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One-Step Synthesis of Cyclodextrin-Capped Gold Nanoparticles for Ultra-Sensitive and Highly-Integrated Plasmonic Biosensors

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Abstract

Plasmonics in conjunction with supramolecular chemistry brings new opportunities for biosensing. Modified with macrocyclic supramolecules, noble metal nanoparticles possess improved biocompatibility and highly efficient biomolecule immobilization and recognition via host-guest interaction. In this work, we synthesize high-quality and monodisperse β -cyclodextrin-capped gold nanoparticles (β -CD-capped AuNPs) in one-step facile and eco-friendly process, where β -CD functions as both reducing and capping agent. Integrated with a microfiber, the β -CD-capped AuNPs deliver promising localized surface plasmon resonance properties and ultralow detection limit of cholesterol molecules of 5 aM. The selective recognition of our proposed biosensor to cholesterol is further verified by interference study and analysis of real human serum samples. This study shows the bright prospects of supramolecular chemistry assisted plasmonics in achieving highly integrated and highly sensitive biosensing with extremely low consumption of clinical samples and small footprint.

Keywords: Localized Surface Plasmon Resonance; Biosensing; Gold Nanoparticles Synthesis; Supramolecular Chemistry; Fiber Optics; Cholesterol

1. Introduction

Gold nanoparticles (AuNPs) capture increasing research attention due to their facile synthesis, chemical stability and unique optical and electronic properties [1]. So far, they have shown promising performance in catalysis [2], nanoelectronics [3,4] and numerous biomedical applications such as imaging [5], disease diagnosis and therapy [6], drug or nucleic acid delivery [7] and optical and electrochemical bioassay [8–10]. One of the most intriguing properties of AuNPs is the localized surface plasmon resonance (LSPR), which associates with free electrons participating in collective oscillation confined in a sub-wavelength particle size [11]. The resonance leads to significant near-field enhancement of electromagnetic wave at AuNPs surface, giving rise to strong light-matter interaction as well as sharp spectral extinction [12,13]. Therefore, the LSPR of AuNPs has become a prominent biosensing technique as it facilitates highly sensitive and label-free detection of biomolecular quantification, binding and dissociation [14,15]. Especially when integrated with fiber-optic probes, it brings the feasibility of miniaturized, remote and even *in vivo* biosensing [16–22]. Both biocompatibility and specific molecular recognition are of crucial importance for biosensors. AuNPs are commonly obtained by the Turkevich method where gold hydrochlorate is reduced to metallic gold colloids stabilized with capping ligand [23]. Despite the inert nature of gold, further surface functionalization of AuNPs to diminish cytotoxicity is often required [6]. Meanwhile, the further surface functionalization of AuNPs should also serve as a selective bio-interface [24]. Approaches like ligand substitution, polymer deposition and biomolecule coating have been proven to offer both biocompatibility and specific molecular recognition [6,24–26], but require strict preparation conditions and complicated processing steps [27]. Another concern is the compromised sensitivity resulting from the complex capping strategies. The plasmonic sensitivity is critically affected by the distance from analyte molecules to AuNPs surface as the near-field enhancement exponentially decays into surrounding medium, hence keeping the thickness of recognition layer down to ~1 nm or less is essential to improve the competitiveness in terms of sensitivity and limit of detection (LOD) [13,24,28]. To address the aforementioned difficulties and challenges, one-step synthesis of macrocyclic supramolecule-modified AuNPs is expected to be a promising solution.

