

Combining Additive and Subtractive Techniques in the Design and Fabrication of Microfluidic Devices

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ABSTRACT

Laminate construction of microfluidic devices offers a means to incorporate both additive and subtractive manufacturing techniques for the development of multiplexed fluidic devices that have, for example, porous membranes formed-in-place (FIP). Important applications for these hybrid devices includes sample preparation, separations, and containment of samples fed by a single inlet and outlet, such as multiple samples of suspended cells in culture contained in their respective wells. We describe the development and performance of a fluidic card for the NASA Ames GeneSat-1 program for autonomous cell culture experiments in space. The fluidic card was constructed as a multi-layer laminate of acrylic and bio-compatible bonding adhesive, using laser ablation to form the channels with porous membranes placed at the inlet and outlet of each sample well to contain individual samples of *E. coli* and prevent cross contamination.

Keywords: cell culture, laminates, microfluidics, form-in-place membranes, spaceflight.

1 INTRODUCTION

Fabrication methods for microfluidic devices, such as embossing, and injection molding can require expensive tooling and long turn around times, making empirical, performance-based modifications to the design expensive and time consuming. These methods also are limited in the materials and functionality that can be incorporated, so that complex, disposable devices that incorporate on-board valves, separation media, membranes, and recirculating pumps, can not be developed without considerable expense in molds and assembly fixtures. This creates a barrier to the development of complex and higher functionality devices where the cost to benefit ratio of incorporating functionality is too risky for the typical laboratory, diagnostic or medical device developer.

The benefits of using laminate techniques to build complex, three dimensional devices include the use of a variety of inexpensive, high quality, bio-compatible materials that easily accommodate the incorporation of other planar structures, such as porous membrane sheets, flexible valves, electrodes, circuits and diaphragm pumps. Because lamination is widely used in several other industries, such as graphic arts and printed circuits board, the availability of manufacturing equipment to scale the production is readily

available. Scalable and reproducible fabrication is an important consideration even at the early prototyping stage; the device itself is part of the analytical system, and it is therefore necessary to fabricate it as reproducibly as possible so that defects and variations in the device are not convolved with experimental data collected. To achieve these advantages, we have developed reproducible, scalable methods of fabrication.

There are several issues that potentially limit the ability of laminates to serve both as prototypes and as commercially viable products. Among them are the concerns about affects of materials and bonding schemes on biological activity, consistent and repeatable flow, and susceptibility to delamination under internal pressure. In this paper we demonstrate the use of commercially available adhesives and materials to reproducibly fabricate a fluidic card that was designed and developed for the GeneSat program at NASA Ames and the National Center for Space Biological Technologies at Stanford. The GeneSat program is a technology demonstration of an autonomous cell culture system housed in a very small ('nano') satellite containing all the required reagents and support systems to continuously monitor the growth of *E. coli* over a period of 96 hours once it is launched into low earth orbit. Details of the program and information on the most recent successful launch can be found at (<http://tia.arc.nasa.gov/genesat1/> and <http://genesat1.engr.scu.edu/dashboard/>)

2 FABRICATION

Laminate prototyping techniques have been used extensively to demonstrate the utility of laminar flow to measure kinetic phenomena in microfluidic devices. Prototyping techniques have been developed that use laser cutting of pressure sensitive adhesives, and thin polyester films (PET) to create elaborate fluidic circuits.

We have independently developed a laminate fabrication technique which has been specifically aimed at incorporating flexible, pneumatically driven valves and porous membranes, and a variety of materials with thicknesses ranging from 12.5 microns up to 2 mm thick, in a scalable, manufacturable format. Our fabrication method bridges the gap from prototyping to volume manufacture, providing high quality devices to support product development and launch. We are able to rapidly and inexpensively create enclosed channels in a solid plastic device, easily multiplex analysis using a single sample that

is 50 μ L or less, and incorporate on-board reservoirs and valves. This has served an unmet need for the laboratory, diagnostic and medical device developer in lowering the risk of incorporating the advantages of microfluidic circuits and functions. We foresee the successful application of our fabrication methods, such as for the GeneSat-1 fluidic card, propelling the development of cost effective, sophisticated fluidic systems for cell culture, medical diagnostic and research applications.

