# Lehigh University

# Annual Progress Report: 2009 Formula Grant

**Reporting Period**

July 1, 2013 – December 31, 2013

**Formula Grant Overview**

Lehigh University received $119,007 in formula funds for the grant award period January 1, 2010 through December 31, 2013. Accomplishments for the reporting period are described below.

**Research Project 1: Project Title and Purpose**

*Development of a Point-of-Care Opto-Fluidic HIV Viral Load Detector* - The intended result of this work is the design and construction of a hybrid platform that uses a combination of filtration and concentration methods in order to quantify the number of circulating HIV virons directly from patient blood samples. The purpose of this project is to perform fundamental studies to gauge the feasibility and performance of the device. After primary feasibility studies, we expect that the device will address the challenges associated with a point of care diagnostic platform that is both portable and easy to use in both resource-limited and general settings.

**Duration of Project**

1/1/2010 - 12/31/2013

**Project Overview**

The purpose of this project is to create a novel, low-cost, globally deployable diagnostic device capable of detecting viral loads in HIV patients. HIV infection affects more than 33 million people worldwide, most of whom live in areas without even the most basic laboratory facilities. Although state-of-the-art technologies based on nucleic acid amplification are sensitive and quantitative for viral load analysis, they are widely accepted to be technically too demanding and too costly, thus severely limiting their applicability at the point of care (POC). The goal of this project is to furnish the device in a usably portable high-performance platform. Since the device will quantify whole viral particles from blood using a combination of filtration and detection, we must first verify the individual components of the technique. There are two main factors in the operation of the device; one being the straining/concentration element and the other being the labeling and detection of the filtered viral particles.

In order to detect the viruses, they must first be isolated from the other blood-borne debris and concentrated significantly in order to be optically detected. The clinically relevant level of circulating viruses can be as low as nine orders of magnitude below even the most sensitive

optical detection techniques. In order to accomplish the necessary concentration enhancement, we will combine nanoporous filtration membranes with an optical concentrator. The filtration membranes will strain and concentrate the viruses by six orders of magnitude. After this step, an optical concentrator will be used to further enhance the concentration. Additionally, a systematic fluorescent labeling of the virions will occur in tandem with the membrane element to allow optical detection.

Thus the isolation of the viruses requires a combination of mechanical and optical techniques, both of which require fundamental research to move forward. In addition to system development, we will also evaluate the system performance using clinical samples obtained from Lehigh Valley Hospital. The funding of this project will support both the fundamental research and clinical evaluation.

**Principal Investigator**

Xuanhong Cheng, PhD
Assistant Professor
Lehigh University
5 E. Packer Ave, Whitaker Lab
Bethlehem, PA, 18015

**Other Participating Researchers**

H. Daniel Ou-Yang, PhD – employed by Lehigh University
Timothy Friel, MD – employed by Lehigh Valley Hospital

**Expected Research Outcomes and Benefits**

The end result of this project will be a compact device capable of quantifying HIV viral loads directly from whole blood. We expect that at the completion of this study, we will have explored the fundamental questions that surround the device; including the performance of the filtration membranes, the effectiveness of the fluorescent labeling and the concentrating power of the optical elements. Once the device has been tested with model systems and clinical trials, we expect that in the future a compact version of the platform will improve the public health by providing a cheaper, faster and more accessible diagnostic method for viral infections.

Although the focus in this project is HIV viral load quantification, the concentrating and detection platform could easily be applied to the detection of other pathogens for clinical diagnosis, homeland security and epidemiological monitoring purposes.

**Summary of Research Completed**

In our prior reports, we fabricated optical sensors for nanoparticle detection and tested their performance. We also demonstrated the concepts of viral processing components. In the past six months, we focused on fabricating and testing microfluidic devices capable of capturing viruses from biological fluids through immunoaffinity isolation. Two types of devices with well

controlled nanostructures were explored: nanoporous monolithic devices and microchannels with incorporated nanofiltration membranes. The well characterized internal nanostructures in these devices allowed fundamental analysis of nanoparticle transport in addition to highly efficient viral isolation. The capture efficiency of both types of devices was evaluated using laboratory stock of HIV viruses.

Methods:
The first viral capture strategy was based on the idea of immunoaffinity matrix. To achieve effective interaction with target analytes, the capture matrix needs to have characteristic dimensions comparable to the target particles. Considering the small dimension of viruses, we attempted to create nanoporous monolith with controlled nanostructures. One such monolith was created by templating anodic aluminum oxide (AAO) membranes. AAO is known to have straight through-membrane pores and regular pore arrangements. In previous reports, we presented a strategy to control the pore size and pore period. Here, the AAO membranes were used as a template mold and methyl-methacrylate (MMA) was polymerized inside the pores and around the membrane through UV-induced free-radical polymerization. After removing the AAO by wet etching, the resulting polymethyl-methacrylate (PMMA) monolith contained high aspect ratio, vertically standing nanoposts. The interpost spacing was measured to be ~300nm by scanning electron microscopy. Diameter and height of the individual pillars matched the pore size and thickness of the AAO template, respectively. The PMMA surface was further functionalized through the *N*-hydroxysulfosuccinimide/1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (NHS/EDC) chemistry to introduce affinity proteins for the HIV virus. As the inter-post distance, 250nm, is comparable to the size of HIV viruses of ~100nm, the monolith offers significant surface area for virus capture. For the application of other viral species, the post geometries can be adjusted by using AAO molds of other geometries, which is readily available through nano-indentation guided aluminum anodization reported in our prior progress reports.

