Microfluidic Device for Extraction of DNA from Selected Cells

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Abstract:

Polydimethylsiloxane (PDMS) microfluidic devices for extraction and purification of genomic deoxyribonucleic acid (DNA) from small cell populations and single cells were developed. Hematopoietic stem cells trapped in a two-dimensional array of micropillars by size exclusion were chemically lysed releasing long strands of genomic DNA which were then immobilized within the microarray by hydrodynamic forces while cellular debris was washed away. The purified DNA was subsequently released from the microarray by enzymatic fragmentation under continuous fluidic flow conditions and collected for off-chip analysis by gel electrophoresis and fluorospectrometry. For a population of less than 100 cells, we have obtained a genomic DNA extraction efficiency of > 95%.

Summary of Research:

Various DNA extraction techniques have recently been implemented in microfluidic systems that provide better handling and manipulation of small sample and reagent volumes in engineered microstructures [1]. Microfluidic devices could perform the analysis automatically in an enclosed system thereby reducing the possibility of human error and cross contamination. These devices may also reduce the time and the cost of analysis by taking advantage of high reaction rates at the microscale and generally provide higher extraction efficiencies by utilizing features with high surface-to-volume ratios for improved DNA extraction, however they generally rely on DNA adsorption to silica or other biochemically functionalized surfaces.

The binding affinity is extremely sensitive to temperature, pH, and buffer composition which requires careful optimization to minimize DNA losses. Even after meticulous optimization, it is difficult to ensure that all the DNA fragments get adsorbed and the whole genome is represented in purified extracts obtained from a few cells and/or a single cell. Fundamentally different approaches to genomic DNA capture need to be explored to improve the extraction efficiency.

Here we explore a novel technique to extract and purify DNA by physically trapping long strands of genomic DNA in arrays of micropillars by hydrodynamic flow.

Figure 1: Photomicrographs of the fabricated PDMS microfluidic devices (Bottom) and of the array of micropillars for cell capture and DNA immobilization (Top).
MO-91 cells (hematopoietic stem cells infected with myeloid leukemia) were injected into the input of the microchannel and drawn under constant flow into an array of micropillars shown in Figure 1 in which they become trapped by size exclusion. The array with progressively decreasing spacing between microposts prevents channel clogging when larger numbers of cells and other debris are present in the growth medium.

The captured cells were lysed with a solution containing 1% sodium dodecyl sulfate (SDS) in Tris-EDTA buffer. Long strands of genomic DNA released from the cells become immobilized in the array of microposts by hydrodynamic flow. Additionally, the pressure driven flow is sufficiently slow as to prevent any shearing of the DNA.

After lysis, the genomic DNA is then rinsed and purified by flowing proteinase K and ribonucleases through the microchannels to remove histone proteins and RNA that may have become intertwined within the DNA. Thorough removal of cellular debris is important for the subsequent single-molecule studies as the labeled protein and lipid fragments can interfere with the analysis.

DNA capture by hydrodynamic forces allows separation of genomic DNA not only from proteins and lipids which are primary components of the cell lysate, but also from other nucleic acids such as mitochondrial DNA and RNA. This cannot be achieved with the alternative extraction methods. DNA strands extracted from four immobilized cells and from a single cell stained with PicoGreen fluorescent dye are shown in Figure 2. Purified DNA is released from the device by enzymatic digestion with restriction endonucleases (Bam HI and Hind III). The digestion process is captured in Figure 3.

All purified DNA is released from the microarray into the collection reservoir within two minutes. The digested DNA was collected into small elution volumes (~ 20 µL) for off-chip analysis with gel electrophoresis and fluorospectrometry (Nanodrop 3300). The cells captured in the array of micropillars were counted and the amount of the DNA was determined by assuming a quantity of 6.6 pg of DNA per cell. These fluorospectrometric measurements indicate that we are collecting and purifying and collecting > 95% of the genomic DNA released from the cells.

References: