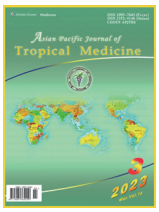

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Tapered optical fiber DNA biosensor for detecting *Leptospira* DNA

Jia−Yong Lam[1], Mohd Hanif Yaacob[2,3], Hui−Yee Chee[1,3]✉

[1]*Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[2]*Department of Computer and Communication Systems Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[3]*Wireless and Photonics Networks Research Centre (WiPNET), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

ABSTRACT

Objective: To establish a DNA detection platform based on a tapered optical fiber to detect *Leptospira* DNA by targeting the leptospiral *secY* gene.

Methods: The biosensor works on the principle of light propagating in the special geometry of the optical fiber tapered from a waist diameter of 125 to 12 μm. The fiber surface was functionalized through a cascade of chemical treatments and the immobilization of a DNA capture probe targeting the *secY* gene. The presence of the target DNA was determined from the wavelength shift in the optical transmission spectrum.

Results: The biosensor demonstrated good sensitivity, detecting *Leptospira* DNA at 0.001 ng/μL, and was selective for *Leptospira* DNA without cross-reactivity with non-leptospiral microorganisms. The biosensor specifically detected DNA that was specifically amplified through the loop-mediated isothermal amplification approach.

Conclusions: These findings warrant the potential of this platform to be developed as a novel alternative approach to diagnose leptospirosis.

KEYWORDS: DNA biosensor; Tapered optical fiber; Leptospirosis; *Leptospira*

1. Introduction

Climate change and recurrent natural disasters have favored pathogens in the environment, causing the emergence of waterborne and vector-borne diseases, such as leptospirosis[1]. Leptospirosis is caused by spirochetes in the *Leptospira* genus[2]. Early diagnosis of leptospirosis is crucial for prompt intervention, as the therapeutic strategies are most effective when initiated during the early phase[3]. However, the nonspecific clinical features during the early acute phase often complicate the diagnosis. The distinctive clinical manifestations of leptospirosis are only observed when the disease has worsened into the severe late phase[4]. As such, leptospirosis is often the cause of undifferentiated febrile illness, particularly in regions where other infectious diseases with overlapping manifestations are endemic[5−7]. Without a robust diagnosis, identifying leptospirosis is often dependent on the suspicion of the clinician but presumptive treatment is common for managing the disease[8].

The gold-standard tests, such as culture and the microscopic agglutination test, are laborious and require proper quality controls;

Significance

The diagnosis of leptospirosis is difficult because of nonspecific clinical manifestations and laborious gold-standard tests. DNA-based detection of the *Leptospira* pathogen remains the fundamental approach to curb such challenges. In this study, a new biosensor was developed that allows for the sensitive detection of *Leptospira*.

✉To whom correspondence may be addressed. E-mail: cheehy@upm.edu.my

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hence, they are often ineffective for an early rapid diagnosis of the disease[9]. Given the potential severity of the disease, molecular detection, such as a DNA-based approach, is a more effective option to detect the etiological agent[4,10]. Detection based on DNA results in higher sensitivity and specificity than the gold-standard or serological tests, particularly during the early phase of the disease[9]. Nucleic acids have been extensively applied in a wide range of biosensors due to their versatile physical, chemical, and biological activities[11]. The biological recognition element in a biosensor, or the bioreceptor, is immobilized on a physical transducer to convert the bioreceptor interaction with its respective analyte into a measurable signal[12]. Hence, the recognition of the bioreceptor for the analyte is based on highly specific complementary binding of nucleotides, allowing for sequence-specific information in the form of measurable signals.

Optical fibers have become an important part of biosensing technology due to their compact size and protection from electromagnetic interference[13,14]. They are often used as transduction elements in biosensors, relying on certain optical transduction mechanisms for detecting the target analyte. In particular, tapered optical fibers have been extensively used to develop systems to detect various biomolecules[15-18]. Unlike a typical optical fiber, where light propagation is confined inside the core with minimal loss and decay in the cladding where reflection occurs[19], the tapering process of a tapered optical fiber allows exposure to the evanescent field. This design allows for an interaction between the transmitted light and the external medium, which, in turn, allows for the evanescent sensing mechanism on the fiber[16]. In DNA biosensing, the evanescent wave interacts with DNA molecules along the sensitive tapered distance of the fiber, causing a change in frequency, phase, or intensity of light with respect to the quantity and configuration of the DNA molecules[16,20,21]. The mathematical principle of biosensing using a tapered optical fiber has been described in a previous study[18] and suggested that changing the refractive index at the external surroundings of the tapered region would affect the phase shift, fringe shift, and output intensity of the light.

