. The concentration of antibody in the supernatant was measured with an ELISA, as shown in FIG. 9*c* (grey). The cells were washed with fresh media twice, checking to ensure that the antibody concentration in the supernatant had decreased to a negligible value, as shown in FIG. 9*c* (green). The density was adjusted to 10×10^6 cells/mL, and the cells were encapsulated. A portion of the emulsion was immediately broken to ensure that there was very little antibody production during the encapsulation process, as shown in FIG. 9*c* (orange). The remaining drops were incubated for 6 hours on the incubation device, and the emulsion was broken. The antibody concentration increased significantly as shown in FIG. 9*c* (red). As a control, the measured results were compared with those obtained from cells cultured on a dish for 6 hours at the same initial density (10×10^6 cells/mL). Nearly identical concentrations were measured, as shown in FIG. 9*c* (blue). Assuming a

typical rate of immunoglobulin secretion by hybridomas of 5,000 molecules/s, it was estimated that the antibody concentration in the supernatant was about 10^{15} molecules/mL after 6 hours. All of the ELISA measurements were performed in a regime where the signal was not saturated by performing additional experiments at ten-fold and one-hundred-fold dilutions. The measured relative concentrations decreased proportionately, verifying the consistency of the results, as shown in FIG. 9*d*. This confirmed that the cells were viable and that the metabolism of the encapsulated hybridoma cells was not degraded by their confinement. It also highlighted a unique feature of these drop-based microfluidic devices: the ability to rapidly attain high concentrations of secreted molecules in the confined volumes of the drops.

[0215] After on-chip incubation, further analysis of the cells and the drop contents was performed with the analysis device. This required transferring the emulsion from the incubation device to the analysis device. A syringe pump was connected by external tubing to the inlet of the incubation device and carrier fluid was used to drive the emulsion through additional external tubing, connecting it to the analysis chip. A flow-focusing geometry was used at the inlet of the analysis chip, with the auxiliary oil channels adjusting the spacing between the drops as shown in FIGS. 6*g* and 6*h*. This leads to a uniform flow of drops, which can then be run into other modules fabricated on the analysis device. Potential examples include drop merging, splitting, detecting, and/or sorting, depending on the assay desired. Alternatively, drops can be loaded onto a microfluidic device designed to store ordered arrays of drops, shown schematically in the bottom of FIG. 6*d*. This allows individual drops to be monitored, as shown in FIG. 6*i*, enabling time-resolved single-cell analysis.

[0216] The drop-based microfluidic system presented in this example was a modular, and therefore a highly flexible, system which combined distinct devices to encapsulate, incubate, and manipulate single cells in small drops (≤ 33 pL), enabling the concentrations of secreted molecules to rapidly attain detectable levels. The advantage of the modular concept is its flexibility, allowing adjustment to specific experimental requirements. The components here were placed on physically separate chips which were connected by means of external tubing. Thus components can be exchanged to address the different experimental demands encountered when varying assays. Moreover, dysfunctional chips can be replaced, mitigating problems caused by clogging or leakage.

[0217] It was shown in this example that antibody production, cell survival, and proliferation upon recovery were ensured despite the encapsulation in the confined geometry of the drops. These represent important preconditions for single cell experiments, such as screening for monoclonal antibodies, using drop-based microfluidics. Indeed, the small volume of the drops means that a single hybridoma cell in a drop secreted detectable concentrations of antibodies in only 6 hours, at least in some cases. The modular design of the devices also allowed for adjustment to many other functional single cell assays where statistical information from large populations of individual cells can be collected while each cell is isolated in its own microenvironment. This can thus separate the encapsulation, incubation, analysis, and sorting steps of assays. For example, drops containing other reagents or elements of a library could be merged with the cell-bearing drops prior to incubation or to sorting.

EXAMPLE 3

[0218] This example describes two complementary drop-let-based microfluidic platforms which allowed fully viable