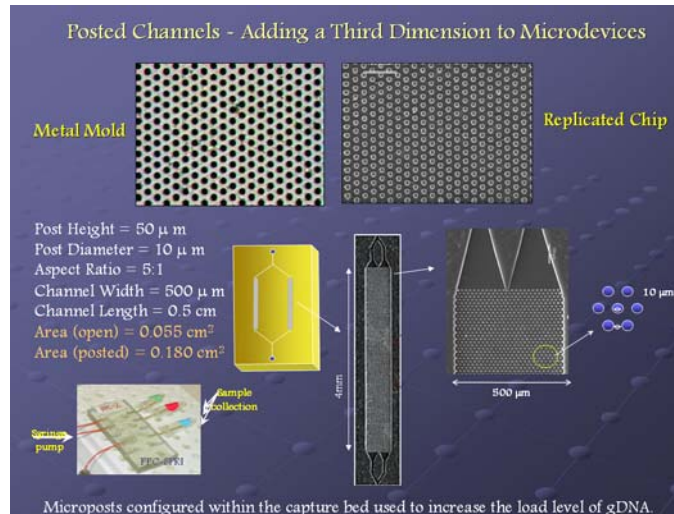


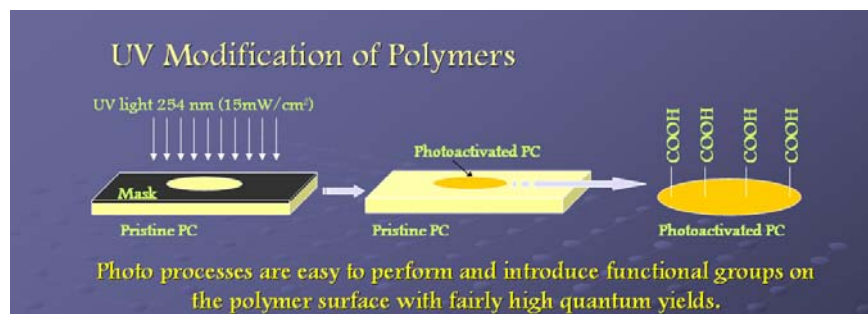
On-Chip Purification of Genomic DNA from Whole Cell Lysates Using Photoactivated Polycarbonate (PPC) Microfluidic Devices

We have used of a photoactivated polycarbonate (PPC) microfluidic device for the solid-phase, surface-immobilization and purification of genomic DNA (gDNA) from whole cell lysates. The

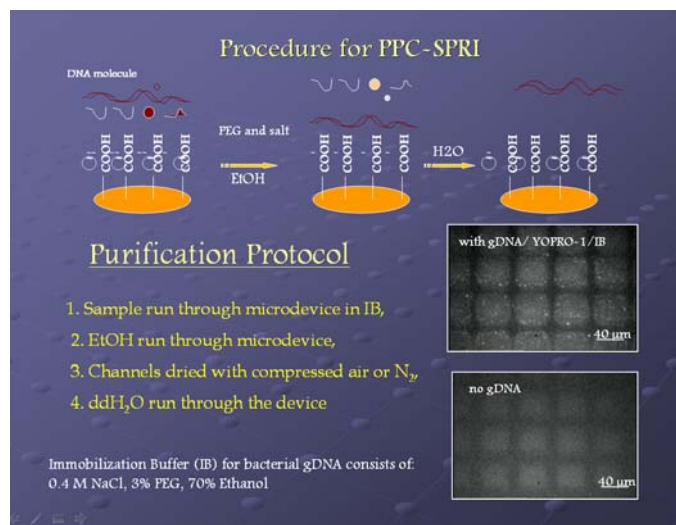


immobilization bed for gDNA consisted of an enclosed microchannel with 10 μm diameter, high aspect ratio microposts, which were fabricated during embossing of the microfluidic channels, and used to increase the available surface area to increase the load of gDNA. The posted-design delivered a 2.6 fold increase in the potential surface immobilization area compared to an open channel of the same physical dimensions.

The surface of the pristine polycarbonate device was activated by ultraviolet (UV) radiation. A photooxidation reaction caused by 254 nm (15 mW/cm²) light resulted in the production of functional scaffolds on the channel surface, consisting primarily of carboxylate groups.



