BBSI Proposal Summer 2008

Towards Understanding the Pathogenesis of Bacteria Vaginosis: Analyzing the Growth, Tolerance, and Adherence of Anaerobes and the *Gardnerella vaginalis* Biofilm

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Introduction

Bacterial vaginosis (BV) is the most prevalent vaginal infection in women of reproductive age, as it affects 8%–23% of women.[1] Complications associated with BV include preterm delivery,[2] an increased risk of pelvic inflammatory disease,[3] and an increased susceptibility to HIV acquisition and transmission.[4] Current treatment, which usually involves metronidazole drug therapy, has not proved sufficient. Even after successful treatment, there is still over a 50% recurrence rate.[5,6] Despite the rate of recurrence and prevalence of infection, current knowledge of the etiology and pathogenesis of BV is limited. While the initiation factor of BV remains a mystery, it has been associated with three characteristics: a rise in pH (>4.5), an overgrowth of pathogenic BV-associated bacteria, and a reduction in lactobacilli, the bacteria present in the healthy vagina. Lactobacilli produce lactic acid and hydrogen peroxide, maintaining the low pH of the vagina and, ideally, preventing the BV-associated bacteria from colonizing. Of these pathogenic bacteria, *Gardnerella vaginalis* has been found to be the predominant species.[7,8] However, *G. vaginalis* on its own does not cause BV, as there are many other anaerobic bacteria associated with the disease, including *Atopobium vaginae*, *Prevotella bivia*, and *Mobiluncus vaginalis*. Of all bacteria identified, only *G. vaginalis* is known to produce a very resilient biofilm.[7] If we can characterize how these pathogenic anaerobes can persist in the vagina, then perhaps we can develop methods to disrupt their colonization and prevent recurring infections. I will be testing two hypotheses with the following specific aims: 1) The *Garderella vaginalis* biofilm creates a more suitable environment for other anaerobes and the biofilm itself to survive in the presence of chemicals present in the vagina: oxygen, lactic acid,

and hydrogen peroxide. 2) *Gardnerella vaginalis* is the initial colonizer in bacterial vaginosis, and allows for other BV-associated anaerobes to adhere to the vaginal epithelium.

Methods

To address the first aim, I will use mathematical modeling and lab experimentation. First I will use a monospecies 2D biofilm mathematical modeling program [9] to test the hypothesis that a *G. vaginalis* biofilm would deplete local oxygen levels sufficiently for survival of strict anaerobes. The program models biofilms based on metabolic and environmental parameters and simulates biofilm growth and oxygen consumption as a function of time. I will also test the hypothesis by growing anaerobes (specifically *Atopobium vaginae*, *Prevotella bivia*, and *Mobiluncus vaginalis*) alone and in the presence of a *G. vaginalis* biofilm, testing their tolerance to oxygen, lactic acid, and hydrogen peroxide in both cases. I will expose the bacteria to incremental amounts of these chemicals and test bacterial cell viability with The BacTiter-Glo™ Microbial Cell Viability Assay (Promega Corp, Madison, WI). This luciferase assay quantitatively measures adenosine triphosphate from the bacterial cells, where light units are directly related to viable colony forming units.

To address the second aim, I will analyze the adherence of anaerobes to vaginal epithelial cells and to a *G. vaginalis* biofilm. For the first part, I will culture immortalized cervical epithelial cells and separately add equal numbers of viable *G. vaginalis* and the other anaerobes to the cells. To quantify relative adherence, I will wash off the non-adherent cells, gram stain the cells, and visualize them with microscopy. For the second part, I will grow a *G. vaginalis* biofilm on cervical epithelial cells and add the other anaerobes. To quantify cell numbers, I will extract bacterial DNA and run real-time polymerase chain reaction (PCR) using primers specific for the 16S rRNA gene of each species.

Possible Results and Implications

The mathematical modeling will provide insight into what biofilm thickness is predicted for anaerobe survival. This information provides a starting point of how developed of a biofilm the anaerobes should be introduced to. It will also provide insight into the time and conditions for this growth. However, it is important to acknowledge the limitations of the program, as any model does not mirror real life completely accurately. It is a simple model and only addresses a monospecies biofilm, when it is possible that a multi-species biofilm could contribute to oxygen depletion. It only addresses one solute species (oxygen), and there may be other biological factors that contribute to biofilm formation.

The lab component of aim one could provide information regarding anaerobe tolerance to a certain concentration of chemicals with and without the *G. vaginalis* biofilm. If the anaerobes do not behave differently with and without a biofilm, then one might suspect that the *G. vaginalis* biofilm does not affect the local environment to the benefit of the anaerobes. However, if anaerobe bacterial viability is higher in a biofilm, then it would seem that the biofilm protects the anaerobes. Also, perhaps optimal viability or a tolerance threshold could be correlated with a certain concentration of chemicals. Another possibility is that the anaerobes could grow worse in the presence of *G. vaginalis* and be out-competed by the biofilm.

In the adherence assay, a possible result could be that the anaerobes do not adhere to epithelial cells alone but do adhere in the presence of the biofilm. This would suggest that *G. vaginalis* could be the initial colonizer. Another result could be no adherence difference between the two tested substances, which would suggest that the biofilm does not create a more suitable surface. It is also possible that there is adherence to both biofilm and epithelial cells, but noticeably better adherence in the biofilm. The latter two possibilities would not disprove *G.*

vaginalis as an initial colonizer, but more experimentation would be needed. For the two wet lab experiments, it is important to note that they are not done *in vivo*, so other biological factors may play a role. Another limitation is that only three BV-associated strict anaerobes are tested, and other anaerobes might behave differently under similar conditions.

Future Possibilities

Do *Gardnerella vaginalis* biofilms contribute to the growth of other anaerobes associated with bacterial vaginosis? If these experiments lead to confirmation of the two hypotheses, then it would provide some insight on the pathogenesis of bacterial vaginosis. If *G. vaginalis* biofilms play an important part in this pathogenesis, then it would explain why *G. vaginalis* does not always cause BV by itself but is usually present in women with BV. Potentially, to prevent colonization of subsequent anaerobes, one could study the properties that allow the anaerobes to adhere to the biofilm. Alternatively, one could target the biofilms directly with enzymes that cause biofilm dissolution but do not harm the vaginal epithelium. Another possibility is using quorum-sensing inhibition to prevent bioform formation and testing whether BV can occur in the absence of the biofilm.

References

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