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Dual-fiber optic bioprobe system for triglyceride detection using surface plasmon resonance sensing and lipase-immobilized magnetic bead hydrolysis

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ABSTRACT

The rapid and accurate detection of triglyceride (TG) plays a valuable role in the prevention and control of dyslipidemia. In this paper, a novel method for TG detection using a dual-fiber optic bioprobe system, which can accurately detect different levels of TG concentration in serum, is proposed. The system employs disposable microprobe-type fiber optic surface plasmon resonance (SPR) biosensors for signal acquisition, providing high stability and portability while avoiding cross-contamination caused by repeated use. The proposed biosensor with a high sensitivity of 1.25 nm/(mg/mL) for TG detection in serum and a tiny diameter of 125 μm, was fabricated using a novel multimode fiber–single-mode fiber–reflector (MSR) structure, which has been scarcely ever reported to the best of our knowledge. In the process of TG detection, lipase-immobilized magnetic beads were introduced to specifically hydrolyze TG, and the relationship between the TG content and the SPR differential signal was obtained from dual-fiber optic bioprobe measurements of the TG sample before and after hydrolysis. The proposed method achieved TG detection in the concentration range of 0–8 mg/mL (including healthy and unhealthy levels of TG concentration in the human body). Additionally, the miniaturized fiber optic biosensors used in this work have the advantages of low sample consumption, high sensitivity, simple operation, label-free measurement, high selectivity, and low cost. This method provides a new pathway for rapid and reliable TG detection and has potential applications in medical research and clinical diagnosis.

Dear editors,

1. Introduction

With the development of the economy and the improvement in human living standards, dyslipidemia caused by unhealthy dietary habits, overnutrition, and other reasons has gradually become a common disease (Peng et al., 2020). However, it is difficult to completely cure dyslipidemia because drugs can only temporarily reduce blood lipid levels. Blood lipid monitoring has become an effective method for preventing and controlling dyslipidemia. Therefore, the development of rapid, accurate, and portable blood lipid detection tools has become an important task in medical research (Gao et al., 2017). Triglyceride (TG) concentrations are routinely assessed in blood lipid tests. Increased TG levels indicate an increased risk for various diseases, such as arteriosclerosis (Farnier et al., 2021), coronary heart disease, kidney disease, and pancreatitis (Park et al., 2020). Decreased TG levels are common in hyperthyroidism, severe liver dysfunction, and malnutrition (Kotwal et al., 2020). Normal and decreased TG levels in the blood correspond to less than 1.5 mg/mL and less than 0.5 mg/mL, respectively. TG levels in the range of 1.51–1.99 mg/mL indicate a borderline concentration, 2–4.99 mg/mL is considered high, and levels above 5 mg/mL correspond to a severe condition (Laufs et al., 2019). Therefore, the determination of TG blood levels plays an important role in clinical diagnosis.

Various methods are available for TG detection, such as chemical

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(Neri and Frings, 1973), chromatographic (Prados et al., 2012), and enzymatic (Pundir and Narwal, 2018) methods. Chemical methods involve a tedious process and sophisticated technology for operation (Klotzsch and McNamara, 1990). Chromatographic methods require expensive equipment and complex sample preparation (Hou et al., 2016). Both chemical methods and chromatographic methods have excellent detection accuracy, but these approaches cannot meet the requirements of simple, fast, and portable detection. The enzymatic method is the most widely used method for TG detection, with the advantages of stable reagents, high sensitivity, and a wide linear range (Kalia and Pundir, 2004). To collect TG detection signals, the enzymatic method uses biosensors, which improve the detection efficiency and accuracy (Pundir and Narang, 2013). Different types of enzyme-based TG biosensors have been reported, including enzyme electrodes (Wu et al., 2014), enzyme field-effect transistors (Vijayalakshmi et al., 2008), fluorescent sensors (F Sun et al., 2018), and fiber optic enzyme (Baliyan et al., 2013) sensors.

Among these biosensors, fiber optic biosensors have the characteristics of small size, low cost, portability, and high sensitivity (Zhao et al., 2019). At present, several types of fiber optic biosensors have been reported for TG detection, such as fiber optic surface plasmon resonance (SPR) (Baliyan et al., 2013), fiber optic localized SPR (LSPR) (Baliyan et al., 2017), and long-period fiber grating (LPG) (Baliyan et al., 2016) biosensors. Fiber optic LSPR biosensors and LPG biosensors both use covalent binding to immobilize lipases on the sensor surface for the specific hydrolysis of TG, whereas fiber optic SPR biosensors employ the gel entrapment method to immobilize lipases. All of these enzyme-based biosensors achieve good detection. However, when preparing these TG biosensors, the enzyme immobilization procedure is complicated, and the introduction of a specific recognition layer may not completely block influences from the serum background. Because it is difficult to separate serum when detecting human TG, it is more meaningful to directly determine the TG concentration in serum. Although some fiber optic sensing methods can be used for the quantitative analysis of TG, it is still challenging to determine the TG level in a serum background.

Lipases can hydrolyze TG into glycerol and fatty acid, which inevitably causes a slight change in the refractive index (RI) of the detection environment. SPR is a widely used surface analysis technology and is sensitive to changes in the surface RI (Homola et al., 1999). Compared with other biological detection methods, SPR technology does not require labeling or amplification and does not affect the activity of biomolecules (Zhou et al., 2019)(Yuan et al., 2020). Moreover, fiber optic SPR biosensors combine the advantages of fiber optic sensing and SPR technology, with a compact structure, high sensitivity, online real-time detection, low reagent consumption, and low cost (Lu et al., 2020). Therefore, the authors believe that fiber optic SPR biosensors have great development potential for TG detection.