The gDNA was selectively captured on this photoactivated surface in an immobilization buffer (IB), which contained 3% polyethylene glycol, 0.4 M NaCl, and 70% ethanol. The process of immobilization and purification consisted of three steps: (i) suspension of crude cell lysates in the IB

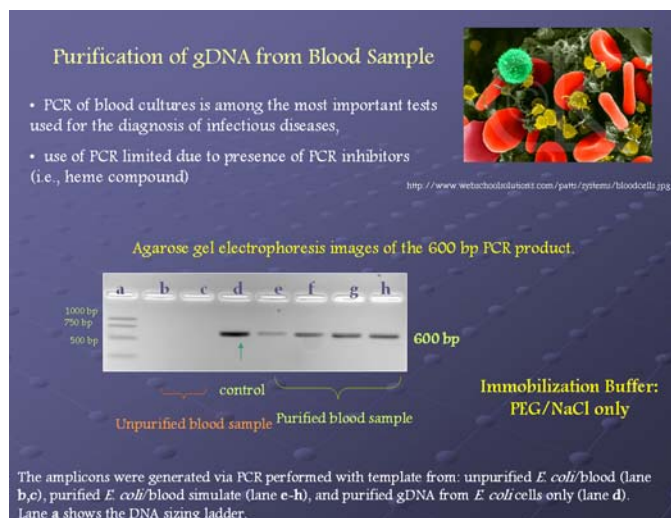


and insertion into the PPC device causing surface-immobilization of the gDNA, (ii) washing with 85% ethanol, and (iii) release from the surface with DI water. The methodology reported herein is similar to solid-phase reversible immobilization (SPRI) in that surface-confined carboxylate groups are used for the selective immobilization of DNA, however, no magnetic beads are required nor a magnetic field. The presence of the purified genomic material in the capture bed was confirmed by the

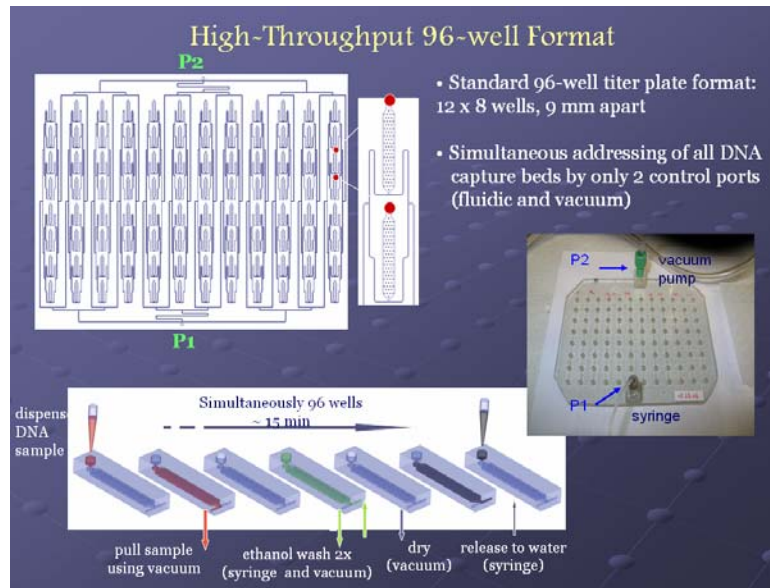
polymerase chain reaction (PCR) with complementary primers to amplify a recognition sequence within the gDNA from the lysed cells. As observed by UV spectroscopy, a load of approximately $7.6 \pm 1.6 \mu\text{g/mL}$ of gDNA was immobilized onto the PPC bed.

Results demonstrated the ability of the method to remove cellular proteins and debris from the cell lysate. The absorbance ratio of I_{260}/I_{280} obtained after purification of whole cell lysates was found to be between 1.7-1.9, indicating that the method produced negligible protein contamination. The immobilization and purification assay using this PPC device was performed within 25 min: (i) DNA immobilization ~6 min, (ii) device washout with ethanol 10 min, and (iii) drying and gDNA desorption ~6 min. In addition, the PPC device could be used for subsequent assays with no substantial loss in recovery and no need for "reactivation" of the PC surface with UV light.

