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Detection of methicillin-resistant staphylococci by biosensor assay consisting of nanoscale films on optical fiber long-period gratings

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ABSTRACT

Methicillin-resistance among *Staphylococcus* species is a major health problem in hospitals, communities, and animals. There is a need for culture-free diagnostic assays that can be carried out rapidly, and maintain a high degree of sensitivity and specificity. To address this need an ionic self-assembled multilayer (ISAM) film was deposited on the surface of a long-period grating (LPG) optical fiber by immersion alternately in poly-allylamine hydrochloride and in poly-1-[p-(3′-carboxy-4′-hydroxyphenylazo) benzenesulfonamido]-1,2-ethandiyl (PCBS), resulting in terminal carboxyl groups on the LPG-ISAM. The terminal carboxyl groups were covalently conjugated to monoclonal antibodies (MAb) specific to penicillin-binding-protein 2a of methicillin resistant (MR) staphylococci. After exposure of the LPG-ISAM to 10^2 colony forming units (CFU)/ml of MR *S. aureus* (MRSA) for 50 min., light transmission was reduced by 19.7%. In contrast, after exposure to 10^6 CFU/ml of methicillin-sensitive *S. aureus* (MSSA) attenuation of light transmission was less than 1.8%. Exposure of the LPG-ISAM to extracts of liver, lungs, or spleen from mice infected with MRSA attenuated light transmission by 11.7–73.5%. In contrast, exposure of the biosensor to extracts from MSSA-infected mice resulted in 5.6% or less attenuation of light transmission. When the sensor was tested with 36 strains of MR staphylococci, 15 strains of methicillin-sensitive staphylococci, 10 strains of heterologous genera (all at 10^4 CFU/ml), or tissue samples from mice infected with MRSA, there was complete agreement between MR and non-MR bacteria determined by antibiotic susceptibility testing and the biosensor assay when the cutoff value for attenuation of light transmission was 6.3%. Thus, the biosensor described has the potential to detect MR staphylococci in clinical samples with a high degree of sensitivity and specificity.

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1. Introduction

Staphylococcus species are responsible for skin, soft-tissue, and systemic infections in humans and animals. In general, one in three (33%) people carry staphylococci in their nose, usually with no illness (Gorwitz et al., 2008). *Staphylococcus aureus* is a dynamic and adaptable bacterium that has a remarkable ability to acquire antibiotic resistance quickly. In the pre-antibiotic era, *S. aureus* was associated with a high incidence of mortality. Penicillin, first introduced in the early 1940s, quickly lowered mortality associated with *S. aureus* infections. However, by the mid-1940s, strains of penicillin-resistant *S. aureus* were detected in hospitals. By 1960, *S. aureus* resistance to penicillin was commonplace in both community-acquired and hospital-acquired strains. Methicillin, a penicillinase-resistant semi-synthetic penicillin, was introduced to treat patients infected with penicillin-resistant *S. aureus*. However, in 1961 methicillin-resistant *S. aureus* (MRSA) isolates were reported (DeLeo et al., 2010). MRSA has become established in healthcare facilities throughout America, Asia, and Europe (DeLeo et al., 2010; Molton et al., 2013). Compared with infections due to methicillin-sensitive (MS) *S. aureus* (MSSA), infections by MRSA strains are associated with increased morbidity and mortality in affected patients (Cosgrove et al., 2003). Once considered predominantly a hospital-acquired infection (HA-MRSA), community-

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acquired MRSA (CA-MRSA) has emerged as a major public health threat (David and Daum, 2010). According to CDC data, there were estimated to be 75,309 cases of MRSA infections in the U.S. in 2011, with 11,285 deaths (CDC, 2012). A delay in initiation of appropriate therapy is associated with increased mortality (Lodise and McKinnon, 2005). Therefore, an assay to detect MRSA infection rapidly with high specificity and sensitivity is highly desirable to initiate appropriate antibiotic therapy.

Many *Staphylococcus* species other than *S. aureus* are opportunistic pathogens in both humans and animals, and cause serious diseases of the skin and other body tissues and cavities (Kloos and Musselwhite, 1975; Scott et al., 2001). The common staphylococcal species recognized as animal pathogens include *S. aureus, S. pseudintermedius, S. hyicus, S. sciuri,* and *S. schleiferi* subspecies *coagulans* (Devriese et al., 2005; Frank et al., 2003; Nemeghaire et al., 2014; Werckenthin et al., 2001). In humans, *S. aureus* is the predominant pathogen, whereas *S. pseudintermedius* and *S. schleiferi* are the primary pathogens in dogs (Devriese et al., 2005; Frank et al., 2003; May et al., 2005; Oehler et al., 2009; van Duijkeren et al., 2011). *S. pseudintermedius, S. schleiferi,* and *S. aureus* have been described as both commensal and pathogenic species of cats (Abraham et al., 2007; van Duijkeren et al., 2011). Pet animals are believed to acquire *S. aureus* infections from humans, and bidirectional transmission of *S. aureus* has been reported (Weese, 2005; Weese et al., 2006). Methicillin resistance also occurs in *S. pseudintermedius* (MRSP), *S. sciuri,* and *S. schleiferi* (MRSS), and the incidence of methicillin resistance in these and other *Staphylococcus* species is increasing (Nemeghaire et al., 2014). Methicillin-resistant staphylococci have also been isolated from domestic livestock (cows, chickens, horses, and pigs) (Leonard and Markey, 2008). As in humans, methicillin-resistant staphylococci colonize skin, nasal, and the oral mucosa in healthy animals, and infections in animals have been associated with risk factors such as hospitalization, surgery, wounds, chronic disease, and immunosuppression.

