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Stable oligonucleotide-functionalized gold nanosensors for environmental biocontaminant monitoring

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ABSTRACT

The global propagation of environmental biocontaminants such as antibiotic resistant pathogens and their antibiotic resistance genes (ARGs) is a public health concern that highlights the need for improved monitoring strategies. Here, we demonstrate the environmental stability and applicability of an oligonucleotide-functionalized gold nanosensor. The *mec*A ARG was targeted as model biocontaminant due to its presence in clinically-relevant pathogens and to its emergence as an environmental contaminant. *mec*A-specific nanosensors were tested for antibiotic resistance gene (ARG) detection in ARG-spiked effluent from four wastewater treatment plants (WWTPs). The *mec*A-specific nanosensors showed stability in environmental conditions and in high ionic strength ($[MgCl_2]$ < 50 mM), and high selectivity against mismatched targets. Spectrophotometric detection was reproducible with an LOD of 70 pM ($\approx 4 \times 10^7$ genes/μg), even in the presence of interferences associated with non-target genomic DNA and complex WWTP effluent. This contribution supports the environmental applicability of a new line of cost-effective, field-deployable tools needed for wide-scale biocontaminant monitoring.
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Introduction

Biological contaminants such as those contributing to antibiotic resistance are a complex and pervasive public health threat that calls for immediate and coordinated global attention (Laxminarayan et al., 2013; Woolhouse and Farrar, 2014). Although clinical sources are most often associated with the emergence and dissemination of antibiotic resistance, environmental sources represent a potentially large and diverse reservoir of antibiotic resistance genes that can be horizontally transferred to pathogens of concern to public health (Allen et al., 2010). ARGs can be disseminated from reservoirs of non-pathogenic bacterial hosts and even by uptake of naked DNA (Skippington and Ragan, 2011), both of which advocate for direct monitoring – particularly in environments that are likely nodes for transfer and amplification (*e.g.*, surface, waste, potable, and reclaimed waters; crop soil; hospitals; and human and animal hosts) (Forsberg et al., 2012; Marti et al., 2014; Pruden et al., 2013). On the other hand, direct monitoring of pathogens, especially in their resistant forms, represents a more direct public health protection strategy due to their imminent risk to public health. Accordingly, fast and direct detection strategies are needed in order to monitor and control environmental dissemination of biocontaminants in their different forms.

Wastewater treatment plants (WWTPs) are of special importance as they not only represent a point of biocontaminant convergence, transfer and amplification, but they are also relied

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upon for inactivation or removal of contaminants prior to returning treated waters to the environment (Laxminarayan et al., 2013; Pruden, 2014). However, because WWTPs were not specifically designed to treat contaminants of biological nature (*i.e.*, which can replicate and be transferred), their potential to serve as hot-spots for antibiotic resistance proliferation is of particular concern (Luo et al., 2014; Mao et al., 2015; Rizzo et al., 2013). In this context, monitoring WWTPs and their effluents for biocontaminants of concern can aid in elucidation of their fluxes into the environment and help identify promising treatment technologies.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a notorious antibiotic resistant pathogen with the ability to infect multiple tissues and produce toxins. Multidrug resistant MRSA and highly virulent strains, such as USA300, are widespread (Carrel et al., 2015; Mine et al., 2011); while *S. aureus* strains resistant to the antibiotic of last resort, vancomycin, have been reported (Lindsay, 2013). *mec*A is the most common ARG detected in MRSA and confers resistance to methicillin and other β-lactam antibiotics (Peacock and Paterson, 2015; Shore and Coleman, 2013). *S. aureus* strains that express this gene are resistant to most, if not all, β-lactam antibiotics. Furthermore, MRSA is commonly known to harbor a broad range of ARGs corresponding to various antibiotic classes (Deleo and Chambers, 2009; Nikaido, 2009). Reported MRSA incidences range from 30 to 90% (Pathare et al., 2016; WHO, 2016). Specifically, one study from India reported 45% MRSA incidence with an alarming 10% resistance to vancomycin, an antibiotic of last resort (Mendem et al., 2016). The rise in community acquired MRSA infections (Klein et al., 2013; Stryjewski and Corey, 2014) highlights the public health relevance of this particular pathogen, whereas the recent detection of MRSA strains in WWTP influents and effluents (Borjesson et al., 2010; Goldstein et al., 2012; Gomez et al., 2016; Thompson et al., 2013) highlights its environmental relevance and potential for dissemination *via* the anthropogenic water cycle.

