ABSTRACT

In this paper, we report the development of a micropatterned co-culture technique for co-culture of bacteria and eukaryotic cells for investigating host-pathogen interactions in pathogen infections. A key feature of the proposed model is that pathogens are exposed to an environment similar to the human gastrointestinal tract. This work can be used for developing a fundamental understanding of interkingdom communication and signal recognition during pathogenic infections.

KEYWORDS: Micropatterned Co-Culture, EHEC infection, Quorum sensing signals, Chemotaxis

INTRODUCTION

The intestinal tract is colonized by hundreds of non-pathogenic (commensal) bacterial species, including those belonging to the genus Escherichia, that are important in maintaining normal gastrointestinal (GI) tract function [1]. Colonization of the GI tract by pathogenic bacteria such as Escherichia coli O157:H7 (EHEC) proceed in three distinct steps: (i) migration of the pathogen to intestinal epithelial cell surface, (ii) colonization, and (iii) injection of virulence factors.

While the driving forces underlying EHEC infections are not fully understood, it is becoming increasingly clear that soluble signal-mediated interactions, both between different bacteria (intra-kingdom) as well as between bacteria and eukaryotic cells (inter-kingdom), are important in EHEC infections.

EXPERIMENTAL

Recent work from our lab [2, 3] has shown that phenotypes relevant in EHEC infections - chemotaxis, colonization, and attachment – are differentially regulated by eukaryotic hormones in the GI tract (e.g., norepinephrine) and bacterial quorum sensing signals (e.g., indole). These results underscore the importance of GI tract signals in EHEC infections.
The goal of this work is to develop microfluidic models for systematically investigating EHEC migration and colonization in the presence of different GI tract signals. Microfluidic devices are ideal for this problem as they enable generation of stable gradients of signaling molecules (for migration studies) and spatial patterning of bacteria (for colonization studies) so that in vivo conditions can be mimicked. Figure 2A shows the microfluidic chemotaxis model in which two streams, containing either a GI tract signal or blank buffer, are used to generate a gradient of signal concentrations. Bacteria expressing green fluorescent protein (GFP) are introduced to the device and immediately encounter the signal being tested. The relative migration towards or away from the signal is monitored at different locations downstream of the cell inlet with respect to dead (red) bacteria which do not demonstrate any chemotactic migration. Figure 2B shows E. coli migrating away from a known repellant.

We are also using micropatterning to manipulate the local microenvironment that EHEC encounters during colonization in order to determine its role in EHEC infections. This model is shown in Figure 3. Conventional cell culture methods for studying EHEC attachment do not account for localization of different bacteria and the signals that they produce on EHEC colonization as it is difficult to localize bacteria in specific locations among eukaryotic cells. Using easy to operate pneumatic valves, we have fabricated several commensal bacterial “islands” ranging in diameter from 20 to 100 microns and surrounded them with eukaryotic cells so as to create...

Figure 2: Microfluidic chemotaxis. (A) Schematic of device. (B) Representative image showing migration of bacteria from Figure 3: Hypothetical model for EHEC colonization and infection.

Figure 4: Micropatterned infection model.

Figure 5: Localizing dyes in specific islands.
an in-vivo like microenvironment (Figure 4). Microvalves are used to localize the introduced bacteria to the islands (Figure 5A). The feasibility of our localization procedure in specific regions is demonstrated in Figure 5B using sequential introduction of dyes. A similar approach was used with bacteria. Commensal bacteria were introduced initially into the islands. Any bacteria outside the island regions were removed by exposure to penicillin/streptomycin for 10 h. The chamber was thin coated with fibronectin and epithelial cells (HeLa) are seeded and allowed to attach for 24 h.

RESULTS AND DISCUSSION

Figure 6 shows patterning of bacteria in specific locations. Current work focuses on optimizing the effect of commensal island size and bacterial and HeLa cell attachment time, as these two parameters are expected to directly influence the concentration of signaling molecules released into the microenvironment. EHEC can also be introduced into the micropatterned chamber and its colonization to specific locations studied.

CONCLUSIONS

We have developed a micropatterned co-culture model of bacteria and eukaryotic cells for investigating host-pathogen interactions. Our results show bacteria can be cultured in specific locations among eukaryotic cells; thereby, mimicking the human GI tract microenvironment.

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