In this paper, a miniaturized fiber optic SPR biosensor with a diameter of 125 μm is introduced for TG detection. By combining this biosensor with lipase-immobilized magnetic bead hydrolysis technology, different levels of TG concentrations in serum were successfully distinguished. The proposed biosensor was fabricated using a novel microprobe-type hetero-core fiber structure, i.e., a multimode fiber–single-mode fiber–reflector (MSR) structure. Compared with traditional fiber optic SPR biosensors, which are hundreds of microns in diameter, the proposed fiber optic MSR-SPR biosensor has been significantly improved in size and flexibility. In addition, a dual-fiber optic MSR-SPR bioprobe detection method was proposed to ensure accuracy and specificity in TG detection. For convenience, the "biosensor" described in this paper is also denoted as a "bioprobe."

2. Principles and methods

2.1. Dual-fiber optic MSR-SPR bioprobe detection method

Enzymes can catalyze a specific target substance, changing the amount of the substance to be measured in the environment. For example, lipases can hydrolyze TG into glycerol and fatty acid (Cheng et al., 2021) as follows:

$$\begin{array}{l} CH_2OCOCH_2R_1 \\ | \\ CH_2OCOCH_2R_2 \\ | \\ CH_2OCOCH_2R_3 \\ \text{Triglyceri des} \end{array} + 3H_2O \xrightarrow{\text{Lipase}} \begin{array}{l} CH_2OH \\ | \\ CH_2OH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array} + \begin{array}{l} R_1CH_2COOH \\ | \\ R_2CH_2COOH \\ | \\ R_3CH_2COOH \\ \text{Fatty Acids} \end{array} \quad (1)$$

The solution is free of TG after the TG is fully hydrolyzed by lipases. Two highly consistent fiber optic MSR-SPR bioprobes were used to detect the state of TG solution before and after hydrolysis, and the changes in resonance wavelength measured by the two sensors were recorded. The TG concentration was analyzed on the basis of the difference in solution status before and after hydrolysis, according to the SPR differential signal of the two sensors. However, lipases must be in full contact with TG without contaminating the sensor; thus, a lipase carrier must be introduced. To achieve full dispersion in the TG solution during the reaction and rapid separation from the TG solution after the reaction, immunomagnetic beads are an ideal choice. Micron-sized magnetic beads with a large specific surface area are suitable for immobilizing large amounts of lipases and for achieving uniform dispersion in solution and rapid separation from solution under an external magnetic field.

The proposed dual-fiber optic MSR-SPR bioprobe detection method requires no modification on the sensor surface. Instead, lipases are immobilized on magnetic beads for the hydrolysis reaction. This approach not only avoids any influence from modifications on the sensor surface, but also renders the hydrolysis reaction more efficient and the detection more specific.

2.2. Fiber optic MSR-SPR biosensor

Fiber optic SPR biosensors excite surface plasmon waves (SPWs) in a metal sensing layer through a surface evanescent field, thus enabling detection of the RI and biomass. These biosensors are usually made from large-core silica fibers with easily removable coating and cladding (Zheng et al., 2020). To reduce the sensor size and improve its flexibility, a hetero-core fiber SPR biosensor with a diameter of 125 μm has been proposed (Mai et al., 2019). In this paper, a more compact and portable microprobe-type fiber optic MSR-SPR biosensor is proposed, as shown in Fig. 1 (a). The biosensor consists of three components: a multimode fiber (MMF) for guiding light injection, a single mode fiber (SMF) as the sensing region, and a reflector on the end face of the SMF. An Au sensing layer is deposited on the cylinder surface of the sensing region for SPR excitation. During biosensor operation, most of the incident light is coupled into the cladding of the SMF from the core of the MMF, attributed to the mismatched cores of the MMF and SMF. The light then enters the sensing layer in the form of an evanescent wave. Once the SPR excitation condition is satisfied, that is, once the parallel component of the wavevector of the incident light (k) is equal to the propagation constant of the SPW (k_{SP}), SPR occurs (Maier, 2007). k and k_{SP} are expressed as follows:

$$k_{SP} = \frac{2\pi}{\lambda}\left(\frac{\varepsilon_s \cdot \varepsilon_m}{\varepsilon_s + \varepsilon_m}\right)^{1/2} \quad (2)$$

$$k = \frac{2\pi}{\lambda} \cdot n_c \cdot \sin\theta \quad (3)$$

where ε_s and ε_m are the dielectric constants of the external medium and metal layer, respectively, n_c is the RI of the fiber core, and θ is the incident angle of the incident light. To convert the biosensor into a portable bioprobe, a reflector is designed on the end face of the SMF for reflecting the sensing signal, causing the incident light to participate in SPR sensing twice through back and forth transmission. Therefore, the

