EXTRACTION AND PURIFICATION OF GENOMIC DNA VIA ENTRAPMENT IN AN ARRAY OF MICROPOSTS

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ABSTRACT

A novel microfluidic device was developed to extract and purify genomic DNA from a small number (from a single to hundreds) of cells using an array of microposts. Following chemical-induced lysis, separation occurs when large genomic DNA strands became physically entrapped within the post array while other smaller cellular components were flushed out of the device. The purified DNA was extracted by a continuous flow enzymatic digestion process which fragmented the trapped DNA into smaller sizes. Using this strategy, we have obtained high DNA extraction efficiencies (~ 85 to 90%) for low elution volumes ($\leq$ 20 μL).

KEYWORDS: DNA extraction, DNA entrapment, micropost array

INTRODUCTION

The current field of DNA purification in microfluidic devices is primarily based on solid-phase extraction techniques which employ various silica-based surfaces such as beads, particles, and pillars. However, because the binding affinities of the various cellular components are extremely sensitive to the process conditions (e.g., temperature, pH, and buffer composition) this technique suffers from significant shortcomings in terms of low extraction efficiencies and contamination from co-elution of other cellular species [1]. Previous studies have used non-functionalized post-arrays to study the dynamics of DNA molecules in both electrophoretically [2, 3] and hydrodynamically driven flow fields [4]. The primary motivation for these studies was to improve the design of lab-on-a-chip devices and thus they used pre-purified (typically bacteriophage-λ) DNA as model biological macromolecules.

Here we describe a novel technique to extract and purify DNA directly from mammalian cells by physically trapping genomic DNA fragments in an array of non-functionalized microposts. This approach is versatile for a range of input samples (i.e., from a single to hundreds of cells) and has been shown to achieve very high DNA extraction efficiencies (~85 to 90%) while requiring only very low elution volumes ($\leq$ 20 μL).

EXPERIMENTAL

The microfluidic device (see Figure 1A for details) was fabricated with polydimethylsiloxane (PDMS) via standard soft lithography and mould-replica techniques and then plasma bonded onto a fused silica wafer. An array of microposts (5 μm wide, 20 μm tall) was designed with a spacing gradient to create a depth barrier for cell capture with the closest spacing between the microposts being 1.5 μm. The progressively decreasing spacing between microposts prevents channel clogging when larger numbers of cells and other debris were present in the cell media. The total internal volume of the microfluidic device was approximately 50 nL. The wafer was mounted onto the stage of an inverted fluorescence microscope with a CCD camera. A syringe pump was used to load the desired number of hematopoietic stem (HS) cells into the device and to introduce the different solutions that were used for the subsequent cell lysis, purification, and DNA extraction steps. The content and quality of the extracted DNA were determined from fluorospectrometry measurements and gel electrophoresis analysis respectively. Additional details are provided below.

RESULTS AND DISCUSSION

Figure 1A displays the brightfield microscopy image following the loading of seventy HS cells into the microfluidic device at a flow rate of 50 nL/min. Higher flow rates (~500 nL/min) caused the cells to rupture upon contact with the PDMS posts. The array of microposts promotes a uniform distribution of cells within the capture region, an important factor for the subsequent processing steps. The cells were chemically-lysed following the introduction of a 1% SDS solution into the microfluidic device. The very large genomic DNA strands became physically entrapped within the post array while the other smaller cellular components (e.g., proteins, RNA, cellular debris) were flushed out of the device resulting in a purified DNA product. Hydrodynamically induced shearing of the DNA fragments was minimized by operating at low flow rates (~50 nL/min). We propose that the trapping phenomenon is primarily due to inter-strand interactions between the 'micropost-hooked' DNA fragments [3]. The trapped genomic DNA fragments were fluorescently-stained using the nucleic acid intercalating dye PicoGreen and then visualized by fluorescence microscopy (see Figure 1B). The background fluorescence of the device was dramatically reduced by removing the unbound dye.

