366g Two Dimensional Micro Gel Electrophoresis Device with Integrated Removeable Capillary Insert (Rci) for Macro-Micro Interface and Post Separation Sample Manipulation

Swomitra Mohanty, Javier Atencia, Jack Gorski, and David J. Beebe

Previous research conducting high speed electrophoresis separations of biological material on the microscale is well established [1-3]. Conducting such analysis on the micro scale has the advantage of high speed, parallel high throughput analysis with reduced reagent volumes. Many of these devices have focused on the separation and detection of biological material on chip, however post separation sample handling off chip is not as well discussed. In cases where separation of an unknown DNA sample is conducted, a specific band of interest is present which needs to be extracted for further analysis (for example southern blot, PCR, DNA sequencing). In conventional gel electrophoresis, protein or DNA bands can be simply cut out of the gel mechanically and removed from the gel using digestion or electric fields. In the microscale alternative techniques must be employed. One group has looked at selective extraction DNA samples using an electrophoresis scheme involving two intersecting orthogonal gel filled channels [4]. In this case, once the band of interest is within range of the intersection, a field is applied and the band is moved off into the orthogonal channel where it is collected in a reservoir. However, given the small volumes used in microfluidic systems, interfacing with the macro world can be difficult and the details of simple off chip sample manipulations are rarely discussed. This paper presents a method of integrating a removable capillary insert as part of the μFluidic Tectonics (μFT) platform [5] for easy manipulation at these small volumes. This paper also demonstrates the first electrophoretic separation in a μFT fabricated device. The sample manipulation scheme presented here makes products analyzed using microchips simple to interface with the macro world as well as the micro world. A methodology for sample collection and manipulation after electrophoretic separation of DNA or proteins is reported. The device integrates a 2D micro gel electrophoresis device with a removable capillary insert (RCI). A micro scale device separates DNA based on size and nucleic acid sequence. Separation in one direction is based on the size of denatured DNA, while separation in the other direction is based on renatured secondary structure. The denaturing and renaturing environments are achieved by localized cooling and heating on chip. One channel is heated locally using a resistive heat pad to create a denaturing environment. The second channel is cooled locally using a chemical cooler to create a renaturing environment for the second separation to occur. The RCI serves as part of the separation path during the separation process but is a removable component. This allows use of the electric field to not only perform separations, but to also move biological material into the insert. After migrating into the RCI, the biological material is then removed by the user to be used in post separation analysis. This was done two ways using an RCI with and without an electrode. The electrode in the capillary serves as the second electrode for separation and can also be used to subsequently remove biological material from the RCI via electrokinetic methods by interfacing it with a second compatible microfluidic chip with the necessary electrodes. This is useful for transferring material to a small volume analysis region off chip (or to another chip) where high concentrations are needed. Biological material moved into the RCI without an electrode is simply removed with the bulk solution in the capillary insert. This can be delivered to any compatible microfluidic device or macro scale container such as an eppendorf tube for traditional analysis. Demonstration of this was done on a micro 2D gel electrophoresis device using a BlueRanger pre-dyed colorimetric protein marker visible under normal light. Initial separation was done in one dimension followed by choosing a band of interest and moving it in a second dimension for sample collection. Figure 1 shows a schematic of two micro electrophoresis devices with different types of RCI components. Figure 2 shows images of the micro device before and after the addition of the RCI components. Figure 3 shows the protein band reaching the orthogonal intersection where it is then sent to the RCI for collection. References: 1. Woolley AT, Mathies RA, Anal Chem 1995, 67, 3676-3680 2. Effenhauser CS, Manz A, Widmer HM, Anal Chem, 1993, 65, 2637-2642 3. Gottschlich N, Jacobsen SC, Culbertson CT, Ramsey JM, , Anal Chem 2001, 73, 2669-2674 4. Lin L, Burke DT, Burns MA, Journal of Chromatography A, 1010 (2003) 255-268 5. Beebe DJ,

Moore J, Liu Q, Liu RH, Kraft ML, Jo B-H and Devadoss C, Proc of the Natl Acad of Sci, 2000. 97:25, 13488-13493. 6. Kovar HG, Jug H.A, Skern T, and Blass D, Nucleic Acids Res., 1992, 19:13, 3507-3510.