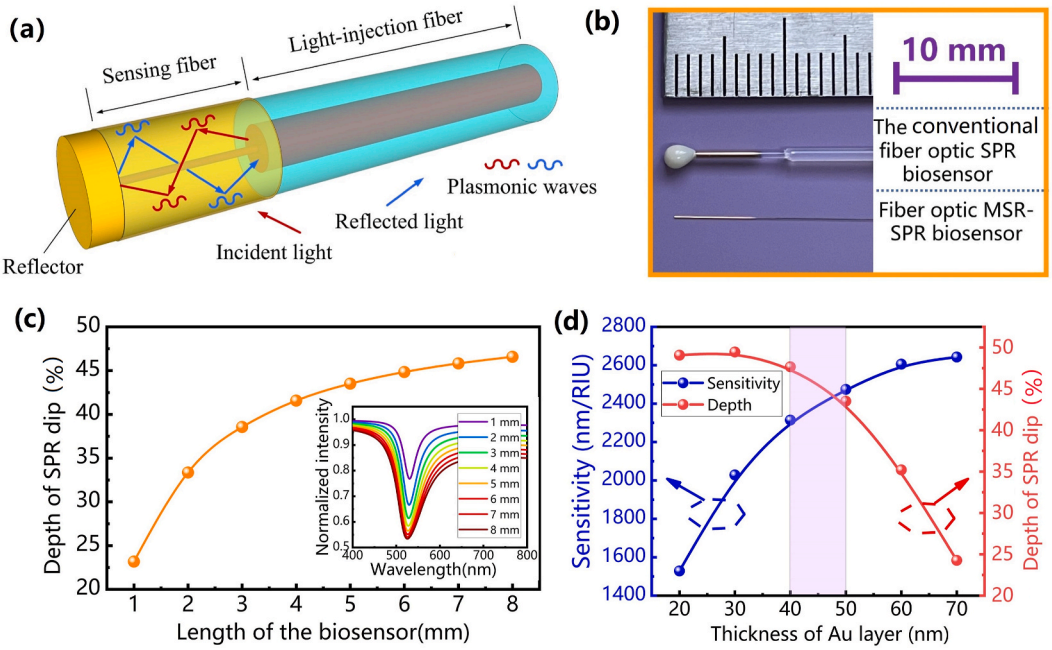

Fig. 1. (a) Structure of the fiber optic MSR-SPR biosensor. (b) Physical comparison of the lab-prepared fiber optic MSR-SPR biosensor and a conventional large-core silica fiber SPR biosensor. Optimization of (c) the length of the sensing region and (d) the thickness of the Au layer. The inset diagram in (c) represents SPR spectrum evolution as the length of the sensing region increases.

length of the proposed bioprobe is reduced to half of that for a conventional hetero-core fiber SPR biosensor, while maintaining the same level of performance. In addition, with this probe form, the proposed biosensor is easy to use and can operate in a smaller sample. A physical comparison between the lab-prepared fiber optic MSR-SPR biosensor and a conventional large-core silica fiber SPR biosensor is shown in Fig. 1 (b).

To optimize the fabrication parameters of the proposed bioprobe, simulations were performed. The principle of the simulations is based on our previous work (Chen et al., 2020). Notably, the sensing efficiency of the proposed biosensor in this work is twice that reported in the literature (Chen et al., 2020) because of the reflector. The short length of the sensing region (L) is beneficial for both ease of use and reduction of sample consumption. However, an excessively short L would reduce the SPR excitation, resulting in an insufficient depth of the SPR dip (D), which can easily be blocked by noise. Fig. 1 (c) presents simulation results of the relationship between L and D. Here, the thickness of the Au sensing layer (T) is set to 50 nm, and D is sufficient when L is set to 5 mm. To determine T, Fig. 1 (d) shows the relationship between the sensor sensitivity (S) and T and the relationship between D and T. Clearly, the trend of S is opposite to that of D with increasing T. Considering the common optimal value of S and D, T is designed to be 40–50 nm. On the basis of the simulation results, we experimentally prepared the proposed biosensor with the optimized parameters.

3. Materials and methods

3.1. Materials

Lipase, glucose, 4-morpholineethanesulfonic acid hydrate (MES), and fetal bovine serum (FBS) were purchased from Macklin. Arabic gum and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Trilaurin, which was used for the preparation of TG samples, was purchased from Xiya Reagent. Urea was purchased from Sinopharm Chemical Reagent Co., Ltd., and magnetic beads with N-hydroxysuccinimide (NHS) groups, which were used for the preparation of lipase-immobilized magnetic beads, were purchased from Enriching Biotechnology, Ltd. Phosphate-buffered saline (PBS, 10 mM, pH 7.4, 1.76 mM KH_2PO_4, 10.14 mM $Na_2HPO_4{\cdot}12H_2O$, 136.75 mM KCl) was prepared with ultra-pure water. All chemicals used in this experiment were of analytical reagent grade. The SMF (9/125 μm) and MMF (62.5/125 μm) were purchased from Yangtze Optical Fiber & Cable Co., Ltd. A 4N-grade high-purity Au target was purchased from ZhongNuo Advanced Material (Beijing)Technology Co., Ltd.

3.1.1. Fabrication of the fiber optic MSR-SPR biosensor

A disposable fiber optic MSR-SPR biosensor was prepared for TG detection in this experiment and the fabrication steps are shown in Fig. 2 (a). First, a 5-mm SMF with two flat end faces was cut off by a fiber optic cleaver and fused to the flat cutting end face of the MMF with a fusion splicer (Fitel S179, Furukawa Co., Ltd.) to form the hetero-core fiber structure. After sufficient cleaning, the side cylinder of the hetero-core fiber was wrapped with a tin foil, and the exit end face of the SMF was left exposed for reflector deposition. Then, a 300-nm Au reflector was deposited on the exit end face of the SMF by magnetron sputtering. Next, the tin foil was removed to expose the clean fiber side cylinder for spin deposition (Chen et al., 2015). Finally, a 45-nm Au sensing layer was uniformly deposited on the side cylinder of the SMF. A JGP450A magnetron sputtering fiber coating device (Sky Technology Development Inc.) was used for the deposition of the fiber-end reflector and side-cylinder sensing layer. This preparation method ensures high consistency among the fiber optic MSR-SPR biosensors (as described in Section 3.1) and feasible TG detection by the dual-fiber optic MSR-SPR bioprobe method.

3.1.2. Preparation of lipase-immobilized magnetic beads

The lipase-immobilized magnetic beads can be uniformly dispersed in the TG solution and sufficiently hydrolyze TG molecules. They can also be rapidly separated from liquid using a magnetic field to avoid influencing the detection of the samples by the proposed biosensor. Fig. 2 (b) illustrates the process for preparing lipase-immobilized magnetic beads. First, magnetic beads with a diameter of 25 μm were used as lipase carriers. The surface of the magnetic beads was modified with NHS groups, which can covalently bond to the primary amine groups of lipase. Then, 0.2 mg magnetic beads (magnetic removal supernatant) were dispersed in 2 mg/mL lipase solution dissolved in 500 μL coupling