Indeed, macrocyclic supramolecules are excellent candidates for AuNPs surface functionalization. Firstly, common macrocyclic supramolecules such as cyclodextrins (CDs), pillararenes, calixarenes and cucurbiturils function as the host molecules to form specific inclusion complexations with their corresponding guest molecules via hydrophobic association, van der Waal force, electrostatic attraction, π -stacking interaction, etc., which is the so-called host-guest interaction [29]. Encouragingly, the host-guest interaction effectively improves the recognizing and capturing capabilities compared with conventionally adopted biomolecule-ligand binding [30]. Secondly, the macrocyclic supramolecules exhibit good biocompatibility and stabilizing performance [30–32]. Thirdly, the heights of macrocyclic cavities are less than 1 nm [33–35], which hardly compromises the plasmonic sensitivity. Although several attempts have been carried out to save post-processing surface modification by capping AuNPs with macrocyclic supramolecules during the synthesis, the majorities introduce harsh reducing reagents (e.g. NaBH_4 , NaOH , thiols) which would be detrimental in biomedical practices [36–43]. The completely “green” synthesis has only recently been reported that CD serves as both reducing and capping agent [44]. In this work, we explore the plasmonic biosensing potential of macrocyclic supramolecule-modified AuNPs in highly integrated device. To be more specific, we demonstrate one-step synthesis of biocompatible, high-quality and monodisperse β -CD-capped AuNPs. β -CDs have been proven to be highly efficient biomolecular recognition elements on AuNPs that can form complexation with various biomolecules and drugs [44,45]. Furthermore, we decorate the synthesized nanoparticles on the surface of a microfiber to

construct an ultra-sensitive and highly integrated biosensing platform. To validate the constructed biosensor, we apply it to the detection of cholesterol, which is one of the typical guest molecules of β -CD and of great diagnostic significance for various disorders including coronary heart disease, atherosclerosis, hypertension, nephrosis, etc. [46–48]. So far, various cholesterol biosensors based on different sensing mechanisms have been demonstrated, such as fluorometric [49], colorimetric [50,51], high-performance liquid chromatography (HPLC) [52], gas-liquid chromatography [53], electrochemical [47,48,54–59] based cholesterol sensors, etc. However, these sensing mechanisms either consume relatively large amount of samples meanwhile requiring sample pretreatments or component separations that complicate the operation procedure and increase measurement time [47,56], or demand tedious nanomaterials functionalization [57–59]. In comparison, a cholesterol biosensor based on β -CD capped AuNPs decorated microfiber shows the merits of high sensitivity, high integration and simple fabrication. Our proposed biosensor reaches an ultralow cholesterol LOD of 5 aM, which is the most sensitive among the state-of-the-art cholesterol detections [51]. Such low LOD is almost 10^{15} times lower than the physiological cholesterol level in human serum, implying the huge practical advantage in testing clinical samples where strong matrix effects exist and sample dilution is often required [60]. Also, we can achieve extremely low consumption of clinical samples benefited from the superior LOD and high integration of microfiber based device. In addition, our proposed biosensor is easy-to-use. No pretreatment other than the dilution of human serum samples is needed before the measurement. Meanwhile, we carry out an interference study of cholesterol sensing with the existence of common interfering substances in human serum and recovery experiments to evaluate the reliability in analysis of real human serum, which prove the good selectivity of our proposed biosensor.

2. Experimental Section

2.1 Reagents and Apparatus

β -CD (99%) was purchased from Tokyo Chemical Industry. D_2O , tetrachloroauric(III) acid ($HAuCl_4 \cdot 3H_2O$, 99.9%), cholesterol, L-Glutamic acid, L-Cysteine, L-Ascorbic acid, dopamine hydrochloride, human serum albumin (HSA), human serum sample (from human male AB plasma, hemoglobin ≤ 20 mg/dL) were purchased from Sigma-Aldrich. $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ were obtained from Merck KGaA. The phosphate buffer solution (PBS, 0.1 M, pH 7.0) was prepared by dissolving 1.0478 g $NaH_2PO_4 \cdot H_2O$ and 2.2071 g $Na_2HPO_4 \cdot 2H_2O$ in 200 mL deionized (DI) water.