In this study, a DNA detection platform was developed based on a tapered optical fiber to detect *Leptospira* spp. The selectivity of the biosensor toward *Leptospira* was ensured by using a DNA probe immobilized on the surface of the fiber that specifically targeted the *secY* gene, a housekeeping gene present in all *Leptospira* spp. The biosensor demonstrated a concentration-dependent response upon exposure to *Leptospira* genomic DNA and was capable of detecting a low concentration of *Leptospira* genomic DNA.

2. Materials and methods

2.1. DNA probe design

The *secY* gene sequences from various *Leptospira* serovars (Serovars: Pomona, Canicola, Bataviae, Icterohaemorrhagiae, Hardjo-prajitno, Lai, Djasiman, Autumnalis; Accession numbers: EU357943.1, EU357947.1, EU357956.1, EU357961.1, EU357983.1, EU357997.1, EU358012.1, and EU358013.1), as well as non-*Leptospira* microorganisms [*Vibrio cholerae*, *Escherichia* (*E.*) *coli* O157: H7, *Salmonella enterica*, *Campylobacter jejuni*, *Clostridioides* (*C.*) *difficile*; Accession numbers: NZ_CP043554.1, NC_002695.2, NC_003197.2, NC_002163.1, and NZ_CP076401.1] were retrieved from the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). Multiple sequence alignment was performed on the sequences using Clustal Omega software (https://www.ebi.ac.uk/Tools/msa/clustalo/) to identify the conserved region of the *secY* gene. A DNA probe with the sequence 5′-CTT GTT CCT GCC CTT CAA A-3′ was designed and synthesized based on the alignment data (Integrated DNA Technologies, Coralville, IA, USA).

2.2. Extraction of the genomic DNA

Genomic DNA of the *Leptospira* reference strains and other non-*Leptospira* bacteria were extracted from their respective pure cultures using the Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA) according to the manufacturer's procedure. The extracted DNAs were quantified for concentration and purity using the NanoDrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and stored at 20 ℃ until further use.

2.3. Fabrication of the tapered optical fiber and experimental setup

Single-mode optical fibers (SMFs) (Lucent Technologies, New Providence, NJ, USA) with a standard core and cladding diameters of ~8 μm and 125 μm, respectively, were utilized to fabricate the tapered optical fibers in this study. The tapered fibers were fabricated using the Vytran GPX-3400 glass processing workstation (Vytran, Morganville, NJ, USA) by modifying the optical fiber dimensions using the heat and pull principle. The real-time control system of the workstation enables users to control the dimensions and uniformity of the fibers, by ensuring that the pulling speed remains constant at 1 mm/s, and heat power at 42 W. The initial cladding diameter of the fiber was tapered down to 12 μm, with a symmetric up-and-down transition of the taper at the length of 5

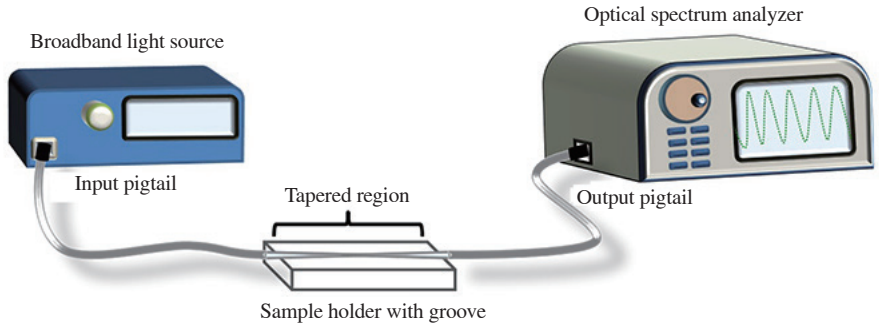

Figure 1. Experimental setup of the tapered optical fiber biosensor and the detection system.

mm, and a tapered waist length fixed at 15 mm. The dimensions of the tapered optical fiber were further validated using the machine's microscope and camera. The dimensions of the tapered fibers used in this study have been optimized and reported in previous studies[15,18].

The fabricated tapered optical fiber was secured onto a custom-made sample holder with the tapered region positioned within the straight sample well-groove. The reagents and analytes were introduced to the tapered region by pipetting into the vacant groove. Both ends of the optical fiber were spliced into SMF pigtails using an optical fiber fusion splicer (Sumitomo Electric, Osaka, Japan). The input end of the pigtail was connected to the single-mode FC/PC patch cord of the Amonics ALS 18-B-FA broadband light source (Amonics, San Po Kong, Hong Kong), while the output end was connected to the Yokogawa AQ6331 optical spectrum analyzer (OSA) (Yokogawa, Tokyo, Japan). The transmission output spectrum from the OSA was recorded into a computer using LabView in-house software.