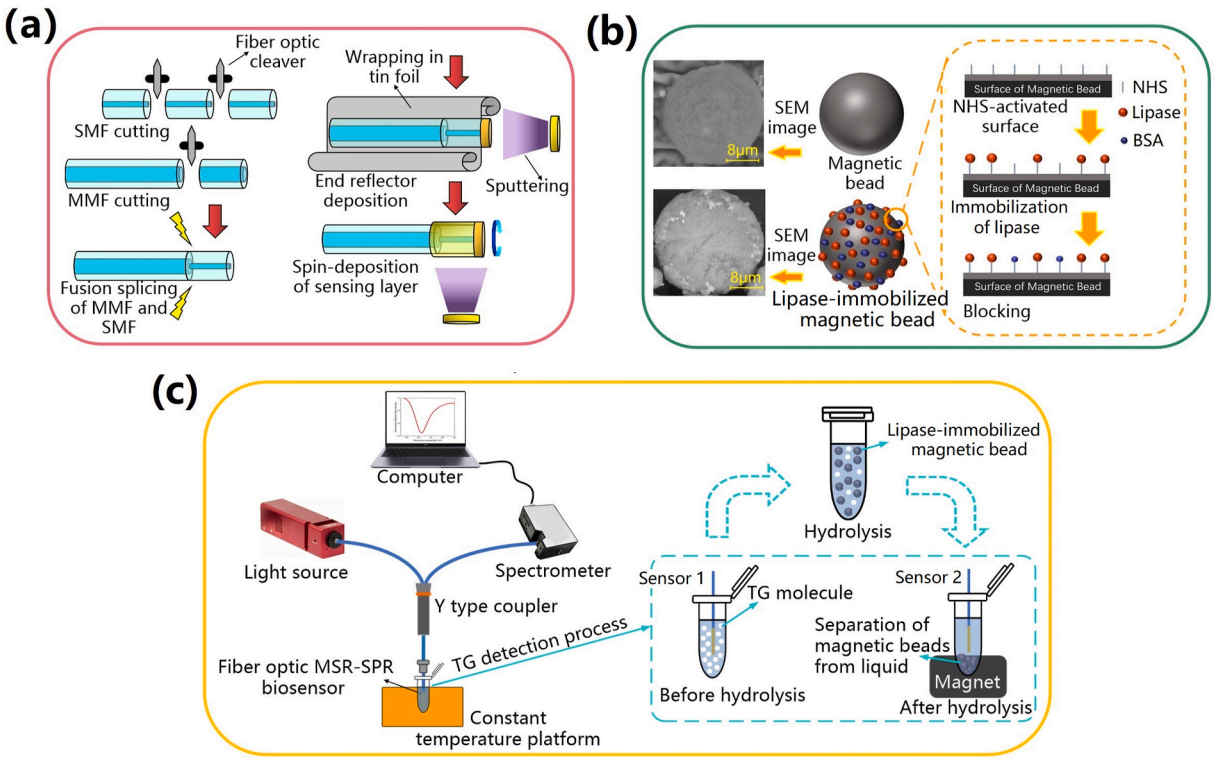

Fig. 2. (a) Fabrication steps for obtaining the fiber optic MSR-SPR biosensor. (b) Preparation process and scanning electron microscope (SEM) images of lipase-immobilized magnetic beads. (c) Sensing system.

buffer (pH 6.0, 0.1 M MES, 0.15 M NaCl) and mixed by a vortex mixer at room temperature of 25 °C for 2 h to achieve lipase immobilization on the surface of the magnetic beads. After a thorough cleaning step with PBS to remove any surface residues, the modified magnetic beads were blocked with 0.2% BSA and sufficiently washed again with PBS. According to SEM images acquired before and after modification, the smooth surface of the magnetic beads became rough, indicating that lipases (and some blocking BSA) had been successfully immobilized on the surface of the magnetic beads.

3.1.3. Experimental setup and methodology

The sensing system and TG detection process are shown in Fig. 2 (c). In this system, broadband light emitted by a white light source (SLS201/M, Thorlabs) passes through the MMF and a Y-type coupler and then enters the fiber optic MSR-SPR biosensor. The transmission light is modulated by the external solution in the sensing region, is reflected by the reflector, then enters the sensing region for a secondary modulation. Finally, the modulated light is collected by a spectrometer (USB 4000, Ocean Optics). After normalization, the SPR spectrum reflects the solution state.

The performance of the proposed biosensor was first studied by measuring NaCl solutions with different RIs. Then, the feasibility of TG detection by the dual-fiber optic MSR-SPR bioprobe method was evaluated. Two highly consistent biosensors were used for TG detection. Sensors 1 and 2 detected the TG in the sample before and after hydrolysis by the lipase-immobilized magnetic beads, respectively. The TG concentration in the sample was analyzed from the SPR differential signal of the two sensors. In the process of TG hydrolysis, the lipase-immobilized magnetic beads were uniformly dispersed in the sample by a vortex mixer, which increased the contact area between the enzyme and substrate for enhanced hydrolysis efficiency. After the hydrolysis reaction, the lipase-immobilized magnetic beads were separated from the sample by an external magnetic field to eliminate any influence of lipase on Sensor 2.

On the premise of confirming the feasibility of the proposed method, we optimized the hydrolysis time. Determining the optimal TG hydrolysis time can reduce the detection time needed for fully hydrolyzed TG. The highest TG concentration of 8 mg/mL was adopted here. The lipase-immobilized magnetic beads and TG sample were mixed evenly, then solid–liquid separation was performed every 5 min. Each separated sample was measured by a disposable bioprobe.

After optimizing the TG hydrolysis time, the TG detection and selectivity of the proposed method were investigated. Twelve TG concentrations (0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, and 8 mg/mL) were prepared in the PBS buffer for the TG detection. These concentrations included ultra-high, high, abnormally low, and normal concentration ranges in the human body. The selectivity was investigated by performing control experiments. TG, glucose, and urea samples with the same concentration of 2 mg/mL were prepared in PBS buffer containing 8 mg/mL Arabic gum for selectivity tests.

Finally, TG detection in serum buffer was studied. Because TG is mainly found in serum, not urine, and it is difficult to separate TG from serum in the detection of human TG, it is more meaningful to directly determine the TG concentration in serum. Here, TG samples were prepared in FBS buffer, and the TG serum samples were then diluted tenfold in PBS buffer. The diluted TG concentrations were 0, 0.1, 0.2, 0.4, and 0.8 mg/mL, corresponding to undiluted concentrations of 0, 1, 2, 4, and 8 mg/mL, which covers various levels of TG concentration in the human body.