A biosensor detects, records, and transmits information regarding a physiological change or the presence of various biological and chemical materials in the environment. Specifically, biosensors are probes that integrate biological components (such as enzymes, antibodies, nucleic acids, etc.) with a physico-chemical transducer (optical, electrochemical, thermometric, piezoelectric) to yield a measurable signal (Ramsden, 1997). Key advantages of optical fibers for use as biosensors include light weight, long interaction length, low cost, and the ability to excite the target molecules and capture the emitted light from the targets. In biosensing applications, an optical fiber can produce a signal that is proportional to the concentration of a chemical or biochemical to which the biological element reacts. Optical fiber grating devices, in which a periodic variation is induced in the refractive index of the optical fiber core, operate by inducing a large decrease in the transmittance of light through the fiber at a specific wavelength. This wavelength (or in the case of turnaround point long-period gratings, the transmitted power) can be modified by temperature, pressure, or binding events (Kersey, 1997).

We have previously shown that optical fibers with long-period gratings (LPGs) exhibit exceptional sensitivity to adsorption of ionic self-assembled multilayers (ISAMs) on the surface of the fiber cladding (Wang et al., 2005a, 2005b). ISAM films (also commonly referred to as layer-by-layer (LBL) films) are a class of materials that allow detailed structural and thickness control at the nanometer level, combined with straightforward manufacturing and low cost (Decher, 1997; Decher and Schmitt, 1992). The ISAM method simply involves the alternate immersion of a charged substrate into an aqueous solution of a polycation and an aqueous solution of a polyanion at room temperature. The LPG causes a strong attenuation at a specific wavelength in the single-mode fiber transmission spectrum due to coupling of light from the fundamental guided mode to a high loss higher order cladding mode. Since LPGs couple light into the cladding, the coupling wavelength can be highly sensitive to material external to the cladding. The ISAM film adsorbed onto the cladding surface causes large wavelength shifts of >1.7 nm per nm of film thickness (Wang et al., 2005a, 2005b). The sensitivity of the sensor has been increased by orders of magnitude by utilizing novel turnaround point (TAP) LPGs. In addition to their exceptional sensitivity, TAP-LPGs offer the additional attractive feature that they exhibit a broadband attenuation that shifts in magnitude rather than wavelength (Wang and Ramachandran, 2003). TAP-LPGs have strong, broadband attenuation peaks that are highly sensitive to changes on the exterior of the optical fiber cladding, providing a highly sensitive, robust, inexpensive biosensor platform where the presence of target materials is detected simply by changes in the transmitted intensity at a particular wavelength. The ISAM film amplifies the sensitivity by providing a high refractive index, large surface area, and a nanoscale coating on the cladding that can be readily coupled to a vast array of receptor molecules such as antibodies and DNA.

We have previously demonstrated that the ISAM TAP-LPG biosensor platform can be used to measure the binding of streptavidin to biotin immobilized on the ISAM surface (Wang et al., 2009). We now present results utilizing a nanoscale-film optical fiber sensor (NOFS) consisting of an optical fiber with nanoscale self-assembled film coatings bound to monoclonal antibodies (MAb) specific to penicillin-binding-protein 2a (PBP2A), which is specific for MR staphylococci. Our results show that the NOFS system provides a promising, culture-free assay for rapid and specific detection of MRSA and other methicillin-resistant staphylococci.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains used and their sources are listed in Tables 1 and 2. All experiments with cultures were carried out in biosafety level (BSL)-2 facilities in an approved biosafety cabinet. Bacteria were grown in brain heart infusion (BHI) (Becton-Dickinson and Sigma-Aldrich, St. Louis, MO) broth, or on BHI agar supplemented with 5% sheep blood (BHIB). Bacterial strains were cultured from -80 °C milk stock suspensions into BHI broth or onto BHIB agar, and incubated at 35 °C. For culture in broth, strains were grown with shaking (175 rpm) in BHI broth at 35 °C. Bacteria grown to mid-log phase in broth cultures were washed once with phosphate buffered saline (pH 7.4) (PBS) and resuspended in PBS to a Klett unit absorbance value of 120, which corresponds to 10^9 colony forming units (CFU)/ml. Serial dilutions of cultures in PBS were streaked onto BHIB agar and incubated overnight at 35 °C to confirm the number of CFU/ml.

All of the animal isolates were obtained from the Veterinary Teaching Hospital at Virginia Tech, and were confirmed to be resistant or sensitive to methicillin by procedures outlined by the Clinical Laboratory Standards Institute (CLSI, 2013). Methicillin-resistant isolates of *S. aureus* (MRSA), *S. epidermidis* (MRSE), *S. haemolyticus* (MRSH), *S. pseudintermedius* (MRSP), and *S. schleiferi* (MRSS) were used. Bacterial species that were *mec*-negative were used to determine the specificity of the assay. The *mec*-negative bacterial species included MSSA, MS *S. hyicus* (MSSH), MS *S. pseudintermedius* (MSSP), *Streptococcus pyogenes, Enterococcus faecalis, E. faecium, Pseudomonas aeruginosa, Pasteurella multocida,* and *Escherichia coli* (Table 1).

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