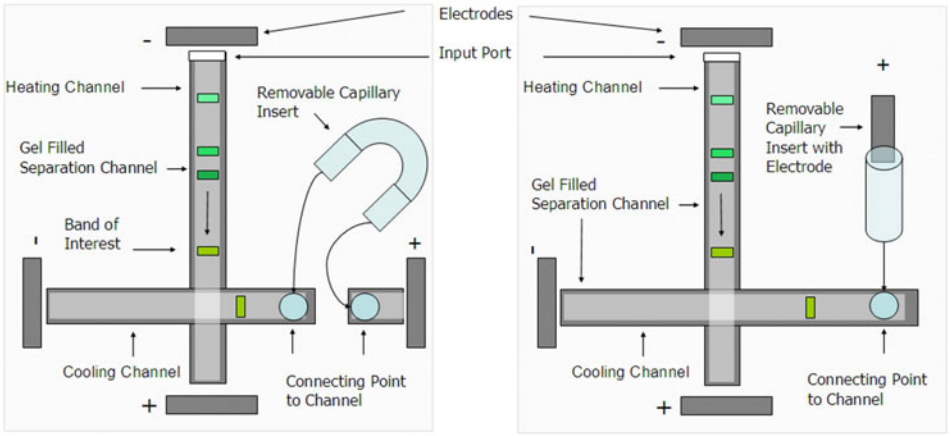

Figure 1 (Left) Schematic of a micro 2D electrophoresis device with removable capillary insert (RCI). Two orthogonal gel filled intersecting channels were fabricated using μTectonics[5]. Once the protein or DNA is loaded into the channel, a field is applied along with heat causing separation based on size to occur in one dimension. Next a band of interest is identified, and is removed from the first dimension at the intersection point by shutting off the first field and applying a second field for manipulation into the second dimension. The channel is cooled to create a renaturing environment. The RCI is filled with buffer and completes the circuit between the two electrodes. This allows the band of interest to be run into the RCI which can then be removed from the device. The content of the RCI is emptied by applying pressure at one end. This is useful for depositing material for traditional analysis methods. (Right) Schematic of a micro two dimensional electrophoresis device with RCI containing an electrode. Attachment of the fluid filled RCI completes the circuit allowing biological material to move in. The RCI is then removed and transported to a compatible device with electrodes so the DNA or protein is extracted using electrokinetics. This methodology is particularly useful for moving samples between different microfluidic devices.

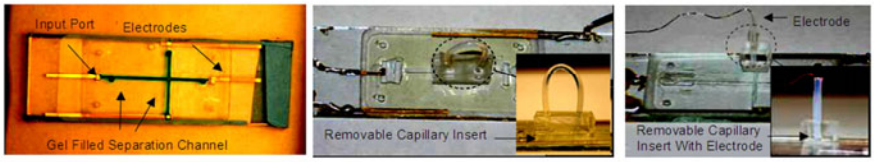

Figure 2 Images of micro 2D electrophoresis device fabricated using μTectonics. (Left) Micro electrophoresis device before addition of RCI component. Channel is dyed green for imaging. (Center) Micro electrophoresis device with integrated RCI temporarily attached using a PDMS connector. (Right) Device with integrated RCI containing second electrode for electrophoresis. Once a protein or DNA is within the RCI, it is removed from the device for post separation analysis.

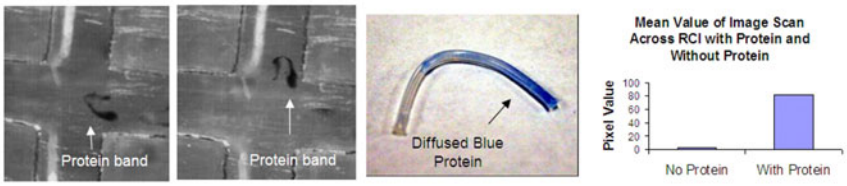

Figure 3 Demonstration of sample separation and recovery using BlueRanger Protein. (Left) Protein band of interest moving in the first separation dimension approaching intersection for removal. (Center) Band of interest entering second separation dimension where RCI is located. (Right) Color image of RCI removed from device after protein migration. The protein marker is dyed blue and can be seen diffusing into the capillary from the right side. The histogram shows intensity measurement of the RCI Image with and without protein to demonstrate successful band capture.