All assays in this paper were performed by using disposable fiber optic MSR-SPR biosensors at room temperature of 25 °C, and each measurement was repeated at least three times.

4. Result and discussion

4.1. Performance of the fiber optic MSR-SPR biosensor

The performance of the fiber optic MSR-SPR biosensor determines

the effect of TG detection. First, the RI response and detection consistency of multiple biosensors were studied. As shown in Fig. 3 (a), the proposed biosensor exhibits obvious SPR dips when measuring NaCl solutions with an RI range of 1.3331–1.3777, and the SPR dip has a sensitive redshift with increasing RI. Fig. 3 (b) illustrates the corresponding relationship between the SPR shift and RI. Each data point was obtained by averaging the detection values of 10 biosensors, and the error bar represents the detection standard deviation (SD) of the 10 biosensors. A sensitivity of 2321.1 nm/RIU was obtained from linear fitting of the data points. The slightly increased SD in the high-RI region is due to the broadening of SPR dips with increasing RI. In biological detection, the RI variation caused by a biochemical reaction is usually in the range of 1.33–1.35 (Chen et al., 2016), corresponding to the orange region in Fig. 3 (b). In this RI range, the relative SD (RSD) of the biosensor is less than 6%, indicating good consistency among the biosensors. The RSD value is the quotient of the SD and the average shift of the SPR dips:

$$RSD = \frac{SD}{\Delta\lambda_{average}} \times 100\% \quad (4)$$

The experimental results show that the proposed biosensor is not only sensitive to the RI, but also has good consistency, rendering this disposable bioprobe suitable for detection.

4.2. Feasibility of TG detection by the dual-fiber optic MSR-SPR bioprobe method

In the first step, the direct response of the proposed biosensor to different concentrations of TG samples was studied. TG samples with different concentrations were prepared in a PBS background, and the bioprobe was directly placed in the samples for detection. As shown by the red line in Fig. 4 (a), the experimental results exhibit no corresponding relationship between the SPR shift and TG concentration, and the change value is chaotic. This result is due to the uneven distribution of TG, which is hydrophobic in nature. To resolve this problem, Arabic gum was introduced into the background solution as an emulsion stabilizer to obtain a uniform dispersion of TG in the PBS buffer (The background signal of Arabic gum was evaluated in **Section S3** of the supporting information). The blue line in Fig. 4 (a) shows the results obtained after the addition of 8 mg/mL Arabic gum, demonstrating a significant improvement. The SPR shift increases with increasing TG concentration, and the trend is stable. After background optimization, the bioprobe can directly measure different concentrations of TG samples (The stability study in measuring different concentrations of TG samples is shown in **Section S4** of the supporting information). In subsequent experiments, Arabic gum was added to the background solution before detection.

In the second step, TG molecules were hydrolyzed by lipase-immobilized magnetic beads. First, TG samples with different concentrations were prepared in the PBS buffer, and then 0.04 mg lipase-immobilized magnetic beads was added to 500-μL TG samples with different concentrations. Next, the samples were fixed on the vortex mixer, which allowed the uniform dispersion of magnetic beads for hydrolyzing TG. After 50 min of shaking, the lipase-immobilized magnetic beads were separated from the hydrolyzed TG samples by an external magnetic field to facilitate detection by the bioprobe. With the introduction of lipase-immobilized magnetic beads, the stability of the lipase immobilization must be confirmed, as well as a lack of other influential substances. The leaching rate of lipase was tested by the bioprobe. Lipase-immobilized magnetic beads were added to the PBS buffer without any TG and then mixed and separated. The change in SPR shift of the bioprobe was recorded for PBS before and after the addition of lipase-immobilized magnetic beads. No change was observed in the repeated experiments, confirming that the lipase-immobilized magnetic beads had no effect on the bioprobe.

In the third step, the TG concentration was obtained by the dual-bioprobe method. Sensors 1 and 2 were used to detect TG samples before and after hydrolysis in "the second step", respectively. The experimental results are shown in Fig. 4 (b), where the blue line represents the SPR shifts of TG samples before hydrolysis, as detected by Sensor 1, and the red line represents the SPR shifts of TG samples after hydrolysis, as detected by Sensor 2. Under different TG concentrations, the results for multiple sensors used as Sensor 1 follow the blue line in Fig. 4 (a), while the results for multiple sensors used as Sensor 2 are basically the same, with only acceptable error fluctuations. Therefore, the SPR differential signal between Sensors 1 and 2 can specifically reflect the TG concentration. To make the detection signal of the dual bioprobes more intuitive and convenient for study, the SPR differential signals of the TG concentrations detected by the dual bioprobes are represented as a histogram, as shown in the inset diagram in Fig. 4 (b). The experimental results show that the lipase-immobilized magnetic beads can effectively hydrolyze TG, and the SPR differential signal increases significantly with increasing TG concentration.

Fig. 4 (c) shows a comparison of the dual bioprobe surfaces after measurements based on SEM images. After the TG measurement before hydrolysis, irregular attachments occur on the surface of Sensor 1, attributed to non-specific adhesion of TG molecules. Following the TG measurement after hydrolysis, there is only a trace PBS residue on the surface of Sensor 2 because the TG in the sample was hydrolyzed into fatty acids and glycerol under lipase catalysis.