The trapped DNA was released by introducing the restriction enzyme BamHI at a flow rate of 50 nL/min and heating the device to 37 ^{o}C to ensure optimal enzyme activity. The fragmentation reaction occurred rapidly with the majority of the DNA released from the microfluidic device within two minutes (see Figure 2).

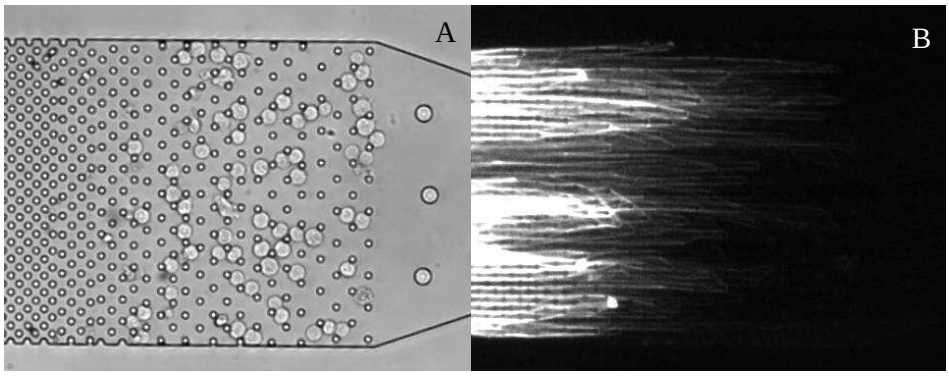

Figure 1: Panel A - Brightfield micrograph of seventy HS cells captured in array of microposts. Panel B - Fluorescent micrograph of PicoGreen stained genomic DNA obtained via chemical lysis of HS cells in panel A.

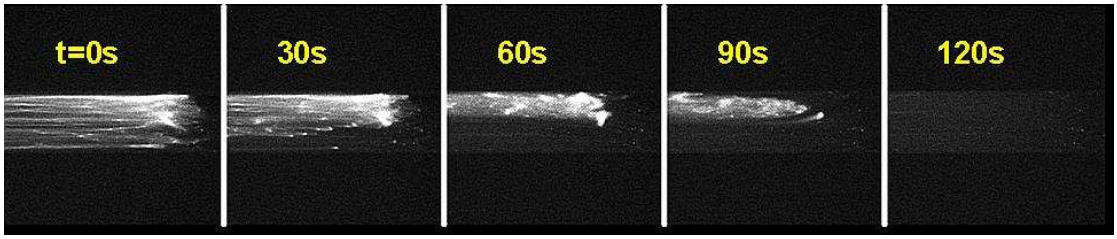

Figure 2: Time series images of genomic DNA undergoing enzymatic fragmentation at 37 ^{o}C using BamHI. The direction of flow within the device was from right to left.

Although it is possible to use elution volumes as low as 3 to 5 μL, the fragmented DNA was collected in 20 μL volumes to facilitate the subsequent off-chip analyses. Figure 3 compares the amount of DNA extracted from six devices that were loaded with a different number of HS cells. Each extraction sample was diluted with an equal amount of PicoGreen and the fluorescence intensity measured using a NanoDrop fluorospectrometer. This instrument only uses a very small amount of sample per measurement (1 – 2 μL) and thus multiple measurements could be taken for each sample. The fluorescence intensity signal was directly proportional to the number of cells (results not shown). From a calibration curve prepared with bacteriophage λ DNA standards, the total mass of extracted DNA (shown as symbols in Figure 3) was calculated from the fluorescent intensity signal. For comparison, the solid line in Figure 3 shows the expected amount assuming genomic DNA content of 6.6 pg per cell. The extraction efficiency is calculated by comparing the ratio of the two; for example the extraction efficiency from 156 HS cells was 84 ± 11 %.

