INTEGRATED FLUIDIC SYSTEM FOR GROWTH AND FLUORESCENCE IMAGING OF MULTICELLULAR ORGANISMS IN NANOSATELLITE APPLICATIONS

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ABSTRACT

We present here an integrated micro-fluidic system for conducting fully automated, unmanned biological experiments in space. The system allows up to four different biological species to be stored for more than 6 weeks in stasis. It includes integrated fluorescence-based imaging of a live-organism detection chamber, built-in buffer/waste storage compartments, precise temperature control, and automated fluidic handling to grow, transfer, and wash the specimens. The 3-D fluidic system utilizes multi-layer pressure sensitive adhesive bonding technology combining eight different materials. This system demonstrates a new generation of complex fluidic devices for highly automated biological assay experiments in space.

KEYWORDS: Integrated Microfluidic System, Nanosatellite, PSA, *C. elegans*, Fluorescence Imager, Laser Cutting, Space Biology, Astrobiology

INTRODUCTION

Nanosatellites are miniature spacecraft with mass < 10 kg; their small size and mass permit frequent, low-cost space access as "secondary" payloads carried with larger conventional satellites [1]. We present here the integrated fluidic subsystem of the Microsatellite In-Situ Technologies (MisST) nanosatellite science payload instrument (full satellite: 10 x 10 x 34 cm) for conducting automated unmanned biological experiments in space, with the overarching goal of understanding space environmental effects relevant to long-term travel and habitation [2,3]. The system includes wet storage compartments for live biological specimens, a detection chamber for fluorescence-based imaging, a pump and valves for fluid handling, along with integrated medium- and waste-storage compartments. The entire fluidic system (Figure 1) is fabricated using multilayer polymer rapid prototyping methods: laser cutting, traditional machining, die cutting, and pressure-sensitive adhesive (PSA) bonding. It includes eight different materials, eight valves, one pump, and a laser-machined micro-check valve. While the manufacturing approach would need to be altered for mass production, this system demonstrates a versatile rapid prototyping approach to complex fluidic systems for biological applications in whole-organism and cellular studies, drug discovery, diagnostics, and bioanalysis.