2.4. Surface functionalization of the tapered optical fiber

The tapered region must undergo a series of functionalization steps to be immobilized with the DNA probe to capture leptospiral DNA. Functionalization is essential to promote the conjugation between the inorganic surface, such as the optical fiber, to the organic biological elements. Surface functionalization was initiated by incubating the tapered fiber in 0.1 M sodium hydroxide (NaOH) (Sigma-Aldrich, St. Louis, MO, USA) for 45 min. The solution was drained, and the tapered region was rinsed three times with deionized water. Then, 2% v/v (3-aminopropyl) triethoxysilane (APTES) (Sigma-Aldrich) was introduced to the tapered region and incubated for 45 min. The tapered region was rinsed three times with deionized water and left to air-dry at room temperature. A 2% v/v glutaraldehyde solution (VWR International, Monroeville, PA, USA) was applied to the tapered region for 1 hour, followed by the same rinsing and drying steps. A 1 μM probe solution was introduced onto the tapered region of the optical fiber and incubated for 1 hour to immobilize the DNA probe. The surface of the tapered region was rinsed three times with deionized water and air-dried at room temperature. The experimental setup of the biosensor detection system is illustrated in Figure 1.

2.5. Hybridization and detection of the target DNA

The transmission output spectrum of the immobilized DNA probe was recorded *via* OSA as "Probe" before introducing the DNA sample onto the tapered region of the fiber. The genomic DNA was heated at 95 ℃ for 5 min, followed by an immediate cold shock on ice for 1 min to denature the double-stranded DNA into single strands for DNA hybridization[22]. The denatured DNA sample was pipetted onto the tapered region of the functionalized optical fiber and allowed to hybridize for 1 hour. After the incubation, the unbound DNA was aspirated off and the tapered region was rinsed three times with deionized water and left to air-dry at room temperature. The transmission output spectrum was once again recorded *via* the OSA as the "DNA sample." The data were transferred to a spreadsheet for analysis, where the transmission spectra for the "Probe" and the "DNA sample" were compared and the wavelength shift in both spectra was determined.

2.6. Sensitivity and specificity testing of the tapered optical fiber biosensor

The genomic DNA isolated from *Leptospira* (*L.*) *interrogans* was serially diluted ten-fold from 0.1 to 0.001 ng/μL to determine the analytical sensitivity of the biosensor. The diluted genomic DNA was separately tested on each taper profile in triplicate. The genomic DNAs isolated from 17 *Leptospira* reference strains (pathogenic *L. interrogans* serovars Serawak, Canicola, Djasiman, Autumnalis, Australis, Pyrogenes, Lai, Copenhageni, Terengganu, Icterohaemorrhagiae, Bataviae, and Melaka; pathogenic *L. borgpetersenii* serovars Bataviae and Javanica; pathogenic *L.*

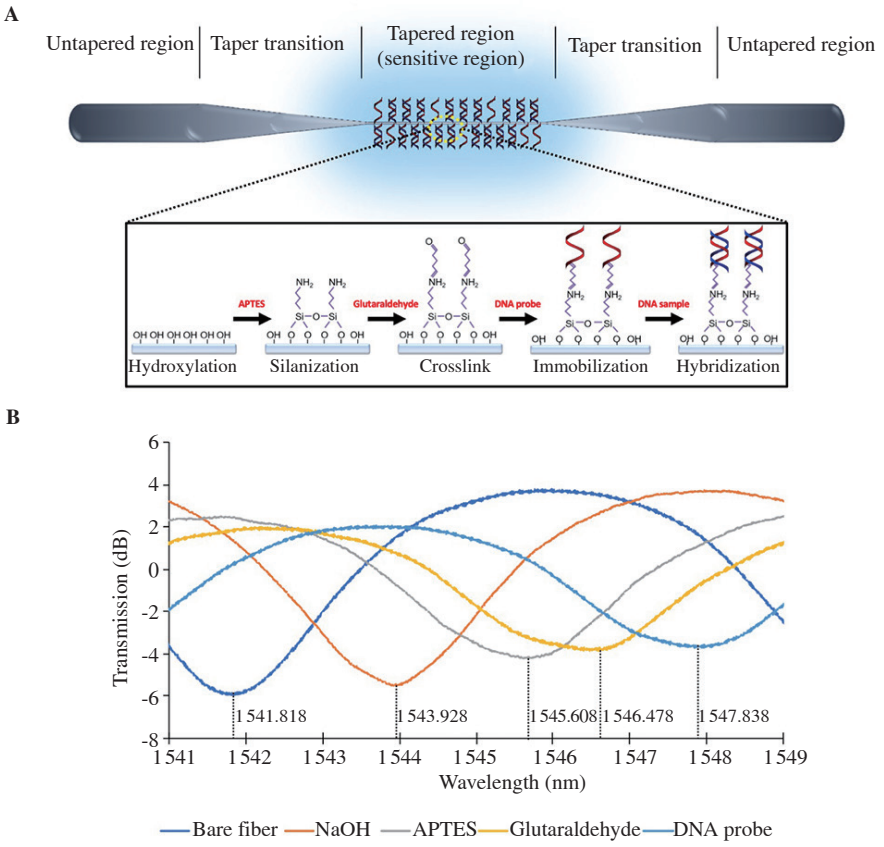

Figure 2. Surface functionalization of the tapered optical fiber biosensor. (A) Schematic illustration of the geometry of the tapered optical fiber and its surface modification during functionalization. (B) Spectral response of the tapered optical fiber indicating a wavelength shift after each functionalization step from NaOH, APTES, glutaraldehyde, and DNA probe immobilization. APTES: (3-aminopropyl) triethoxysilane.