A purification and pre-concentration of DNA is extremely important step in many DNA-based bioassays. We have also developed fast, simple, and effective way for the direct purification of the gDNA from cell lysates and whole blood samples using SPRI in photoactivated polycarbonate (PPC) microfluidic chip. In order to achieve high-throughput purification of DNA samples, 96 capture beds were fabricated into the surface of single polycarbonate sheet using hot-embossing of metal masters fabricated either via high-precision micromilling or LiGA. The capture beds were arranged according to the standardized format of 96-well titer plate. The performance of the device was validated by capture of DNA fragments from 4 different samples, performing the PCR on eluted different size DNA template,



and confirming the presence of PCR products via gel electrophoresis. The 96-well PPC-SPRI chip was



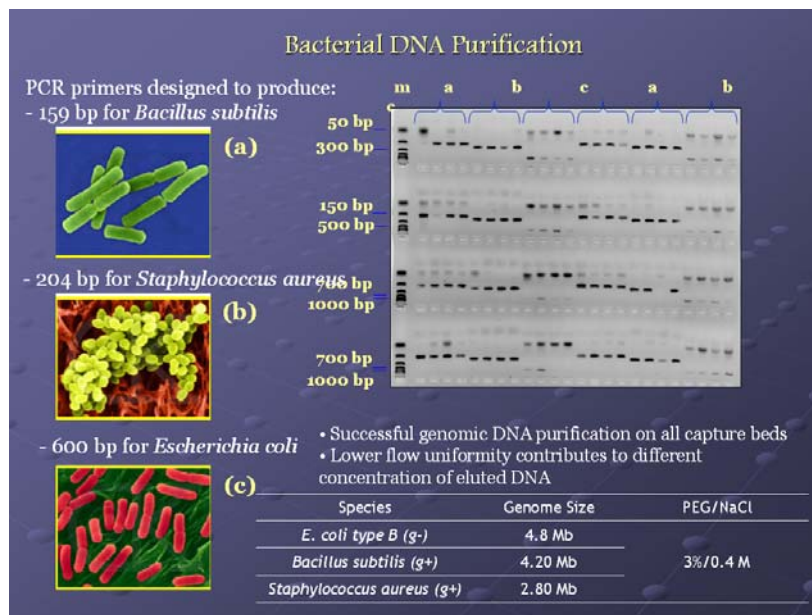
also successfully used for the purification of gDNA from whole bacteria cell lysates, (e.g.: *S. aureus*, *B. subtilis*, *M. luteus*, *E. aerogenes*, and *E. coli*)

The incorporation of a solid phase based purification platform for nucleic acids (NAs) into a microfluidic chip is very appealing because it offers the ability to handle and preconcentrate nucleic acids in a high throughput, automated format and in a closed architecture to

minimize contamination effects. In fact, most microfluidic chips require preconcentration of target species because of the ability of these devices to process only ultra-small volumes of sample (e.g., 10-1,000 pL), making it difficult to detect low bioanalyte concentrations without a preconcentration step. Microfluidic chips are especially attractive for cellular-based assays because many of the sample processing steps can be integrated into a single module, minimizing the dilution of intracellular components following cell lysis. Also, sample handling in microfluidics can be gentle minimizing DNA shearing artifacts, which is important if high molecular weight DNA fragments, such as gDNA, are to be interrogated.

The SPRI-variant used was configured in a microfluidic channel fabricated in PC that was

subsequently exposed to UV radiation, inducing a photo-oxidation reaction creating surface-confined carboxylates, which produce an affinity bed for isolating nucleic acids using a very simple, inexpensive and robust method. The advantages of the PPC-SPRI can be easily recognized. The simplicity of its fabrication is a major advantage. The immobilization bed is fabricated during embossing of the



microfluidic network and activation of the PC surface requires *only* exposure to broadband UV radiation. In addition, with proper selection of components contained within the IB, the selective isolation of intermediate sized nucleic acids can also be envisioned, such as PCR products, mtDNA, clones and RNAs (3-6), making the approach flexible enough to be used in a variety of different applications where the isolation and preconcentration of nucleic acids is necessary prior to downstream processing. Another advantage of PPC-SPRI stems from the fact that these are simple flow-through type reactors. They can be easily implemented into the design of more complex systems performing multi-step bioassays. The fabrication of PPC-SPRI reactors opens the direct route toward low-cost, disposable DNA sample purification units. For example, the cost of each device in material only is a few cents and can be mass produced using injection molding. To further lower the “per purification” cost, SPRI reactors can be fabricated into multi-reactor arrays for parallel preparation of multiple samples. Our group is currently developing a 96-well SPRI card that can be used in conjunction with standard 96-well microtiter plates for high-throughput applications.

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