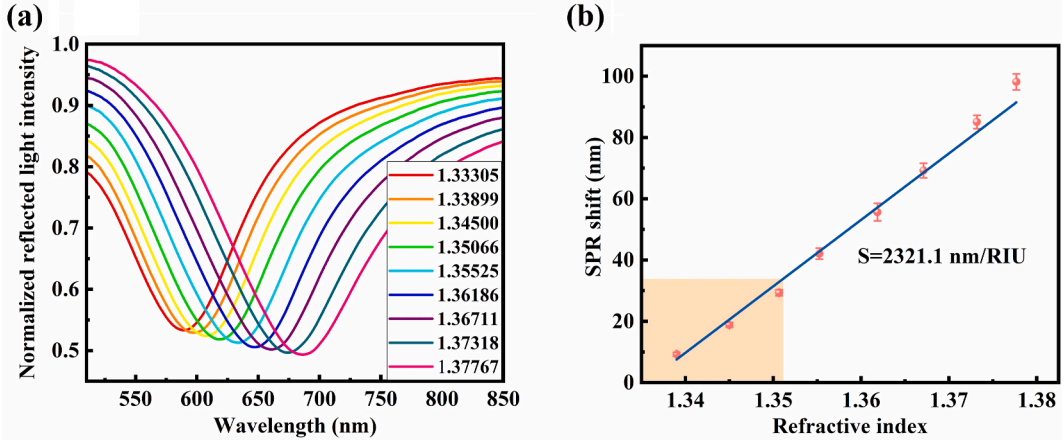

Fig. 3. Performance of the disposable fiber optic MSR-SPR biosensor: (a) RI response, (b) sensitivity and consistency.

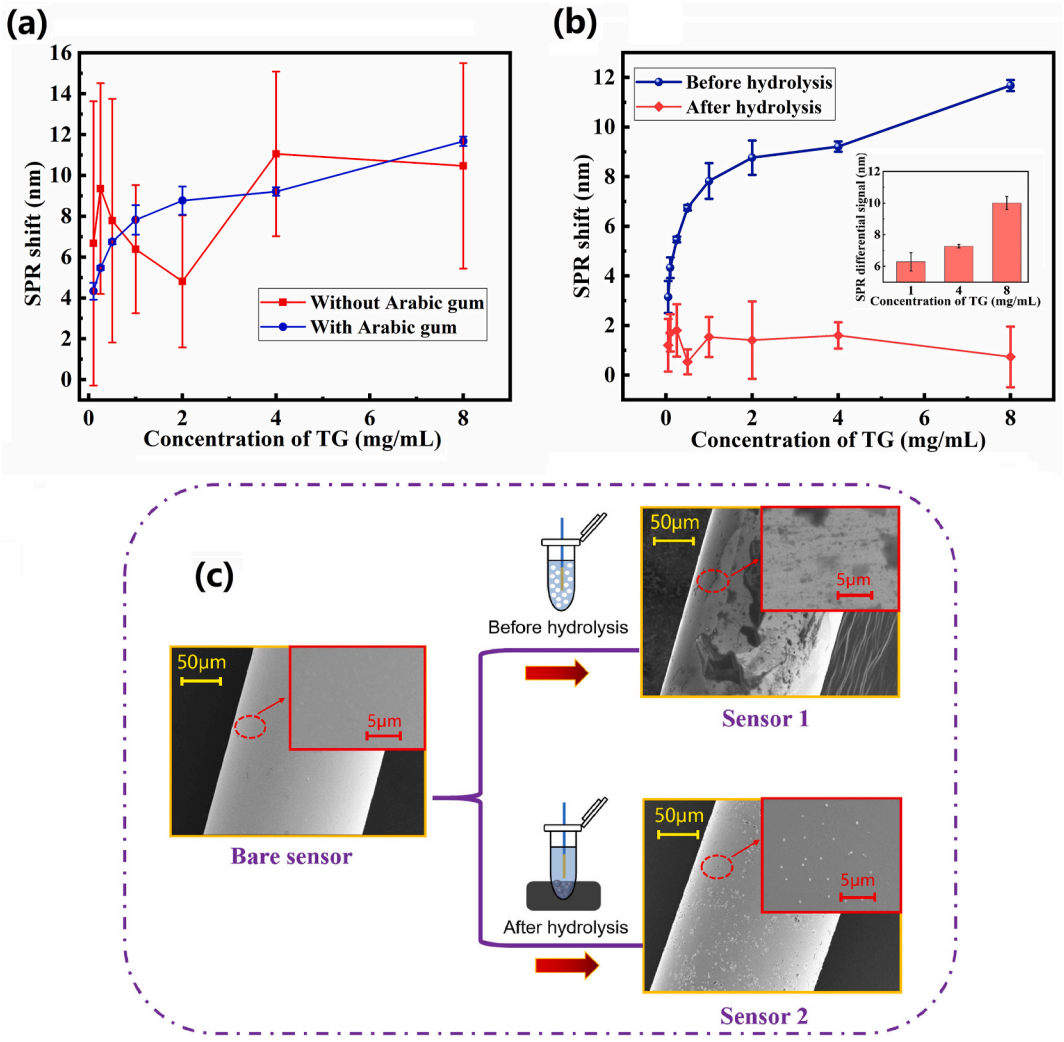

Fig. 4. TG detection by dual-fiber optic MSR-SPR bioprobes: (a) response of the proposed bioprobe to TG samples and the treatment of background solution, (b) results of TG detection by the dual bioprobes, (c) surface comparison of the dual bioprobes after measurements based on SEM images.

4.3. TG detection

4.3.1. Optimization of the TG hydrolysis time

As shown in Fig. 5 (a), the SPR shift decreases with increasing hydrolysis time over the first 20 min. After 20 min, the TG is fully hydrolyzed, and the SPR shift is generally constant, with a small error bar. Thus, an optimal TG hydrolysis time of 20 min was determined and applied in the following experiments.

4.3.2. TG detection by the dual-fiber optic MSR-SPR bioprobe method

The experimental results of TG concentration detection by the proposed method are shown in Fig. 5 (b). Overall, the SPR differential signal of the dual bioprobes is positively correlated with TG concentration, indicating that the proposed method can sensitively distinguish various TG concentrations in the PBS buffer, detecting TG over a concentration range of 0–8 mg/mL.

4.3.3. Selectivity of TG detection

As shown in Fig. 5 (c), the SPR differential signals reach 7 nm for the TG sample, while they are close to zero for the glucose and urea samples, indicating good selectivity of this method.

The proposed method was also applied to characterize urine samples spiked with TG at different concentrations (0, 0.5, 1, 2, 4, and 8 mg/mL). The experimental results are shown in Fig. 5 (d), demonstrating that this method can also be applied in a complex background.