kirschneri serovar Grippotyphosa; saprophytic *L. biflexa* serovar Patoc) and 3 non-*Leptospira* bacteria (*C. difficile*, *E. coli* strain XL10G, and a clinical isolate of methicillin-resistant *Staphylococcus aureus*) were used to assess the specificity of the biosensor, in which 0.1 ng/μL of each genomic DNA was tested in triplicate. All bacterial cultures were obtained from the Microbiology Laboratory, Universiti Putra Malaysia, Malaysia, where they were maintained. As described in a previous study, the *Leptospira* reference strains used by the Microbiology Laboratory, Universiti Putra Malaysia, Malaysia were obtained from the World Health Organization Leptospirosis Collaborating Center, Amsterdam (the Netherlands) and the Institute for Medical Research, Malaysia[23].

2.7. Detection of amplified DNA using the tapered optical fiber biosensor

The tapered optical fiber biosensor was tested for detecting the target DNA amplified using the loop-mediated isothermal amplification (LAMP) method. The LAMP assay was designed to target the *Leptospira secY* gene as described previously, in which the reaction parameters were optimized with slight modifications[24]. The LAMP reaction was assembled in a 25 μL reaction volume comprised of 1× *Bst* ThermoPol® buffer (New England Biolabs, New Ipswich, MA, USA), 6 mM magnesium sulfate, $MgSO_4$ (New England Biolabs), 1.4 mM of each dNTP (First Base Laboratories, Selangor, Malaysia), 0.2 μM each of the F3 and B3 primers, 1.6 μM each of the FIP and BIP primers, 0.8 μM each of the LoopF and LoopB primers, and 8 U of *Bst* DNA polymerase, large fragment (New England Biolabs). Genomic DNA of *L. interrogans* serovar Pomona (3 and 30 ng) was used as the positive control template for the LAMP reactions. Sterile deionized water was used as the nontemplate control in place of the genomic DNA template. The LAMP reaction was performed for 20 min, and then the reaction was terminated at 85 ℃ for 5 min. The reaction products were analyzed by agarose gel electrophoresis, followed by ethidium bromide staining, and visualized on a gel documentation system (Bio-Rad Laboratories Inc., Hercules, CA, USA). A positive amplification reaction was characterized by the presence of a ladder-like pattern. The reaction products were maintained on ice before testing on the tapered optical fiber biosensor using the procedure described in Section 2.5.

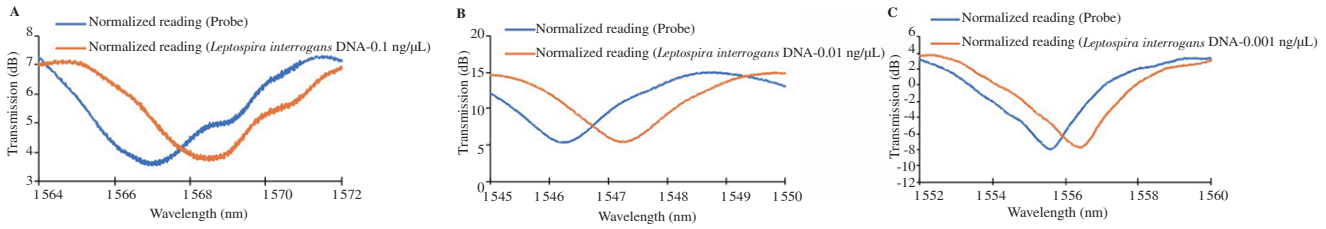

Figure 3. Transmission output spectra of *Leptospira interrogans* genomic DNA at different concentrations. Transmission spectra when tested at (A) 0.1 ng/μL, (B) 0.01 ng/μL, and (C) 0.001 ng/μL of *Leptospira interrogans* genomic DNA.

3. Results

3.1. Functionalization of the tapered optical fiber

The surface of the inorganic tapered optical fiber was modified to allow the binding of biological molecules, such as the DNA probe. Figure 2A depicts the surface functionalization process and the modifications made to the surface of the tapered fiber following a stepwise treatment with chemicals and linkers. Figure 2B shows the transmission output spectra of the bare tapered optical fiber and each of the surface functionalization steps. The transmission spectra demonstrated a red shift after each functionalization reaction. The spectrum shifted 2.11 nm to the right after NaOH hydroxylation. The spectrum continued a rightward shift of 1.68 nm and 0.87 nm following APTES silanization and the glutaraldehyde treatment, respectively. After the DNA probe was immobilized, the spectrum shifted another 1.36 nm, indicating that each of the steps altered the effective refractive index of the surface of the tapered optical fiber, suggesting successful chemical modification of the surface.