The advantages of additive and subtractive fabrication methods are borne out in the development of the fluidic card for NASA Ames' GeneSat program for autonomous biological experiments in space. The stringency of the requirements for these space-borne devices was considerably greater than for earth-bound systems. The need for consistent performance, low power consumption, light weight and flawless performance both pre-launch and in orbit was imperative.

2.1 Fluidic Card Requirements

The GeneSat program technology demonstration goal was to monitor the growth of a non-toxic strain of *E. coli* that was genetically engineered to express GFP in response to metabolic activity. The fluidic card had twelve wells on 18-mm pitch with ten samples and two controls. The growth was monitored using both optical forward scattering and fluorescence. Optical scattering was used to follow the growth of the colony in each well, while the expression of GFP was monitored with a fluorescence detector. An overview of the optical system is described in reference [1].

The specific performance requirements for the card included:

- 1) Simultaneous filling of 10 wells, 110 μ L volume each, through a single inlet to fill all 10 wells, and a single outlet to collect the effluent from all 10 wells;
- 2) Retain individual samples of *E. coli* in their respective wells;
- 3) Provide optical windows for fluorescence and scattering measurements;
- 4) Provide a CO₂/O₂ permeable layer;
- 5) Incorporate alignment features to interface reproducibly with the optical, thermal, and mechanical systems;
- 6) Ensure flatness across the entire surface of $\pm 0.003''$ to ensure proper interface to the heating component;
- 7) Allow independent access to each well for sample introduction, then seal and fill without trapping air bubbles.

Specific fabrication requirements included:

- 1) Use of non-cytotoxic materials to bond the layers
- 2) Alignment to $\pm 0.002''$ (50 microns)
- 3) No delamination of layers at pressures up to 25 psi

- 4) Seating of a 0.45 micron porosity nylon membrane (130 microns thick) at the inlet and outlet of each channel that contained the cells in their individual wells, and provide even flow across all ten wells.
- 5) Minimal contact of the fluid with the bonding adhesive layers.
- 6) Rapid modification of the design and production of functional prototypes for evaluation with the optical assembly and for pre-flight controlled biological experiments.

Several design iterations were tested during the course of the 24-month development program. GeneSat-1, launched in December of 2006, had ten fluidically connected wells, and two controls. An initial version of the GeneSat fluidic card, tested extensively in the lab, was constructed using a stack of 13 individual layers, seven bonding layers made from two different commercially available adhesives that are stocked by ALine, Inc., and 6 cast acrylic layers with thickness tolerances of $\pm 0.002''$ and superior optical quality, available from ALine, Inc. The channels and features were cut into the individual layers using a modified CO₂ laser with a beam diameter of 25 microns traveling on an x-y stage. Figure 1 shows the top view of the GeneSat fluidic card with the hose barb connectors seated in the inlet and outlet ports. The GeneSat flight card required 9 layers rather than 13. This was accomplished by forming the inlet and outlet channels that fed each of the wells into the surface of the acrylic, again using the CO₂ laser system to create the channel structure.

Fluidic Card for Cell Culture



Figure 1. GeneSat-1 Fluidic Card. Membranes at the inlet and outlet of each well provide containment of the *E. coli* sample. Biocompatible adhesives and optical quality cast acrylic were used; and a CO₂/O₂ gas permeable, 50 μ m film sealed the device after sample addition.

In both designs, the membranes were seated at the inlet and outlet of the well in a pocket formed into the acrylic layer. The individual membranes were glued into each location using a UV cure medical grade adhesive that was evaluated for cytotoxicity and found to be non-cytotoxic

Figure 2 shows an edge view of the fluidic card with the stack-up of the layers near the port containing the hose barb connections.

