

only 80% and 68% survived for the five- and ten-fold increased cell density, respectively. Not wishing to be bound by any theory, insufficient gas exchange likely did not contribute to this effect since equally dense cultures in ordinary tissue culture flasks did not survive longer: using a density equal to one cell in a 660 μ l drop ($\sim 1.5 \times 10^6$ cells/ml) the number of viable Jurkat cells remained above 87% for the first two days before decreasing to 51% after four days and no surviving cells after 9 days (data not shown). Therefore the encapsulated cells may have died due to the lack of nutrition or the accumulation of toxic metabolites rather than because of compartmentalization-specific factors such as the oil and surfactant.

[0224] In parallel to encapsulating cells into aqueous drops of a water-in-oil emulsion, a system was established in which aqueous plugs spaced by immiscible oil within a piece of tubing served as a culture vessel. This approach allowed the generation of aqueous microcompartments big enough to host small cell populations and even multicellular organisms. This cannot be achieved by simply increasing the drop size of a given emulsion. First, the maximum size of a drop generated on a microfluidic chip is limited by the channel dimensions. Second, as the size of the drops increases they become less stable resulting in uncontrolled sample coalescence. These problems can be circumvented by alternately aspirating aqueous plugs and immiscible oil into a holding cartridge (e.g. a capillary or a piece of tubing). This approach was used to encapsulate several thousand cells into single microcompartments.

[0225] First, holding cartridges made of different materials were assessed for their suitability to host living cells. For this purpose 660 μ l plugs each hosting 3300 Jurkat cells were generated. While gas-permeable PTFE tubing allowed cell survival for several days, the use of glass capillaries and vinyl tubing (all with an inner diameter of 0.5 mm) resulted in cell-death within 24 hours (data not shown). Live/dead stains revealed that when using PTFE tubing, the survival rate of Jurkat cells remained at approximately 90% for the first two days before decreasing gradually from 69% after three days, to 38% after five days and finally 6% after 14 days (FIG. 13A). The total number of recovered cells increased from 69% after 1 hour to 194% after 5 days indicating roughly 1-2 cell divisions (FIG. 13C). When repeating the experiments with adherent HEK293T cells, slightly different results were obtained (FIGS. 13B and 13C). Here, the fraction of viable cells remained above 80% for the first four days before slowly decreasing to 31% after 14 days. The recovery rate increased during the first five days from 83% to approximately 147%. Recultivation experiments demonstrated the recovery of fully viable and normally proliferating HEK293T cells after two days of encapsulation (FIG. 13E).

[0226] To assess the influence of the cell density on cell survival, experiments with 5- and 10-times more Jurkat cells per plug were also performed. Once again an inverse correlation between cell density and survival was obtained. While approximately 69% viable Jurkat cells were recovered after three days when using the initial cell density, only 52% and less than 1% survived when encapsulating five- and ten times more cells per plug, respectively (FIG. 13D). Not wishing to be bound by any theory, this massive decrease in cell survival may be due to the fact that higher cell densities directly resulted in more cells per plug (even at the lowest density all plugs were occupied), whereas when encapsulating single

cells into drops the proportion of occupied drops was increased first (with a single cell in a drop still experiencing the same cell density).

[0227] In addition, an analysis was performed to determine whether the plugs were subjected to evaporation during the incubation period. For this purpose, the mean length of the plugs over time was determined by measuring the size of 30 plugs for each time point using a digital slide gauge and multiplying the mean value by the inner tube diameter to obtain the corresponding plug volumes. No significant decrease in size was observable (FIG. 13F), perhaps due to the fact that the incubation step was performed in a water-saturated atmosphere (at 37° C., 5% CO₂).

[0228] The possibility of encapsulating multicellular organisms was also investigated. Starting with eggs of the nematode *C. elegans*, plugs were analyzed under a microscope at different time points (FIG. 14). After two days, hatched worms had reached the L2-L3 larvae stage. Four days of encapsulation resulted in the growth of adult worms and the birth of the next generation (L1 larvae). Longer encapsulation resulted in plugs hosting up to 20 worms which finally died after 6-9 days. The passing of individual worms into adjacent microcompartments was never observed, even at high flow rates (up to 1000 microliters/h).

[0229] High-throughput cell-based assays require the readout of individual samples after the incubation step (e.g. to screen the phenotype of individual cells within a heterogeneous population). For this purpose, microcompartments stored in a piece of tubing or a reservoir were re-injected into an on-chip readout module after the incubation period. To prove the feasibility of this approach, HEK293T cells were encapsulated within 660 μ l drops. The resulting emulsions were collected, and the samples were incubated for two and fourteen days. Subsequently, the emulsions were re-injected into a chip (same design as for the encapsulation step) and analyzed microscopically. During reinjection of the emulsion after two days of incubation, little coalescence of individual samples was detectable (FIG. 15A). After 14 days of incubation, some degree of coalescence was observable, however the majority of drops (>90%) remained intact. Microscopical comparison of the drops at the time of incubation and reinjection revealed no obvious reduction of the drop size (FIG. 15B). This indicates that the drops were not subjected to significant evaporation during the incubation period (within a water saturated atmosphere).

[0230] To demonstrate that the drops could be analyzed individually after reinjection, a population of HEK293T cells was encapsulated which, two weeks before the experiment, had been incubated in bulk with viral particles (murine leukemia virus pseudotyped with the G-protein of the vesicular stomatitis virus) having packaged the lacZ gene. The fraction of cells stably expressing the corresponding gene product (β -galactosidase) was approximately 13.9% as determined in an X-Gal assay. During the drop production a fluorogenic substrate (1.7 mM fluorescein di- β -D galactopyranoside, FDG) for β -galactosidase was co-encapsulated into the drops and a laser beam (488 nm wavelength) was focused onto the channel (downstream of the nozzle). The emitted light was collected in a photomultiplier (FIG. 15D) to record the fluorescence signal at to. This measurement was performed with the initial population of transduced HEK293T cells and a sample that had been diluted 1:9 with non-transduced HEK293T cells. At the time of encapsulation, no difference in the fluorescence signals was observable, and drops without