3.2. Sensitivity of the tapered optical fiber biosensor

To determine analytical sensitivity, each dilution of the serially diluted *L. interrogans* genomic DNA (0.1-0.001 ng/μL) was separately tested on the functionalized tapered fiber biosensor. Figure 3 shows the pre- and posthybridization transmission spectra for all three concentrations tested. *C. difficile* genomic DNA (0.1 ng/μL) was used as a nontarget control for comparison. The wavelength shift data for the sensitivity test are summarized in Figure 4. The biosensor demonstrated a concentration-dependent response to *L. interrogans* genomic DNA. A two-sample *t*-test revealed that the wavelength shift exhibited by the lowest concentration (0.001 ng/μL) of *L. interrogans* genomic DNA was significantly higher (P=0.000 2) than that of the nontarget *C. difficile* genomic DNA. The increase in the wavelength shift continued with the higher concentrations of *L. interrogans* genomic DNA with greater significance (P<0.000 1 for both 0.01 ng/μL and 0.1 ng/μL).

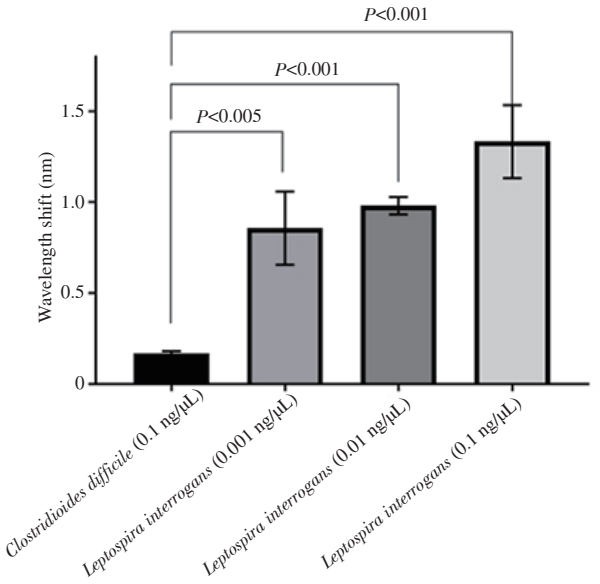

Figure 4. Wavelength shift in the different concentrations of *Leptospira interrogans* genomic DNA on the tapered optical fiber biosensor. Unpaired two-sample *t*-tests were performed to compare the means of the wavelength shift for each tested concentration against the mean wavelength shift of the nontarget *Clostridioides difficile*. The *P*-value is indicated in the figure. Error bars indicate standard deviations.

3.3. Specificity of the tapered optical fiber biosensor

The tapered optical fiber biosensor was tested for analytical specificity on 17 genomic DNAs from *Leptospira* reference strains and 3 from non-*Leptospira* bacteria. As shown in Figure 5A, the spectra pre- and posthybridization procedure of *C. difficile* DNA was comparable, with a minimal wavelength shift, indicating that hybridization did not occur between the probe and *C. difficile* DNA. Hybridization occurred in the presence of the target DNA, resulting in an altered fiber surface refractive index, thereby shifting the spectrum pattern, as shown in Figure 5B. The wavelength shifts for each of the genomic DNAs tested are quantitated in Table 1. The wavelength shifts produced by the *Leptospira* genomic DNAs were compared to the wavelength shifts of the nontarget controls (*C. difficile*, *E. coli*, and methicillin-resistant *Staphylococcus aureus*).

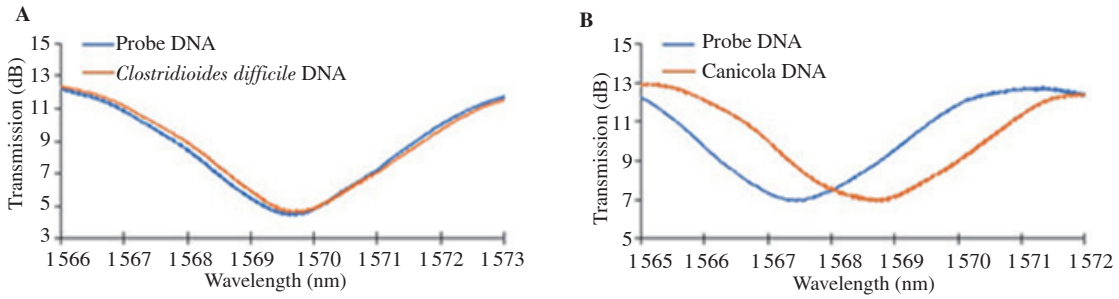

Figure 5. Representative transmission output spectra of non-*Leptospira* and *Leptospira* genomic DNA. Transmission spectra when tested with 0.1 ng/μL of genomic DNA from (A) *Clostridioides difficile* and (B) the *Leptospira interrogans* serovar Canicola.