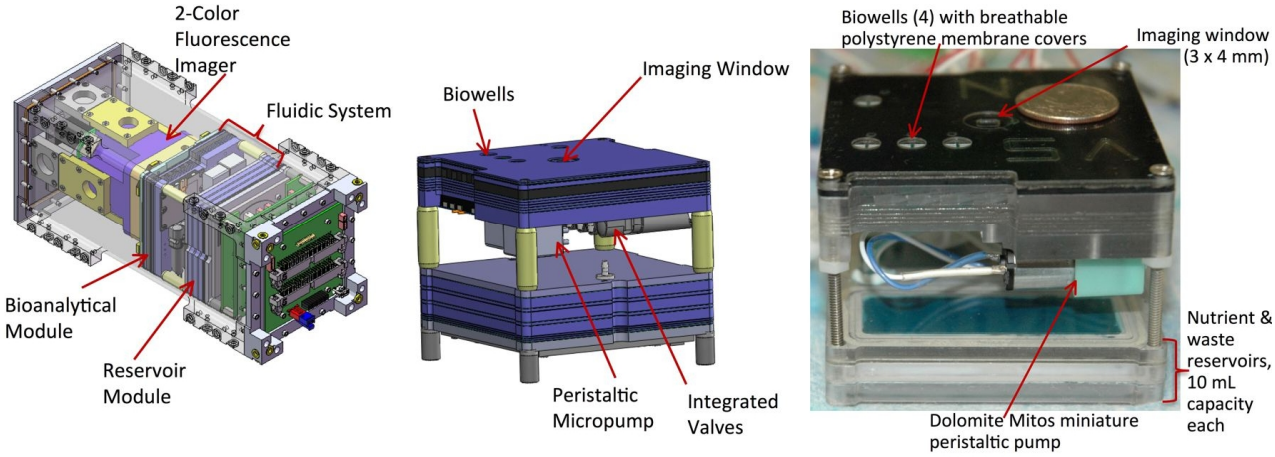

Figure 1: ***Left****: 10 x 10 x 20 cm MisST payload instrument including containment vessel, fluorescence imager, and fluidic system.* ***Center:*** *Fluidic subsystem (7.5 x 7.5 x 6 cm) showing bioanalytical module (top section) and reservoir storage (bottom section).* ***Right:*** *Photograph of a prototype of the spaceflight fluidic system including a US quarter for scale.*

SYSTEM OVERVIEW

The fluidic system includes two modules, the bioanalytical and reservoir modules. The former consists of four wells to store biological specimens, here different strains of fluorescently-labeled *C. elegans* (nematodes). Dauer-state nematodes (a larval stage of stasis) are stored in buffer in the wells during the pre-launch, launch, and transit phases (altogether up to 6 weeks). A 3 x 4 mm window covers an imaging chamber 200 µm tall and is aligned with a custom 2-color fluorescence

imager. Once the nanosatellite reaches stable orbit, nutrient medium is pumped into each well, bringing the worms out of stasis. Worms from the 1st well are pumped into the imaging area for growth and behavioral studies in microgravity for 1 - 2 weeks (worm generational time ~ 4 days). These worms are subsequently washed into the waste reservoir and replaced by a new strain from the 2nd well. Pumping and washing are controlled by eight face-mounted valves (Lee Company, LHD miniature solenoid valves) and a low-power Dolomite miniature peristaltic pump.

FABRICATION

The bioanalytical module is a complex 3-dimensional fluidic network consisting of machined polycarbonate sheets, laser-cut PSA, polycarbonate filters, polystyrene breathable membrane, and integrated face-mount valves. The flow path is defined by laser-cut channels in the PSA and machined vias in the plastic sheets. Integral track-etched membrane filters keep *C. elegans* from flowing or swimming out of the biowells and imaging region; polystyrene membrane sheet allows oxygen to be supplied to the worms while minimizing evaporative loss.

PSA layers, 170 µm thick (3M, 9495LE), are cut using a CO_2 laser (Laser Custom Designs, Hayward, CA). Each PSA layer is then laminated between polycarbonate sheets with machined fluidic through-holes using a precision alignment device (Figure 2a). The use of PSA in the microfluidic block provides the advantages of rapid prototyping and integration of different materials. Since laser cutting and machining are well-established low-cost manufacturing methods, each design iteration requires < 2 weeks from fabrication to assembly, thus allowing rapid testing and design modifications. PSA also serves as a convenient medium for bonding various materials, such as filters and polystyrene film, to the fluidic network at precise locations. As shown in Figure 2c, polycarbonate filters with 10 µm pore size (Sterlitech polycarbonate track-etched membrane disc filter) are bonded to the polycarbonate biowells and detection chamber using PSA.

