

human cells to be recovered with high yield after several days in microcompartments. The volume of each microcompartment can be over 1,000-fold smaller than the smallest volumes utilizable in microtiter-plate based assays, and single, or multiple human cells, as well as multicellular organisms such as *C. elegans*, can be compartmentalized and replicate in these systems. To show the utility of this approach for cell-based assays, automated fluorescence-based analysis of single cells in individual compartments after 16 hours of incubation was also demonstrated.

[0219] The goal of this set of examples was to set up microfluidic platforms for high-throughput cell-based assays. Hence, the technology should allow a) Encapsulation of a pre-defined number of cells per microcompartment (with the option of encapsulating single cells being highly desirable), b) Storage of the compartmentalized samples within a CO₂-incubator, and c) Recovery of the cells from the compartments in a way that does not abolish cell viability.

[0220] The encapsulation step (FIGS. 10A and 10B) was performed on a PDMS chip in which drops of 660 pL volume (corresponding to a spherical diameter of 100 $\mu\text{m} \pm 1.7\%$) were created from a continuous aqueous phase by "flow-focusing" using a perfluorinated carrier oil (Anna et al., 2003). Perfluorocarbon oils are well-suited for this purpose, since they are compatible with PDMS devices, immiscible with water, transparent (allowing optical readout procedures), and have been shown to facilitate respiratory gas-delivery to both prokaryotic and eukaryotic cells in culture. The number of cells per droplet was controlled using on-chip dilution of the cells to regulate the cell density (FIG. 10C). A culture of Jurkat cells, with an initial density of 5×10^6 cells/ml, was brought together with a stream of sterile medium by co-flow immediately before drop formation and the relative flow rates of the cell suspension and the medium were changed, while keeping the sum of the two flow rates constant. The number of cells per drop (k) was in good agreement with a Poisson distribution, and high cell densities at the nozzle ($\geq 2.5 \times 10^6$ cells/ml) made the encapsulation of multiple cells per drop highly likely ($p > 30\%$). In contrast, cell densities of 1.25×10^6 cells/ml and below resulted in low probabilities ($p \leq 7\%$) for the encapsulation of more than one cell per drop (while increasing the probability of finding drops without any cells inside). At the same time, the average number of cells per drop (λ) decreased from approximately two (at 5×10^6 cells/ml) to far below one (at $\leq 1.25 \times 10^6$ cells/ml). Hence, the number of cells per drop can easily be regulated, even allowing the compartmentalization of single cells.

[0221] The generation of stable drops required the use of a surfactant decreasing the surface tension which, for the encapsulation of cells, also has to be biocompatible. For this reason, several perfluoropolyether-derived surfactants (PFPE surfactants) were synthesized, and their effect on long-term cell survival (FIG. 11) was tested. The surfactants differed solely in their hydrophilic head groups, which should be the only part of the molecule in contact with the encapsulated cells. The common perfluorinated tail should be dissolved in the carrier oil and thus be oriented away from the cells. To analyze the biocompatibility, HEK293T cells were seeded on top of a perfluorocarbon oil layer in the presence (0.5% w/w) and absence of different surfactants. While in the absence of any surfactant the cells retained an intact morphology and even proliferated, the ammonium salt of carboxy-PFPE (Johnston et al., 1996) and poly-L-lysine-PFPE (PLL-PFPE) mediated cell lysis. However, polyethyleneglycol-PFPE

(PEG-PFPE) and dimorpholinophosphate-PFPE (DMP-PFPE) showed good biocompatibility, did not affect the integrity of the cellular membrane, and allowed cell proliferation. Since DMP-PFPE generated more stable emulsions than PEG-PFPE (data not shown), it was used for all further experiments.

[0222] As the next step, procedures allowing the recovery of encapsulated cells had to be established. Addition of 15% (v/v) Emulsion Destabilizer A104 (RainDance Technologies) to the emulsions mediated reliable breaking without obvious impact on cell viability. This allowed the determination of the survival rates of suspension (Jurkat) and adherent cells (HEK293T) for different incubation times within drops. For this purpose, cells were encapsulated at a density corresponding to an average of less than one cell per 660 pL drop (1.25×10^6 cells/ml at the nozzle resulting in a λ value of about 0.55 and single cells in approximately 31.7% of all drops) and collected the resulting emulsions in 15 ml centrifugation tubes. After different incubation times at 37 degrees C. within a CO₂ incubator, the emulsions were broken and the cells were treated with a live/dead stain to determine the survival rate and the total number (live and dead) of recovered cells (FIGS. 12A and 12C). During the first four days, the fraction of recovered viable Jurkat cells did not change significantly and was always in excess of 79%. Then the percentage of live cells decreased from 71% after 5 days, to 32% after six days, and finally to 1% after 14 days of encapsulation. The total number of recovered cells divided by the number of initially encapsulated cells (equal to the aqueous flow rate multiplied by the injection time multiplied by the cell density at the nozzle) was defined as the recovery rate and increased from 29% after one hour to more than 55% after two days. This indicates some degree of proliferation within the drops, also supported by the fact that after 24 hours the percentage of dead cells was lower than after 1 hour. During further incubation within drops the recovery rates slowly decreased to just 14% after 14 days. This decrease can be explained by the fact that dead cells ultimately disintegrate (after several days) and thus cannot be stained anymore. This effect is well known and has been analyzed in detail for bacterial cells. However, early time-points and the live stain are not affected by this phenomenon. When repeating the experiments with adherent HEK293T cells, similar results were obtained (FIGS. 12B and 12C). During the first two days, the fraction of recovered viable cells remained constant at more than 90% before slowly decreasing to 58% after five days and 39% after nine days. Finally, after 14 days of encapsulation, 28% of the recovered cells were still alive. The total recovery rate increased slightly from 20% after 1 hour to more than 32% after two days. During further incubation within drops the recovery rates slowly decreased to 23% after 14 days. Not wishing to be bound by any theory, the longer cell survival compared to Jurkat cells may be due to slower proliferation resulting in slower consumption of the available nutrition. Recovered cells could also be recultivated (instead of stained) after incubation for two days within droplets, resulting in normally proliferating cells (FIG. 12E).

[0223] In a further experiment, the effect of the cell density on survival rates was assessed. For this purpose five- and ten-fold higher densities of Jurkat cells were used compared to the amounts used initially. Comparison of the cell survival after three days showed that the cell density was inversely correlated with the survival rate (FIG. 12D). While almost 90% viable cells were recovered using the initial cell density,