Table 1. Means of the wavelength shifts and statistical analysis of the non-target controls.

Serovars	Wavelength shift (mean±SD, nm)	*P*-value*		
		Clostridioides difficile	*Escherichia coli*	MRSA
Leptospira biflexa serovar Patoc	0.875±0.072	<0.01	0.020	<0.01
Leptospira interrogans serovar Copenhageni	0.997±0.025	<0.01	<0.01	<0.01
Leptospira kirschneri serovar Grippotyphosa	1.055±0.340	<0.01	0.030	0.013
Leptospira interrogans serovar Serawak	1.194±0.253	<0.01	<0.01	<0.01
Leptospira interrogans serovar Canicola	1.236±0.089	<0.01	<0.01	<0.01
Leptospira interrogans serovar Autumnalis	1.250±0.241	<0.01	0.011	<0.01
Leptospira interrogans serovar Lai	1.375±0.009	<0.01	<0.01	<0.01
Leptospira borgpetersenii serovar Tarassovi	1.600±0.513	<0.01	<0.01	<0.01
Leptospira interrogans serovar Icterohaemorrhagiae	1.518±0.830	<0.01	0.063	0.045
Leptospira interrogans serovar Australis	1.672±0.217	<0.01	<0.01	<0.01
Leptospira interrogans serovar Bataviae	1.655±0.149	<0.01	<0.01	<0.01
Leptospira interrogans serovar Melaka	1.638±0.495	<0.01	<0.01	<0.01
Leptospira interrogans serovar Terengganu	1.727±0.474	<0.01	<0.01	<0.01
Leptospira interrogans serovar Pyrogenes	2.494±1.389	0.027	0.046	0.038
Leptospira interrogans serovar Pomona	2.120±0.807	<0.01	0.012	<0.01
Leptospira borgpetersenii serovar Javanica	2.683±0.139	<0.01	<0.01	<0.01
Leptospira interrogans serovar Djasiman	3.051±0.063	<0.01	<0.01	<0.01

*P-values obtained from unpaired two-sample t-tests *vs*. the non-target controls. MRSA: Methicillin-resistant *Staphylococcus aureus*.

The findings indicate that the biosensor significantly discriminated the 17 tested *Leptospira* genomic DNAs from the 3 non-*Leptospira* genomic DNAs (P<0.05), except the *L. interrogans* serovar Icterohaemorrhagiae compared to *E. coli*.

3.4. Detection of LAMP-amplified DNA using the tapered optical fiber biosensor

In the present study, the biosensor was also tested with LAMP-amplified DNA. The LAMP assay was performed on the nontemplate and positive controls under optimized conditions before detecting the respective wavelength shifts on the biosensor. Figure 6A shows the transmission spectra obtained when the biosensor was tested on the LAMP reaction products. The nontemplate control exhibited a minimal wavelength shift, which correlated with the absence of a ladder-like pattern on the agarose gel. The ladder-like pattern on the gel is characteristic of a positive DNA amplification reaction (Figure 6B). As such, the wavelength shifts observed for the positive control (3 and 30 ng DNA template concentrations) correlated with the appearance of the ladder-like pattern signifying amplification of the target DNA, and the shifts were significant compared to the nontemplate control (Figure 6C).

4. Discussion

In the present study, a DNA biosensor based on a tapered optical fiber was developed to detect *Leptospira* DNA. The tapered optical fiber was functionalized through a cascade of chemical and linker treatments to allow the binding of the target analyte. The surface of the fiber was first modified with hydroxyl groups (-OH) by sodium hydroxide (NaOH) hydroxylation. These hydroxyl groups are vital for attaching the silanols for crosslinking reactions[15,25]. The silanes from APTES are affixed to the surface through the siloxane (Si-O-Si) linkage *via* the hydroxyl groups introduced earlier. The resulting surface is now modified with amine groups, which readily react with the bifunctional molecule glutaraldehyde. As such, a linker is formed to allow the covalent bonding of the DNA probe to the surface of the