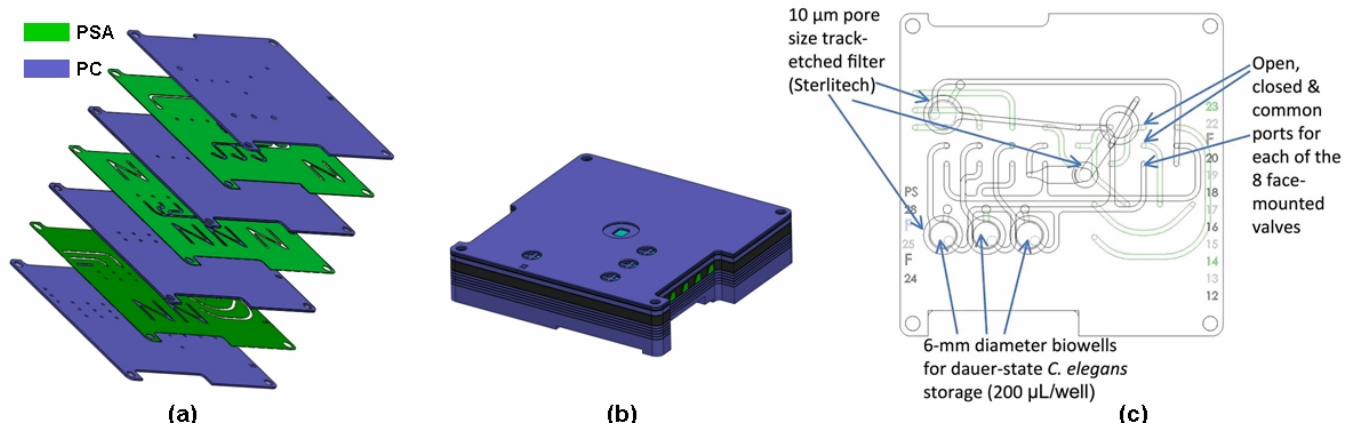

Figure 2: *Design of the multilayer 3D bioanalytical module. (a) Part of the multi-layer PSA-polycarbonate fluidic network, with channels cut through the PSA. (b) The 24-layer assembled bioanalytical module. (c) Top view of the fluidic card with the fluidic channels on 5 different levels, showing placement of integrated filters at the biowells and detection chamber.*

The reservoir module consists of two separate fluidic compartments for *C. elegans* maintenance medium (CEMM) and waste storage. One medium (CEMM) is used for feeding, transport, and washing purposes. The liquid flows always from the medium bag into the waste compartment under the control of the valves. Each compartment consists of a plastic housing with soft silicone sheets forming a flexible bottom. The capacity of the compartment is 1 mL when the silicone membrane is not extended. As more liquid is added, the elastomeric membrane stretches to accommodate it, and up to 10 mL of liquid can be added with less than 3 kPa (0.5 psi) of back pressure. As shown in Figure 3, the two compartments share a common chamber allowing the flexible membranes to defect as CEMM solution is pumped from one compartment into the other. The reservoir module is connected to the bioanalytical module using 2 flexible pieces of tubing (CEMM and waste line).

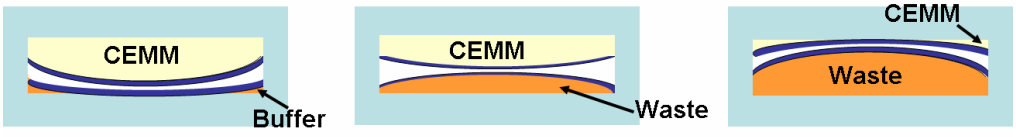

Figure 3: *Schematic diagrams of the reservoir module showing two liquid storage compartments and the deflectable silicone membrane at the various stages of the experiment.* ***Left:*** *The reservoirs are initially loaded with 10 mL of CEMM and 1 mL of buffer.* ***Center:*** *During the experiment, CEMM is used for growth of* C. elegans *in the bio-wells, and for transferring and washing in the detection chamber. CEMM volume decreases and waste compartment expands.* ***Right:*** *When the experiment is completed, the waste compartment is fully extended to 10 mL and 1 mL of CEMM remains in the retracted compartment.*

To minimize bubbles and leakage in the CEMM compartment during the initial filling process, a check valve is also integrated into the CEMM compartment using PSA and polycarbonate layers. The check valve and valve seat are fabricated in two 700-µm-thick polycarbonate sheets using ultra-short pulse laser ablation (Raydiance, Petaluma, CA). CEMM solution is injected into the compartment through the valve using a pressurized syringe. The syringe is then removed from the unit and the valve forms a leak-free seal in the CEMM compartment.