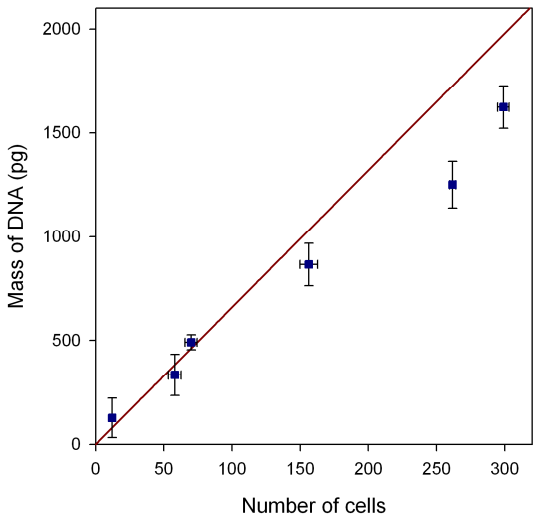

Figure 3: Amount of DNA collected from different number of HS cells in microfluidic device. The solid line corresponds to the expected genomic DNA content of 6.6 pg per cell.

Figure 4 displays the image of a SYBR Gold stained agarose gel used to assess the quality of the extracted DNA. The largest and smallest fragments in the linear DNA ladder (loaded in Lane 1) were 48 and 0.5 kilobase pairs (kbp) respectively. An additional size reference was loaded into Lane 3 corresponding to 2 ng of 165 kbp T4 DNA. The sample in Lane 5 was collected from the lysis and extraction of DNA from approximately five-hundred HS cells trapped in the microfluidic device. As shown by the bright band near the top of the gel, the majority of the DNA is extracted as very large fragments (~50 to 200 kbp) and thus should be amenable to further analysis/processing steps. Note that the faint 'smear' below this band indicates that a distribution of smaller fragments is also present in the sample.

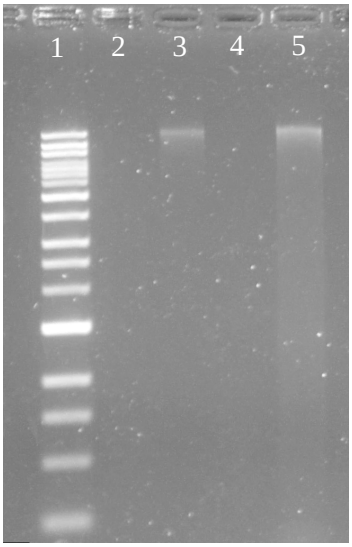

Figure 4: Agarose gel electrophoresis image of linear DNA ladder (lane 1), T4 DNA (lane 3), and DNA extracted from approximately five hundred HS cells (lane 5). Lanes 2 and 4 were intentionally left empty.

CONCLUSION

The entrapment and chemical-induced lysis of mammalian cells in an array of microposts was shown to be an effective technique for collecting and purifying genomic DNA from selected cells. A continuous-flow enzymatic digestion process was effective at releasing the purified DNA fragments. This strategy was shown to be effective for a range of input samples while achieving high DNA extraction efficiencies (~85 to 90%) for very low elution volumes ($\leq$ 20 μL). Although not shown here, this strategy has proven effective for a variety of cell types and sources. We propose that this strategy would be amenable for assessing the epigenetic 'states' of very small populations of cells and even a single cell by using methylation sensitive restriction enzymes for DNA methylation analysis.

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REFERENCES

[1] J. Wen, L.A. Legendre, J.M Bienvenue, and J.P. Landers, "Purification of nucleic acids in microfluidic devices" *Analytical Chemistry*, 80 (2008) 6472-6479.

[2] W.D. Volkmuth, T. Duke, M.C. Wu, and R.H. Austin, "DNA electrodiffusion in a 2D array of posts" *Physical Review Letters*, 72 (1994) 2117-2120.

[3] W.D. Volkmuth, T. Duke, R.H. Austin, and E.C. Cox, "Trapping of branched DNA in microfabricated structures" *Proceedings of the National Academy of Sciences of the USA*, 92 (1995) 6887-6891.

[4] N.P. Teclemariam, V.A. Beck, E.S.G. Shaqfeh, and S.J. Muller, "Dynamics of DNA polymers in post arrays: comparison of single molecule experiments and simulations" *Macromolecules*, 40 (2007) 3848-3859.

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