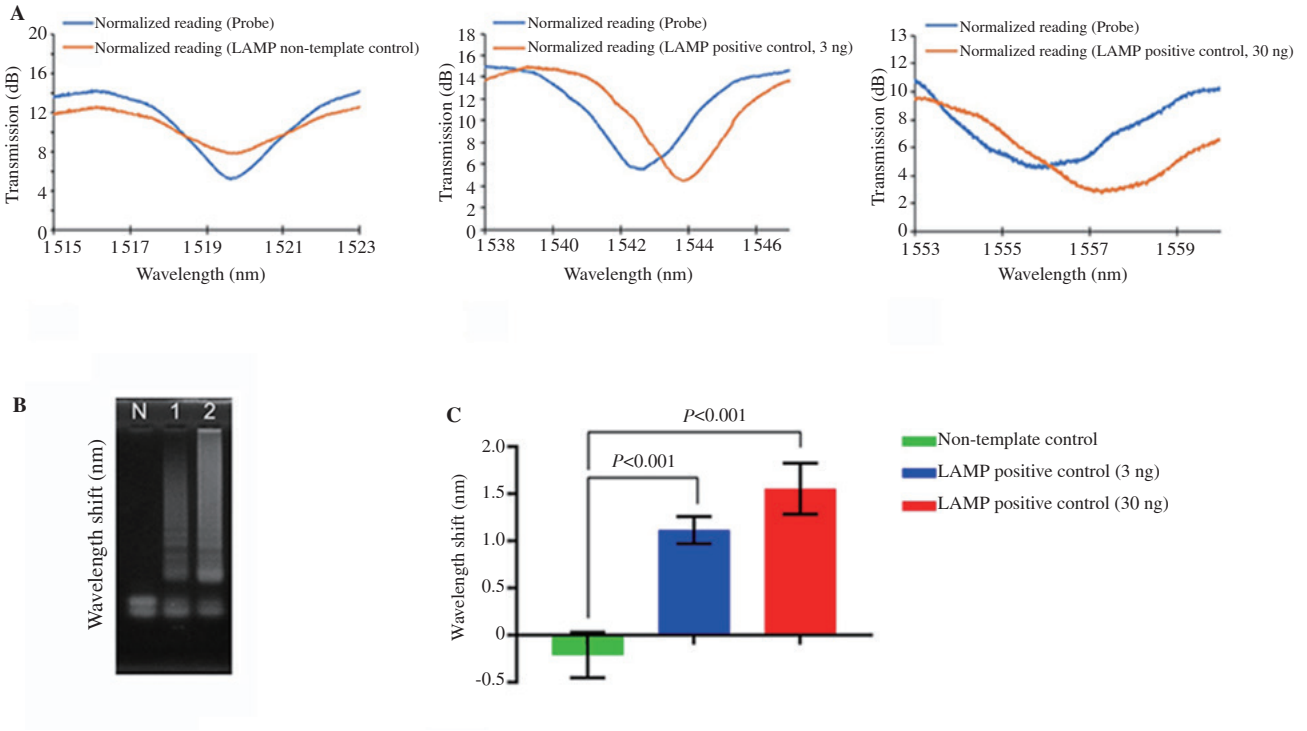

Figure 6. Testing of the tapered optical fiber biosensor for detecting loop-mediated isothermal amplification (LAMP)-amplified target DNA. (A) Spectral response when tested on (from left to right) the LAMP reaction nontemplate control, LAMP amplicons from a 3 ng positive control template, and a 30 ng template. (B) The outcome of 1.5% agarose gel electrophoresis on the LAMP reaction products, where N represents the nontemplate control and lanes 1 and 2 represent the LAMP reaction products from the 3 and 30 ng positive control templates, respectively. (C) Wavelength shift in the LAMP reaction products when tested on the tapered optical fiber biosensor. Each bar represents the mean wavelength shift and error bars indicate standard deviations. The *P*-value from the unpaired two-sample *t*-tests is indicated in the figure.

fiber. With the DNA probe in place, the target DNA is captured based on Watson-Crick base pair matching, and hybridized into a duplex formation[21].

In this study, the DNA probe was designed to target the leptospiral *secY* gene as opposed to the rrs gene probe used in a previous study[18]. Located in the S10-spc-α locus containing genes for ribosomal proteins, the *secY* gene encodes the leptospiral preprotein translocase, a processive enzyme[26]. The *secY* and *rrs* genes are *Leptospira* housekeeping genes and are ubiquitously expressed across all leptospiral species, thereby allowing the detection of pathogenic and saprophytic species of *Leptospira*[27,28]. The unusual presence of saprophytic *Leptospira* has been reportedly detected in suspected leptospirosis patients[29]. As such, a detection platform that focuses only on the pathogenic strains may lead to false-negative results. A recent meta-analysis on the diagnostic accuracy of various leptospiral genetic markers, such as *secY*, *rrs*, *lipL32*, and *flaB*, revealed that nucleic acid-based assays targeting the *secY* gene are promising for detecting pathogens[30]. Therefore, the *secY* gene was selected as the target gene in this work to enable extensive detection of leptospiral DNA regardless of its pathogenicity.