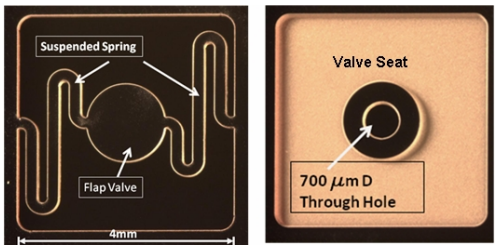

Figure 4: *Photograph of a check valve and valve seat fabricated using Raydiance Smart Light 50™ ultra-fast laser ablation system in 0.7-mm-thick polycarbonate sheet. This check valve is used in the reservoir module for leak-free loading of* C. elegans *growth medium. When placed over the valve seat, the valve flap forms a seal barrier over the through hole that can be opened with pressurized liquid during the loading process.*

RESULTS

Fluid flow is controlled by the integrated valves and pump. There are two main subroutines in the pump-and-valve control program that are crucial in the fluidic sequence: (1) transferring biological specimens from a storage well to the imaging area; (2) washing the detection chamber to prepare for subsequent injection of the next species. The valve flow direction and fluidic flow path for these are demonstrated in Figure 5. The two subroutines are repeated allowing fully automated sequential introduction of the biological species into the detection chamber with minimum cross contamination.

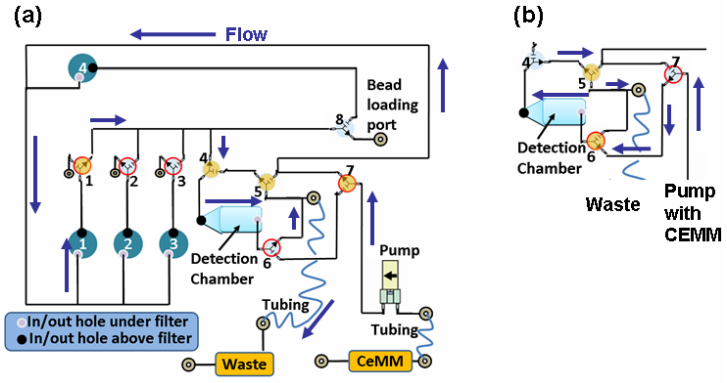

Figure 5: *Schematic diagrams of the fluidic system showing valves (1-8), peristaltic pump and biowells (dark blue, 1-4). The integrated valves control flow during loading and washing of the imaging chamber and prevent cross contamination between biowells. Valves highlighted in yellow are energized, i.e. set at the ON position (opposite to their initial OFF position shown in light blue). (a) Flow direction and valve setting when worms are transferred from Well 1 into the detection chamber. Valves 1, 4, 5 and 7 are on. (b) Flow direction and valve setting during the washing subroutine with valves 5 and 6 switched on.* C. elegans *in detection chamber are washed away through valve 4 and 5 into waste.*

During the experiment, real-time fluorescence imaging of the worms confirms that they are transferred and trapped by the filter in the detection chamber during the 1st sequence, and washed away during the 2nd sequence (Figure 6).

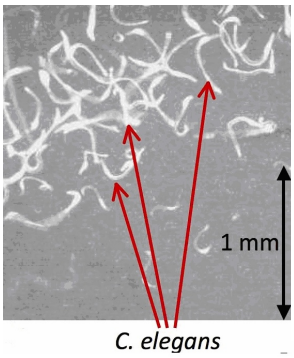

Figure 6: *Fluorescence image of green-fluorescent-protein-labeled* C. elegans *inside imaging zone of prototype bioanalytical module using custom-designed miniature imager. (imaging system: L. Timucin, G. Minelli)*

CONCLUSION

We have demonstrated here a fully integrated fluidic system for studying of *C. elegans* growth in space in a 10 x 10 x 34 cm nanosatellite. The complex fluidic network is formed through a multi-layer laser-cut PSA and plastic sheets structure. Fluorescence imager and liquid storage reservoirs are integrated with the fluidic network.

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