**Embeddable fiber-based biosensors for bacterial vaginosis.**

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**Statement of Purpose:** The immediate goal of this project was to develop a simple colorimetric fiber-based sensor suitable for early detection of bacterial vaginosis (BV). BV is the most common vaginal infection in women of childbearing age, and it is usually caused by a change of normal vaginal bacterial flora, characterized by profuse vaginal discharge. In the United States, 29% of women between the ages of 14 and 49 years and as many as 16 % of pregnant women have BV. In pregnant women, the presence of BV is strongly associated with preterm birth, a risk that is mitigated by early diagnosis and treatment with oral clindamycin. Thus, it is highly desirable to have a simple non-invasive self-diagnostic test that would enable a patient to monitor her BV status on a regular day-to-day basis.

**Methods:** The current "gold standard" in the diagnosis of BV is the Amsel criteria. This test is considered positive if three out of the following four criteria are satisfied: (i) presence of abnormal vaginal discharge, (ii) elevated vaginal pH (>4.5), (iii) positive amine odor, and (iv) presence of clue cells on Gram stain or saline prep of vaginal secretions. This test is laborious and requires extensive lab skills, often leading to poor sensitivity for BV.

One approach to the diagnosis of BV is based on the detection of the enzyme sialidase produced by bacteria that are not normally present in the vagina. A number of colorimetric tests, such as recently FDA-approved BVBlue®, have been proposed for detection of sialidase. Use of these tests, however, requires patient's visit to a doctor's office.

Our project focuses on the fabrication and testing of surface-modified fibers suitable for early colorimetric detection of BV. Such fibers can be incorporated into feminine hygiene products such as panty liners for self-diagnosis or used for point-of-care analyses.

High surface area Nylon yarn (Middleburg Yarn Company) was modified to obtain positive surface charge in 3 steps: first, fibers were treated with radio frequency plasma to form surface reactive groups such as –COOH and –OH, after that poly(glycidyl methacrylate) polymer was attached, and finally fibers were modified by polyethylenimine with positive amino groups. The procedure resulted in the formation of ultra thin layer of positively charged polyelectrolyte on the fiber surface which serves as the anchor for electrostatic attachment of the negatively-charged sialidase substrate. We have used the cyclohexylammonium salt of 5-bromo-4-chloro-3-indolyl-□-D-N-acetylneuraminic acid (BCIN) as a sialidase substrate. Binding efficiency was measured by reading optical density of BCIN solution at 280 nm before and after incubation with modified fibers. It has been established, that we have reached approximately a monolayer BCIN coating on fibers.

**Results:** Our experiments show that colorimetric sialidase substrate can be readily and with high yield immobilized on polymeric fibers surface-modified by covalent attachment of a cationic polymer. In vitro study shows that the fibers change color from white to bright blue in the presence of sialidase. Modified fibers were incubated with 0.06 U of sialidase in PBS buffer for 2 hours to show that the fibers change color from white to blue. In distinction with BVBlue® test this reaction we did not have to add an alkaline solution to reveal the colorimetric signal, which makes them much more suitable for the proposed applications than those treated by BVBlue® reagent.

A clinical study with human subjects was conducted in association with Greenville Hospital System. In this procedure the vaginal fluid of the patients (total 44 patients) were incubated with small piece of fiber (~1 inch of length) and change in color was detected. Standard methods of detection of BV such as Nugent and detection of clue cells (wet prep) were also carried out. The pilot data obtained using modified fibers showed very high sensitivity (75%) and specificity (100%) to BV *(Fig. 1)*.

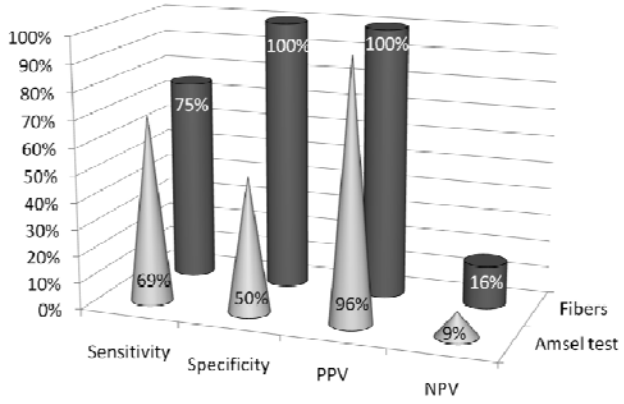


Figure 1. The results of pilot clinical study showed high sensitivity and specificity of fiber-based sensors.

**Conclusions:** In conclusion, the simple colorimetric sensor was developed based on positively charged polymeric fibers modified by electrostatically immobilized colorimetric sialidase substrate BCIN. These fibers change color from white to bright blue in the presence of sialidase and have been successfully used to diagnose BV in human patients. More clinical trials need to be made to collect statistical data on the sensitivity of the fibers. More broadly, the results obtained during this study will serve as the springboard for the development of a novel family of active biosensors embeddable in everyday household items.