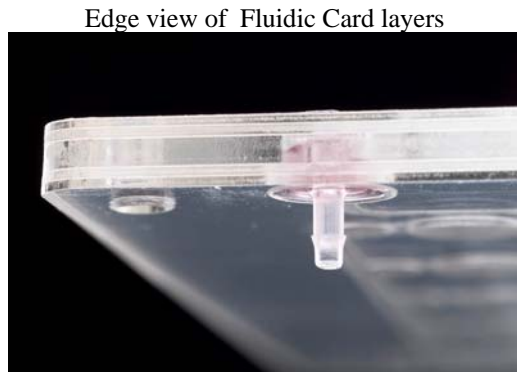


Figure 2: An end view of the 13 layer GeneSat fluidic card with the hose barb connector. This view shows the layers used to construct the card. Each layer is bonded with a pressure sensitive adhesive. The adhesive is along the edges of the channels only, and never forms the top or bottom of the channel.

A cross section of the GeneSat card is shown in Figure 3. For proper filling and exchange of the stasis media for the growth media, the inlet port to the well is at the bottom, and the exit port is at the top of the well. The two ports are situated on opposite sides of the well, so that fluid passes through the membrane and into the bottom of the well, fills and then exits out the top and through the membrane on the exit side of the well. This arrangement provided better than 90% replacement of the stasis media within ~ 30 min. The stasis media, a salt solution, suspended growth of the *E. coli* until the satellite was launched and all the experimental systems functional.

Cross Section of GeneSat-1 Fluidic Card Sample Well

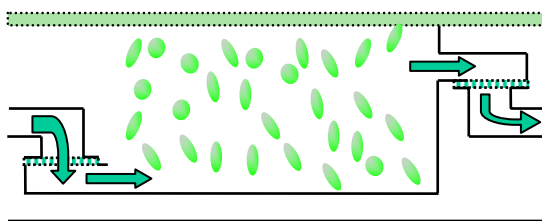


Figure 3: Top bar is the gas permeable layer; the bottom is the optical acrylic. The well measures 6.5 mm wide and 3.5 mm tall. The dark arrows show the fluid movement from the inlet channel, through the 0.45 micron nylon membrane and out through the top of the well, through a second 0.45 micron nylon membrane to the outlet channel. The oval shapes represent the *E. coli* in the bio-zone.

Once the system reached stable temperature and humidity, the stasis media was replaced with the nutrient growth media. This exchange marked the beginning of the growth experiment.

Analogous to printed circuit board assembly, in which repeat patterns of the individual features of each layer is arranged on a sheet and fabricated simultaneously, the GeneSat fluidic cards were fabricated batch-wise. To achieve registration between the layers, alignment fiducials are created as part of the fabrication for each layer and are arranged to provide tolerances of $\pm 0.001''$ along the x and y surface in the entire 12 x 24 fabrication footprint.

2.2 Fluidic Card Performance

The fluidic card was tested for even filling of all ten wells by using the pumping mechanism for flight which was a set of two helical springs mounted between a fixed and sliding plate. The sliding plate provided even pressure across the surface of the media bag. The procedure for filling the cards involves connecting tubing to the fluidic card inlet hose barb connection, and seating the fluidic card in a fixture at about 45 degrees with the inlet side of the device at the bottom so that filling occurs from the bottom edge of the wells. Once the membranes on the inlet side are wet, the fluid flow is stopped and the wells are filled with 10 μ L of solution containing cells. Once the samples are added, the lid assembly with the gas permeable layer is secured with pressure, ensuring the edges are well sealed before the card is completely filled. Key to successful filling is to make sure the outlet membranes are kept dry so that air is not trapped in the well before filling is complete. The filling was tested and found to be even within the requirements for the experiment. This meant that during the exchange of the stasis medium, all ten wells would receive a nearly complete exchange with growth medium so that nutrients do not differentially limit in the growth of the cells in one well relative to another.

Performance of the entire system and results from experiments in December, 2006 is described in reference [2].