4.4. TG detection in serum buffer

The experimental results for the proposed method of detecting TG in serum samples are shown in Fig. 6 (a). The SPR differential signal is positively correlated with the TG concentration, indicating that the method is suitable for detecting TG in serum. The average sensitivity of TG detection was obtained as 1.25 nm/(mg/mL). When processing the detection signal, we found an interesting phenomenon:

I. When the background solution is PBS or urine, the SPR shift of Sensor 1 is higher than that of Sensor 2.
II. When the background solution is serum, the SPR shift of Sensor 2 is higher than that of Sensor 1.

We consider that this result may arise from the complex composition of the background buffer. In Case I, TG molecules are the only non-specific adhesion substances in the solution, and they are removed after hydrolysis. In Case II, there are other non-specific adhesion substances besides TG molecules. Before hydrolysis, TG molecules and other non-specific adhesion molecules together adhere to the surface of Sensor 1. After hydrolysis, the remaining non-specific adhesion molecules adhere to the surface of Sensor 2. To verify that the influence of other

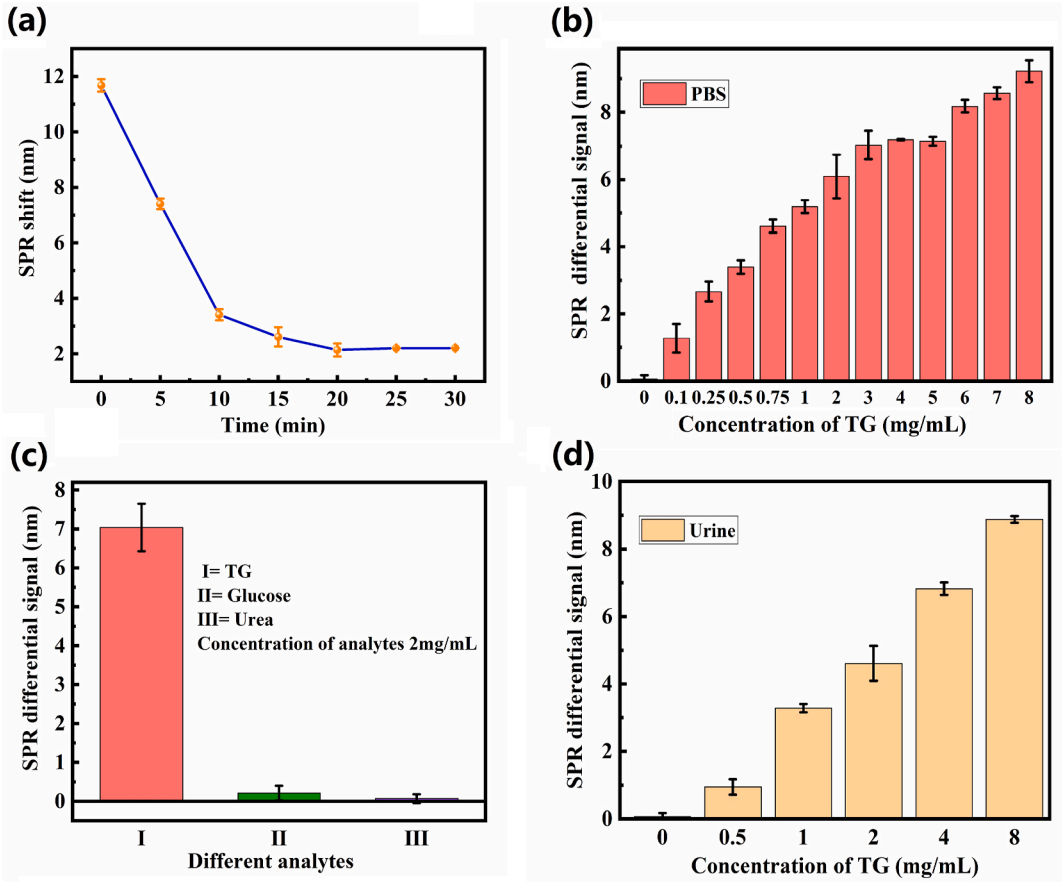

Fig. 5. TG detection by the dual-fiber optic MSR-SPR bioprobe method: (a) optimization of the TG hydrolysis time, (b) TG concentration detection in PBS buffer, (c) selectivity of TG detection, (d) TG concentration detection in urine samples.

non-specific adhesion molecules on TG detection is consistent and can be eliminated, another control experiment was performed. In this case, 2 mg/mL (a very high concentration) of non-specific BSA was added to TG samples in the PBS buffer, and the mixed samples were measured using the proposed method. As shown in Fig. 6 (b), the SPR differential signal indicates the TG concentration, and similar to the detection in serum, the SPR shift of Sensor 2 is higher than that of Sensor 1.

Fig. 6 (c) illustrates the difference in TG detection in these two cases. The explanation for Case I is shown in the blue box: TG molecules attach to the surface of Sensor 1, and thus the SPR shift is higher than that of Sensor 2, which does not have TG molecules on the surface (the SEM images in Fig. 4 (c) confirm these surface conditions). The explanation for Case II is shown in the red box. Before hydrolysis, TG molecules and other non-specific adhesion molecules in the serum simultaneously adhere to the surface of Sensor 1. After hydrolysis, TG molecules are no longer present in the sample, and the surface of Sensor 2 is covered with other non-specific adhesion molecules. In other words, the original positions of TG molecules on the sensor surface are occupied by other non-specific adhesion molecules. In Case II, the TG concentration is reflected by the number of other non-specific adhesion molecules replacing TG molecules, as well as their mass difference. This result is intuitively reflected in the difference in detection signals from the dual bioprobes, namely the SPR differential signals.

Finally, we also compare the performance of different types of TG biosensors, which are listed in Table 1.