Another monolithic material was created by templating tightly packed silica beads. In this case, a suspension of 1μm silica beads was deposited and compacted in a polydimethylsiloxane (PDMS) chamber together with 100nm polystyrene particles. After the solvent was evaporated, the polystyrene beads were melted and silica beads were etched, leaving behind a continuous and porous polystyrene matrix with spherical and interconnected pores. The pore shape was defined by the silica beads and the interconnection was formed from bead contacts. The pore necks were measured to be ~ 250nm, which allows the passage of HIV viruses. Other pore geometries could be obtained by selecting appropriate silica and polystyrene beads as the templating mold. The polystyrene surface was further modified by physiosorption of affinity proteins, so the matrix can be used for specific isolation of HIV viruses from a suspension.

A different strategy to capture virus is based on the idea of nanofiltration. The device with the filtration membrane was made by sandwiching commercial polycarbonate membranes between two PDMS channels. Polycarbonate membranes with a pore diameter of ~30nm were selected to allow liquid to drain through, but retain and enrich HIV viruses. Affinity proteins were physiosorbed on the membrane for specific viral captured. The microchannels above and below the membranes allow both tangential flow and filtration of the biological fluid: the tangential flow removes the fouling layer on the membrane and prevents cake formation, while the

filtration flow enhances the interaction of viruses with the capture surface for a high capture yield.

Results of Data Generated and Analyzed (Figures are not provided pending publication):
We first optimized the surface chemistries on different materials to form a monolayer of affinity proteins for virus capture. Next, we compared the virus capture efficiency in microchannels with and without the nanoporous structures. Without the nanostructures, a flatbed microchannel captures minimal amount of viruses, due to the low concentration of viruses in biological fluids and a corresponding low opportunity of virus collision with the functional surface. The two nanostructured monoliths, on the other hand, increase the surface area by 4 orders of magnitude in the same channel geometry. This greatly enhances the virus-surface collision and promotes viral capture. As the viruses are specifically immobilized in the matrix and the non-specific components are flushed out, the viruses are also concentrated and purified from contaminants in the original sample. These facilitate downstream viral detection, since the presence of non-specific nanoparticles interferes with the optical detection strategies reported in our prior progress reports.

When the two monolithic nanostructures are compared, obvious differences were observed. The two monoliths contain high aspect-ratio nanopost arrays and spherical interconnected nanopores, respectively. Although they have comparable internal surface area, the nanopillar array has a capture yield of ~100% from 400μL of viral suspensions at a concentration of 1 million HIV virions/mL. The monolith with spherical pores, on the other hand, captures only 70% of the HIV virions. The sacrificed capture yield in the spherical pore materials can be explained by large dead volumes. In our test condition, the Peclet number are much greater than 1, thus convection dominates over diffusion. The particles mostly follow the streamlines, which concentrate around the pore neck in the spherical pore structure, but are scarce away from the neck. As a result, particles have a high collision rate with the wall near the pore neck only, but have little chance to interact with the curved pore wall away from the neck. In the nanopillar device, the streamlines distribute much more uniformly in all dimensions, as indicated by fluid dynamics simulation using the COMSOL program. This leads to more active capture area in the nano-pillar device. In addition to the difference in active capture area, the capture yield in the spherical devices is further compromised by the relatively high wall shear stress around the pore neck compared to that on the surface of the nanopillars. The wall shear stress counteracts the affinity binding and reduces initial interaction of viruses with the spherical pores.

The aformentioned two nanostructured materials have only one characteristic dimension, which is comparable to the size of viruses. Thus they can be clogged when large size contaminants exist in the original viral sample. To process more complicated mixture of viruses, we fabricated another type of device based on the concept of nanofiltration. This device contains a nanoporous membrane sandwiched between two PDMS channels. The membrane is functionalized with affinity molecules to specifically isolate target viruses. To prevent membrane clogging, we operated the device in a tangential flow mode: a shear flow tangential to the membrane reduces cake formation, while a through-membrane flow drives viral particles to the functional surface for their capture. The relative fraction flowing tangential and through the membranes are controlled by resistance channels connected to the tangential fraction. In our preliminary tests, the resistance channel is selected to allow 20% tangential flow and 80% filtration. The capture

yield on the membrane is not only controlled by the filtration fraction, but also the sample flow rate. When the flow rate is low, non-specific binding from the sample could coat the membrane and prevent the interaction between target viruses and the affinity coating. On the other hand, specific interactions are interrupted at high flow rates or great wall shear stress. The optimized the flow rate was identified that corresponds to a wall shear stress of ~2dyne/$cm^2$ on the membrane. The capture yield is ~60% at the optimal flow rate. This is lower than the fraction transported through the membrane, but the capture yield holds for suspensions containing different concentrations of virions.