2.3 Form-in-Place (FIP) Membranes

In order for the fluidic card to function properly, the porous membranes that retain the *E. coli* in their individual wells must have the same surface area exposed to liquid, and have the same thickness and porosity. The flight cards for GeneSat-1 used commercially available membranes with adequate thickness and porosity tolerances and the membranes were manually glued using a constant volume dispenser through a 24-gauge needle. Variability in the flux across each of the ten membranes was dependent on the assembler, so that technique was an important component in reproducible fabrication. While volume production of such fluidic cards might entail the use of a pick and place robot to locate the membrane, and robotic dispensing of adhesive to seat it, similar to those used for printed circuit board manufacture, the through-put and variability could prove to be cost prohibitive. An alternative to manual

placement of individual membranes is to fabricate the porous membrane in location in order to create a continuous roll of material with pre-formed membranes in the required location for proper fluidic performance, and to then laminate those layers into the device to achieve volume production of high quality fluidic cards with predictable performance.

While the FIP membrane did not fly with GeneSat-1, ongoing efforts to improve the fabrication technique and explore the use of a variety of standard polymeric membrane materials, such as a hydrophilic nitrocellulose or a hydrophobic polyvinylidene difluoride (PVDF) can provide more breadth in the application of porous membranes for routine tasks required for both space and earth-bound applications while providing a clear path for the volume production of such devices with unique capabilities. These would include sterile air vents to relieve bubble formation, sterile filtration of reagents as well as functional applications as passive valves or fluid stops.

A photo of an experimental membrane formed in this manner is shown in Figure 4. In this example, a sheet of PET, 0.010" thick is first cut with a scalloped pattern around the perimeter of a 4 mm hole. The holes are arranged in the same pattern as the membranes for the GeneSat-1 fluidic card. A fixed volume (10 uL) of a solution of polyester (MW 80,000) in hexafluoropropanol (18% solids) was pipetted into the hole to just fill it and the membrane formed by phase inversion of the polymer in water as it sat on a sheet of glass with a thin film of water covering the surface. In this experiment most of the membranes that were formed shrank as the solvent exchanged with the water and pulled away from the edges. However several formed a complete membrane that appeared uniform in thickness.

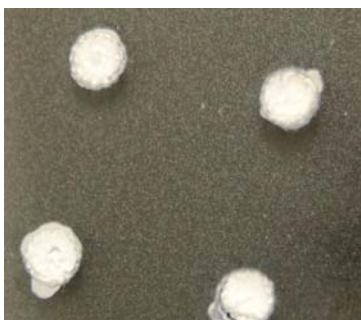


Figure 4: Porous membrane formed in place in 0.010" PET sheet using a scalloped edge to create higher surface area for membrane attachment.

With modifications in the polymer composition, the solvent mixture, the temperature of membrane formation and the cut-pattern, thickness and composition of the supporting layer, a suitable process for membrane formation can be developed that will provide a scalable means for

incorporating membranes with a range of thicknesses and porosities.

The properties of these FIP membranes are readily measured using a constant pressure source of fluid, typically de-ionized water, and measuring the volumetric flow across the membrane for a fixed length of time, a standard method for measuring membrane flux.

Comparison of performance with flat sheet membranes will provide insight into the variability of the FIP method with a standard fabrication procedure in volume production.

The versatility of FIP membranes, where the addition of a feature or component provides unique functionality in microfluidic devices, is being explored further in ongoing fabrication and characterization efforts.

3 SUMMARY

Laminate construction, combined with additive and subtractive manufacturing techniques, has produced microfluidic devices with multiple, membrane isolated cell culture wells, which have been integrated with fluidic pumps, temperature controllers, light sources, and optical and fluorescence sensors. Such a microfluidic device was flown on the NASA Ames Gene Sat-1 mission in December 2006 and supported the successful completion of an autonomous, 96-hour, cell growth experiment in orbit. Variations of these versatile and flight-proven laminated microfluidic devices offer laboratory instrumentation, diagnostic device, and medical equipment manufacturers a new, highly functional, low risk, rapid turn around, disposable, and cost-effective solution for a wide range of chemical, biological, and medical microfluidic applications.

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Acknowledgements: We would like to thank John Hines, NASA Ames, and Tony Ricco, NCSBT, Stanford, for photos, illustrations and edits.