Although previous studies have successfully demonstrated the use of a tapered optical fiber as a DNA biosensing platform, synthesized short oligonucleotides were utilized as the complementary and non-complementary target DNA to determine the sensor's sensitivity and specificity in these studies[18,20,31,32]. While using synthesized short oligonucleotides as target DNA allows flexibility to control the number of mismatches when testing a biosensor's specificity, the downside is that it does not resemble the actual genome of the target organism, which is ultimately the analyte the biosensor was designed to capture. In a previous study targeting the *rrs* gene[18], the complementary and non-complementary oligonucleotides included 20 bases, resulting in a massive difference compared to the actual *Leptospira* genome size at approximately 5 Mb. Henceforth, genomic DNA isolated from a pure bacterial culture was utilized throughout this study to more accurately determine the sensitivity and specificity of the biosensor.

The biosensor demonstrated good sensitivity and detected *Leptospira* genomic DNA down to 0.001 ng/μL, which is lower than the reported limit of detection in a previous study targeting the *rrs* gene[18]. In theory, due to the design of the DNA probe, only *Leptospira* DNA with the *secY* gene can be hybridized to the probe. Thus, when tested on a nontarget sample, such as *C. difficile* DNA, hybridization of the

probe on the *C. difficile* DNA did not materialize, and hence caused no morphological or refractive index changes on the surface of the fiber, which was evident by the minimal wavelength shift observed. In contrast, when tested on the DNA of various *Leptospira* serovars, the biosensor differentiated these DNAs against the three nontarget controls, except one serovar. Technical limitations accounted for the variations in the wavelength shifts across experiments, which may have been contributed during the fabrication and surface functionalization process, as a new fiber was utilized for each test. In particular, the functionalization process is particularly susceptible to variability, as the chemical reactions involved are highly dependent on the temperature and humidity of the surroundings[15,33].

The approach of combining a nucleic acid biosensor with a DNA amplification technique is intriguing due to the improved sensitivity and specificity of the biosensor[34-36]. Such a combination offers exciting opportunities to improve the development of integrated analytic systems, sensor technologies, sensing strategies, as well as analytical instrumentation and procedures[37]. In the present study, we tested the applicability of a tapered optical fiber biosensor to detect amplified target DNA. Here the target DNA was amplified through the LAMP reaction. LAMP is a nucleic acid amplification technique that is completely isothermal within 1 hour[38]. As such, LAMP is the best detection method for various pathogens over other DNA amplification techniques, such as polymerase chain reaction analysis[39,40]. The LAMP assay utilized in this study was developed in a previous study to target the *Leptospira secY* gene[24]. The outcome suggests the applicability of the tapered optical fiber biosensor to specifically detect the amplified target DNA, thus indicating the potential for integrating both techniques into a more robust biosensor.

The development of tapered optical fiber DNA biosensors is still in its infancy, and as such, it is important to address the inconsistencies and the manual nature of the functionalization process. Digital microfluidics has been in the limelight in the fast-evolving field of operation platforms for molecular nucleic acid diagnostics[41]. Reagent consumption is reduced, detection limits are improved, and a faster reaction time is achieved after incorporating a biosensor in digital microfluidics devices[42,43]. More importantly, it is possible to fully automate the process while ensuring controlled chemical and biological reactions, which eventually reduce the overall cost and cross-contamination[44]. This improved device will have potential application as an alternative nucleic acid detection tool for leptospirosis, particularly in countries where the disease is endemic. The nucleic acid-based approach for a biosensor can help with earlier and more accurate interventions and thus will benefit patient management, prevent unnecessary drug use, and reduce overall healthcare costs. The versatility of the LAMP assay developed to detect *Leptospira* in human, animal, and environmental samples will lead to expanded applicability of the biosensor. Given that leptospirosis is an environmentally-associated disease, the application of the biosensor to environmental samples will help with the environmental surveillance of bacteria to monitor, predict, and control disease outbreaks. The tapered optical fiber in the biosensor was a scaffold for the technology to develop biosensors for other diseases where a simple and cost-effective method is needed.

In conclusion, a *Leptospira* DNA detection platform based on a tapered optical fiber was presented herein, as molecular-based detection of *Leptospira* remains an effective diagnostic approach. The biosensor allowed for selective detection of *Leptospira* spp. by targeting the *Leptospira secY* gene, regardless of its pathogenicity classification. The findings demonstrated the detection of amplified DNA using the biosensor and thus suggest its integration in future work. These findings will pave the way for the future development of the biosensor as a new leptospirosis diagnostic paradigm that can be used in field and clinical settings.

Conflict of interest statement

The author declare that they have no conflict of interest.

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Authors' contributions

JYL, MHY and HYC conceptualized and designed the study. JYL collected the data and performed analysis and interpretation of the data. MHY and HYC provided the software for analysis and validated the analysis. JYL wrote the original draft. JYL, MHY and HYC have critically reviewed, revised, and approved the final version of the manuscript. MHY and HYC supervised the project. HYC acquired the funding.

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