The most common method for detection of antibiotic resistant bacteria, including pathogens, relies on direct culturing on selective media followed by subsequent determination of the minimum inhibitory concentration of individual isolates by the antibiotic of interest. On the other hand, direct detection of ARGs is typically achieved *via* polymerase chain reaction (PCR)-based methods, that rely on enzymatic amplification of the target gene. However, performance of the polymerase enzyme can be strongly influenced by environmental contaminants, often requiring sample-specific optimization by skilled personnel to minimize the effects of inhibitors and other factors. Metagenomic sequencing can also be applied to broadly profile the types of ARGs present and provide relative quantification, although at higher detection limits than PCR-based methods. Metagenomics relies on the random sequencing of fragmented DNA extracted from the sample of interest followed by alignment to known ARG sequences available in databases for identification (Ju and Zhang, 2015). Due to the cost and expertise required, however, metagenomics is not practical for routine monitoring. Microarray-based detection has also been reported (Lu et al., 2010; Perreten et al., 2005). Here, target-specific probes are immobilized onto a solid surface, which are then used to trap and detect the target ARGs. However, application of microarrays to environmental samples is also subject to substantial complexity, particularly with respect to multiple steps of preparation, hybridization and labeling that are each subject to sample and target-specific biases and interferences. In all, alternative strategies for biocontaminant detection that may enable economical, accessible, and accurate environmental monitoring scenarios are of interest.

In reference to sensing technologies, gold nanoparticles (AuNPs) are a promising sensing substrate due to their ideal optical properties, stability, and ease of functionalization. Localized surface plasmons (LSPs), or collective oscillations of conduction electrons on the nanoparticle surface, are an important property inherent to metallic nanoparticles. Due to interactions between LSPs and incident light, these particles exhibit extinction cross-sections that are higher than expected based on their size alone. LSP frequency is highly dependent on nanoparticle size and aggregation state and this property can be exploited in aggregation-based detection schemes by monitoring the wavelength of the LSP resonance band (λ_{LSPR}). Furthermore, functionalization of AuNPs with thiol-functionalized oligonucleotides has been previously described and optimized (Hurst et al., 2006; Zhang et al., 2012), thereby facilitating their application as gene-specific biosensors.

Here, we applied a sequence-specific target-nanoprobe hybridization assay (Fig. 1) to demonstrate the stability and environmental applicability of the oligonucleotide-functionalized nanoprobes for the detection of the *mec*A ARG, a model biocontaminant of concern, in four different treated wastewater effluents. This assay requires minimal sample preparation, does not rely on enzymatic gene amplification, has multiplex application potential, and presents an adaptable platform for environmental monitoring of biocontaminants.

1. Experimental

1.1. Solutions

All buffers and solutions were prepared using autoclaved nanopure (>18 MΩ·cm) water and molecular biology grade reagents. Phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) was prepared as a stock solution and autoclaved and diluted as necessary. Chloroauric acid ($HAuCl_4{\cdot}3H_2O$) and sodium citrate dehydrate (99+%) were purchased from Sigma-Aldrich. Citrate buffer (250 mM, pH 3) was prepared by dissolving sodium citrate in water and adjusting the pH using HCl. 5× stock hybridization buffer consisted of 25% formamide, 20% dextran sulfate, and 5 mM $MgCl_2$ (Storhoff et al., 2004; Wetmur, 1975).

1.2. Probe, target and control sequences

*mec*A-specific probe, single-stranded *mec*A target oligonucleotide and nonspecific control oligonucleotide sequences were reported by Storhoff et al. (2004). Purified and/or thiolated oligonucleotides were purchased from IDT and are described in Table S1. Thiolated probes were activated by incubation in 500 mM DTT in TE buffer.

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