2.21 Microfabricated Genomic Analysis System

FLIGHT DATES:
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PRINCIPAL INVESTIGATOR:
Steve Gonda, NASA/Johnson Space Center

CO-INVESTIGATOR:
René Elms, Texas A & M University

GOAL:

Assess the suitability of utilizing a microfabricated platform for gel electrophoresis of DNA for spaceflight applications.

OBJECTIVE:

Assess the integration of a microfabricated electrophoresis device with the microfluidics system, and the feasibility of a microscale electrophoretic approach for separation of DNA in low gravity.

INTRODUCTION:

Genetic sequencing and many genetic tests and assays require electrophoretic separation of DNA. In this technique, DNA fragments are separated by size as they migrate through a sieving gel under the influence of an applied electric field. In order to conduct these analyses on-orbit, it is essential to acquire the capability to efficiently perform electrophoresis in a microgravity environment. Conventional bench top electrophoresis equipment is large and cumbersome and does not lead itself to on-orbit utilization. Much of the previous research regarding on-orbit electrophoresis involved altering conventional electrophoresis equipment for bioprocessing, purification, and/or separation technology applications.

A new and more efficient approach to on-orbit electrophoresis is the use of a microfabricated electrophoresis platform. These platforms are much smaller, less expensive to produce and operate, use less power, require smaller sample sizes (nanoliters), and achieve separation in a much shorter distance (a few centimeters instead of 10's or 100's of centimeters.) In contrast to previous applications, this platform would be utilized as an analytical tool for life science/medical research, environmental monitoring, and medical diagnoses. Identification of infectious agents as well as radiation related damage are significant to NASA's efforts to maintain, study, and monitor crew health during and in support of near-Earth and interplanetary missions. The capability to perform genetic assays on-orbit is imperative to conduct relevant and insightful biological and medical research, as well as continuing NASA's search for life elsewhere. This technology would provide an essential analytical tool for research conducted in a microgravity environment (Shuttle, ISS, long duration/interplanetary missions.) In addition, this technology could serve as a critical and invaluable component of a biosentinel system to monitor space environment genotoxic insults to include radiation.

METHODS AND MATERIALS:

The Microfluidics system developed by Chris Culbertson of Kansas State University was utilized to accomplish DNA separations in a UV-polymerized sequencing quality acrylamide matrix using a microfabricated electrophoresis device. Detection was achieved by labeling the DNA with an intercalating dye (POPO-3) with an excitation/emission profile of 534nm/570nm. The DNA sample used was a 100 bp ladder. Separations were conducted by application of 30-60V across a distance of approximately 3 centimeters. A green laser was used for excitation of the DNA. A 100 μm pinhole was used for focusing of emitted light prior to entrance into the photomultiplier tube (PMT.) Also, prior to the PMT entrance is a 595 ± 30 nm filter. The Microfluidics system was controlled and data collected via LabVIEW. Following each set of parabolas, the system was opened and a new device placed into the system.

Device Fabrication and Assembly

The microfabricated electrophoresis device (microdevice) used during flight was designed by Burns and colleagues and measures 7 mm by 35 mm. Standard photolithographic techniques are used for fabrication of the device. The silicon substrate contains the electrodes, heaters, and temperature sensors, which are subsequently wire bonded to a PC board. The etched glass channel is bonded to the silicon substrate. In this research, it was necessary to use a glass substrate in order to facilitate alignment of the channel with the detection system. A photo-initiated polyacrylamide gel was used. This gel is a denaturing gel and is the same used for high resolution sequencing. Gels are cast by first filling the channel with the monomer/crosslinker mixture. Next, an opaque mask is placed on top of the loading ports and the gel polymerized by

UV illumination. After illumination, the mask is removed and the unpolymerized reagents are removed from the loading ports. The resulting gel interface is well defined, flat, and precisely positioned.

Microfluidics system

The Microfluidics system is housed in a metal enclosure. Fluorescently labeled test analytes are detected as they pass through a laser. The fluorescent emission from the analytes is collected using a microscope objective and detected with a channel photomultiplier (PMT). The signal from the PMT is sampled using a multiDAC card attached to a neighboring laptop computer. The current in each of the channels is monitored continuously and accelerations of ± 2g in the X, Y, and Z planes are recorded by an integrated accelerometer.

Device Preparation

The devices were attached to an interchangeable mount and the glass channels aligned prior to gel casting. A 5% Long Ranger acrylamide solution was used and crosslinking is UV-initiated, allowing for specific positioning of the gel interface.

RESULTS:

Three separations yielded usable data. This data from the flights did show separation of the DNA did occur throughout all gravity regimes. It is apparent not all 10 bands in the ladder were resolved. In all three separations, it appears that multiple bands were detected simultaneously. Similar results were achieved during ground studies.

DISCUSSION:

After further investigation, it seems that potentially the primary reason for the simultaneous detection of bands is a large plug width. Plug width is indirectly related to resolution. Plug width can be decreased and controlled on-chip by use of electrode-defined injection, increasing resolution. The Microfluidics system was not configured to allow for electrode-defined injection. Instead, the same pair of electrodes was used for injection and separation, resulting in a large plug width and loss of resolution.

CONCLUSION:

Separation of dsDNA was accomplished during all gravity regimes experienced onboard the KC-135. However, not all 10 bands in the 100 bp DNA ladder were resolved. It is believed this is a result of using a single pair of electrodes for both sample injection and separation, resulting in a large plug width. In order to increase the achievable separation resolution, it is suggested that electrode-defined injection and electric field directed DNA collection be integrated into the Microfluidics system for future microdevice DNA separation experiments. It appears from these experiments that the 1) microdevice integrated well with the Microfluidics system, and 2) a microscale electrophoretic approach for separation of DNA is feasible and functional in low gravity.

PHOTOGRAPHS:

JSC2004E43037 to JSC2004E43040
JSC2004E43978 to JSC2004E44046

VIDEO:

- Zero-g 09/28 to 10/01/04 , Reference Master: 718802, 806

Videos available from Imagery and Publications Office (GS4), NASA/JSC.

CONTACT INFORMATION:

Steve Gonda, Ph.D.
Johnson Space Center
Mail Code: SJ
Houston, TX 77058
Phone: 281.483.8745
Steve.Gonda-1@nasa.gov

René Elms, Ph.D.
Department of Chemical Engineering
Texas A&M University
3122 TAMU
College Station, TX 77843-3122
Phone: 832.259.6333
Rene.Elms@chemail.tamu.edu