Using a Lung Cell Microfluidics Device to Study the Bacteriophage Adherence to Mucus (BAM) Model

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***Short Abstract* — The BAM Model or Bacteriophage Adherence to Mucus Model has been published recently by our lab and shows a novel mechanism of immunity mediated by bacteriophage. Bacteriophage adhere to mucus through Ig-like domains and accumulate in mucus. This produces a barrier to invading microbes and when a microbe attaches it can be infected by the bacteriophage and subsequently lysed. To better study this model we are using a microfluidics device to model the lung. This allows for a more physiological modeling of this system and allows for quantitation using Plaque Assays, Colony Counts, and Flow Cytometry.**

***Keywords* — Phage, Bacteriophage, Mucus, Immune System, Innate Immunity, Ig-like domains, Microfluidics, PDMS, Methods, Lung Cells, Cell Culture, A549, Bacteria, Flow Cytometry, Plaque Assays, Colony Counts.**

I. Purpose

MUCUS is present on many metazoan cellular surfaces and serves as an innate immune system to protect from pathogens [1]. It is composed of mucin glycoproteins and is colonized by bacterial symbionts. Minot et al. recently showed that bacteriophage or phage have many hypervariable proteins in the form of Ig-like domains [2]. These Ig-like domains were not needed for growth in a laboratory so they were thought to have a role in environmental growth instead. After this observation our lab set out to test this both in the environment and took samples of various surfaces [1]. We found there was a higher phage-to-bacteria ratio in mucosal surfaces than the outside environment.

To model this in the lab we used static sterile tissue culture with mucus producing (A549) and non-mucus producing cells (T84, *MUC*$^-$) and checked for phage adherence [1]. There was a significantly higher amount of phage on mucus producing cell lines (A549). In another experiment the cell lines were pretreated with T4 phage and then incubated with *E. Coli* bacteria. There was a significant reduction in cell death with mucus producing A549 T4 phage pretreated cells compared to no phage pretreatment.

Overall this shows a novel link between phage and metazoans and that phage co-evolve with metazoans and adhere to mucus through these Ig-like domains. All the work has been done in static tissue culture and we would like to model this in a more physiological model in which mucus is being shed and there is a flow present. We have developed a microfluidics device, which is similar to the Lung-on-a-Chip where we can better recreate this BAM Model [3].

The device is made from PDMS (Polydimethylsiloxane) a biologically inert polymer that can form micro features when poured onto a mold [4]. The device has one main channel with one inlet and one outlet. For use it is bonded onto a glass slide and holes are punched and tubing is inserted. After bonding, media is added and concentrated A549 cells. The device is then connected to a 10-plex multiplex syringe pump and the cells grow very well for up to 2 weeks and potentially longer in a tissue culture incubator. Experiments can be done on the cells similarly as the published experiments but exact flow rate can be controlled. This allows for better sampling and observation of the BAM Model phenomena.

An easy to follow protocol has been developed to allow for easier use of microfluidics with eukaryotic cells. Using this device it is much easier to do reproducible and more physiologically relevant experiments on tissue culture cells. These techniques and theories will likely have a broad impact across many fields of science including immunology, bioengineering, biophysics, quantitative biology, cell biology, and microbiology.

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