

reference to Example 1, were explored. In addition, control microfluidic devices, one including the herringbone pattern and the other including the flat surfaces, were tested. In addition, the control microfluidic devices were also tested by functionalizing with an irrelevant capture antibody not configured to capture the cancer cells. For both control microfluidic devices, zero cell capture was observed. Similar to the previous results associated with cancer cells in the buffer solution, the capture efficiency with the microfluidic device **400** having the herringbone pattern was better than the microfluidic device **200** having the flat surfaces, for all conditions tested.

Further, a cell line of prostate cancer cells (PC3) was tested due to the reduced EpCAM express. Cancer cells have less EpCAM than regular epithelial cells. The new cell line and their expression level is approximately 40,000 EpCAM molecules/cell. The number of cells spiked into blood were 1,000 cells/ml so that the spiking numbers are more relevant to rare cell detection levels. For the new cell line, experiments were conducted at flow rates of 0.12 ml/hr and 0.24 ml/hr. For the PC3, the EpCAM surface express was decreased by an order of magnitude relative to the cancer cells and the spiking concentration was reduced by a factor of five. Nevertheless, capture efficiencies comparable to the H1650s are observed using the microfluidic device **400**.

EXAMPLE 3

Cell Viability

The effect of flow patterns and subsequent higher shear stress on viability of the captured cells was also studied using traditional Live/Dead assays. Cancer cells spiked into whole blood were captured in the micro-channel **415** of a high throughput microfluidic device **700**, as shown in FIG. 7, having columns of herringbone patterns. The microfluidic device **400** represents a small footprint version that can be used for initial validation studies. The microfluidic device **700** is an example of a scaled-up version of the microfluidic device **400**. To scale up the device, the design of the microfluidic device **400** was repeated and elongated. In some embodiments, the microfluidic device **700** is 2 cm wide and 4 cm long and includes a header region and a footer region. In this example, multiple herringbone patterns were formed by foaming columns of herringbone patterns adjacent to each other in an upper wall of a micro-channel having a larger width than the micro-channel **415**. The volumetric flow rate through the micro-channel **715** is 2 ml/hr. captured cells were stained on the substrate to which the cells were bound with CalceinAM and Ethidium Homodimer. Results indicated that the most (~90%) of the captured cells were viable, demonstrating that the herringbone pattern had limited negative effects on the captured cells under these conditions.

In some embodiments, the cells can be separated from the substrate and cultured separately. To separate the cells from the adherents, the linkage can between the adherents and the cells can be weakened, for example, by dissolving the adherents in a solution that does not affect the cells.

The number of columns of herringbone patterns was limited only by the width of the micro-channel. In some implementations, the microfluidic device **700** includes eight mini-chambers, i.e., eight columns of herringbone patterns. In such implementations, a header design can be incorporated at an inlet of the micro-channel to provide stability and uniform fluid volumes to each column of herringbone patterns. In some implementations, each column of herringbone patterns is positioned next to an adjacent column of patterns such that,

an apex of a “V” shaped groove in the column is aligned with an apex of the “V” shaped groove in the adjacent column. In other words, the apexes of both grooves lie on a line perpendicular to a principal axis passing through the micro-channel of the microfluidic device **700**. If all grooves in a column are equidistantly formed in the micro-channel of the device **700**, then all grooves in the device **700** will be aligned with each other. In some implementations, a column of herringbone patterns can be offset from an adjacent column. For example, the apex of a “V”-shaped groove in the column can be offset by 10 μm from the apex of a “V”-shaped groove in the adjacent column. The offset column design can further promote mixing. In some implementations, the multiple columns in the device **700** can include symmetric grooves **335** and asymmetric grooves **340** randomly interspersed in each column. The forming of interspersed grooves promotes transverse movement of the fluid and the particles suspended in them, thereby increasing the number of cell-micro-channel wall interactions and consequently increasing cell capture.

EXAMPLE 4

Cell Culturing

In another example, a microfluidic device **800** for culturing captured cells as shown in FIG. 8 was used to capture and culture cells. The microfluidic device **800**, was similar to the high throughput design described with reference to FIG. 7, and included a lower substrate manufactured using glass and an upper substrate **810** manufactured using PDMS that included the columns of herringbone patterns as described previously. The flow rates of blood containing the cells to be captured were around 2 ml/hr and were manipulated to cause the cells to contact adhere to the lower glass substrate **810**. In the microfluidic device **800**, the both the lower and the upper substrates were coated with adherent. The lower and upper substrates were reversibly bonded to each other using such that, subsequent to cell capture, the upper substrate **810** could be removed from the lower substrate **805**, for example, by applying mechanical forces. In other embodiments, the lower and upper substrates can be mechanically clamped to form a water-tight seal or by suitable methods that do not damage the bound cells. In some embodiments, the adherent **460** can be disposed on either the lower or the upper substrate. Cells can be captured on the substrate on which the adherent is disposed.

FIGS. 9A-9C are micrographs showing the growth of captured cells on a glass substrate. Following capture of the cancer cells spiked into blood, the upper substrate **810** of the microfluidic device **800** was removed and both upper and lower substrates were placed into a petri dish and incubated at 37° C., 5% CO₂ with the appropriate cell culture media (FIG. 9A). As shown in FIG. 9B, the cells were adhering to the substrate and had started to spread and increase in number within 24 hours. After more than three weeks of cell culture, the cells continued to divide, forming a monolayer on both the lower substrate (glass) and the upper substrate (PDMS). At this point, the cells were removed from the capture surfaces (via trypsinization) and cultured in traditional cell culture flasks. In this manner, successful culture of captured cancer cells for extended periods of time was demonstrated. Thus, the cells were not only viable but also functional and can be grown in culture.

EXAMPLE 5

Phenotype Changes

FIG. 10 shows fluorescence-activated cell sorting (“FACS”) analysis of EpCAM expression on the cells cap-