5. Conclusions

A novel method for TG detection with a dual-bioprobe system, which can accurately detect different levels of TG concentration in serum, was proposed in this work. To the best of our knowledge, the disposable fiber optic MSR-SPR bioprobe with a tiny diameter of 125 μm described in this work, which has high stability and portability and can effectively avoid cross-contamination caused by repeated use, has been scarcely ever reported. During the detection process, TG samples were hydrolyzed by lipase-immobilized magnetic beads. Then, the signal difference for TG samples before and after hydrolysis was measured using a dual-bioprobe method, and the TG content was determined by the SPR differential signal. Experimental results show that the proposed method can achieve TG detection in a concentration range of 0–8 mg/mL and is suitable for TG detection in various complex backgrounds, such as urine and serum. The sensitivity of the proposed biosensor was obtained to be 1.25 nm/(mg/mL) for TG detection in serum. Additionally, the proposed method has the advantages of high sensitivity, compact structure, simple operation, label-free detection, high selectivity, and low cost. Therefore, it is expected to be applicable for clinical TG detection.

In future work, we will further improve the TG detection system. We aim to integrate the dual bioprobes into a flow cell system, with a lipase-immobilized microchannel between the channels of the dual bioprobes to enable full hydrolysis of the TG sample as it passes through the flow cell. In this case, the detection signals before and after TG hydrolysis can be directly read by the dual bioprobes, realizing one-step TG detection.

Here within enclosed is our paper submitted to “Biosensors and Bioelectronics”. The further information about the paper is in the following: The Title: Dual-fiber optic bioprobe system for triglyceride detection using surface plasmon resonance sensing and lipase-immobilized magnetic bead hydrolysis The Authors: Shirong Zhou, Xuejin Li, Jinghan zhang, Hao Yuan, Xueming Hong, and Yuzhi Chen All authors agree with the submission and they claim that none of the material in the paper has been published or is under consideration for

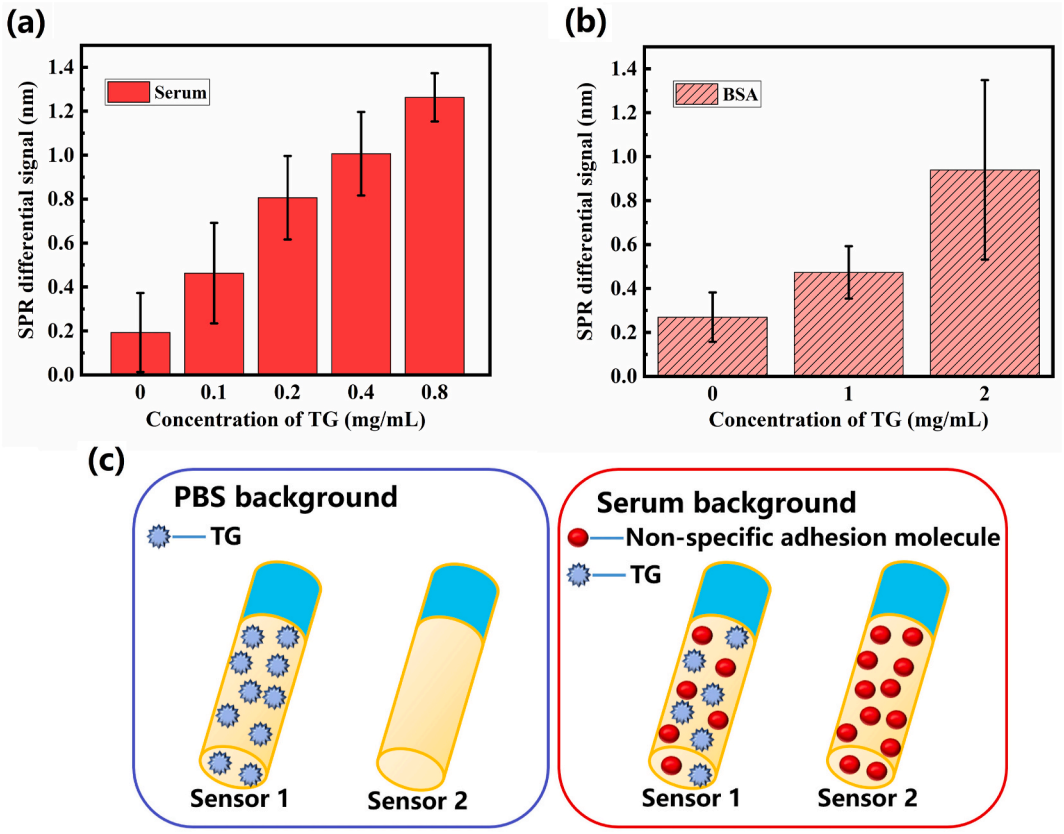

Fig. 6. TG detection by the dual-bioprobe method in complex backgrounds: (a) TG detection in serum solution, (b) TG detection in BSA solution, (c) explanation of SPR differential signal detection in different buffers.

Table 1
Performance comparison of different types of TG biosensors.

Sensor type	TG detection range	Sensitivity	Applicable background	Ref.
Fiber SPR	0.5–7.0 mM	3.17 nm/mM	PBS	Baliyan et al. (2013)
Fiber LSPR	0–7 mM	28.5 nm/mM	PBS	Baliyan et al. (2017)
Long period fiber grating	0–7 mM	0.5 nm/mM	PBS	Baliyan et al. (2016)
Potentiometric analysis	5–30 mM	57.6 mV/pH	PBS	Vijayalakshmi et al. (2008)
Amperometric analysis	50–700 mg/dL	0.05 μA/(mg/dL)	PBS, serum	Narang et al. (2013)
Fiber MSR-SPR	0–8 mg/mL	1.25 nm/(mg/mL)	PBS, urine, serum	This work

publication elsewhere or is reproduced from another source.

All authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted. I am the corresponding author and my address and other information is as follows, Address: College of physics and optoelectronic engineering, Shenzhen University, Shenzhen 518060, China.

Thank you very much for consideration! Sincerely Yours, .

CRediT authorship contribution statement

Shirong Zhou: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Xuejin Li:** Conceptualization, Data curation, Visualization, Supervision, Project administration, Funding acquisition. **Jinghan Zhang:** Software, Resources, Writing – original draft. **Hao Yuan:** Resources. **Xueming Hong:** Resources, Writing – original draft. **Yuzhi Chen:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113723.

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