The scanning electron microscopy (SEM) characterizations were carried out on a Field Emission SEM (JEOL JSM-6340F) under the accelerating voltage of 5 kV. The transmission electron microscopy (TEM) observations were conducted on a Field Emission Electron Microscope (JEOL JEM-2010F). The sizes and zeta-potentials of the β -CD-capped AuNPs dispersions in solution were measured by using a Zetasizer Nano ZS (Malvern Instruments, UK). The absorption spectra of AuNPs solutions were measured using a UV-Visible Spectrophotometer (Hewlett Packard 8453). Fourier-transform infrared spectroscopy (FTIR) spectra were obtained on a Nicolet iS10 spectrometer (Nicolet) with samples cast into thin films on KBr. The X-ray photoelectron spectroscopy (XPS) measurements were performed by Kratos AXIS Supra XPS system. 1H NMR spectra were conducted on a Bruker Avance 300 spectrometer (Bruker BioSpin, Switzerland) operating at 300 MHz (1H) in D_2O .

2.2 AuNPs Synthesis

35 mL of DI water, 5 mL of PBS (0.1 M, pH 7.0), 10 mL of 0.01 M β -CD aqueous solution and 1 mL of 0.01 M $HAuCl_4$ were added to a round bottom flask. Then the mixed solution was heated to 100°C for 30 mins with vigorous stir. To purify the AuNPs, the cooled solution

underwent centrifugation with 8000 rpm for 8 mins. The precipitates were collected and redispersed in 5 mL DI water for the next-step characterizations and optical biosensing.

2.3 Microfiber Surface Functionalization

To immobilize the negatively charged β -CD-capped AuNPs on microfiber surface, we functionalized the microfiber surface with positively charged poly(allylamine) (PAA). The microfiber was stabilized in a flow chamber filled with DI water. 100 μ L 1.0 M NaOH (Alfa Aesar) solution was flowed to the chamber and stagnated for 10 mins. Then we rinsed the microfiber as well as the chamber with DI water for 5 times. 100 μ L 0.05 wt% PAA (Sigma-Aldrich) was then flowed into the chamber and stagnated for 20 mins. Again, the microfiber was rinsed with DI water for 5 times and left with monolayer of positively charged PAA. The purified AuNPs solution was diluted by 3 times for the decoration of microfiber surface. 50 μ L of the diluted AuNPs solution was flowed into the chamber and stagnated for 10 min. Then we rinsed the chamber with DI water for 5 times to flush away the redundant and weakly adsorbed AuNPs.

2.4 Determination of Cholesterol

Before the determination of cholesterol, an aqueous cholesterol solution with concentration of 5.0 μ M was prepared and sonicated for 2 hours to ensure the cholesterol were completely dissolved. Then the cholesterol solution was diluted to different concentrations ranging from 5 aM to 0.5 μ M for later use. For each measurement of cholesterol concentration, 200 μ L of pure cholesterol solution or human serum sample was flowed into the chamber stabilized with β -CD-capped AuNPs decorated microfiber and stagnated for 5 mins, during which the LSPR peak first observably descended and then gradually stabilized. The chamber was then rinsed with DI water. The transmission spectrum of microfiber was barely affected by the rinsing as the cholesterol molecules were stably captured by the β -CD cavities. The transmission spectrum was recorded after the rinsing.

3. Results and Discussion

3.1 Design and Configuration

Figure 1 schematically illustrates the configuration of our β -CD-capped AuNPs based fiber-optic biosensor. Each AuNP is equipped with numerous β -CD molecules through the conjunction of the carboxyl groups of the oxidized β -CDs and the gold surface. The β -CD-capped AuNPs are immobilized onto the surface of microfiber via electrostatic force. A considerable portion of the evanescent field leaks out due to the thin diameter of microfiber and excites strong LSPR of the AuNPs. The cholesterol molecules are attracted to the macrocyclic cavities of β -CDs via host-guest interaction and perturb the surface plasmons, thus resulting in the variation of the transmission spectrum. There are three common formations of CD, α -, β - and γ -CD, which are composed of six, seven and eight D-glucose units linked by α -1,4-glucose bonds, respectively [61]. β -CD has been proven to provide the highest quality of the synthesized particles [44]. The host-guest interactions between CDs and guest molecules are mainly driven by the hydrophobic associations [33,62]. The toroidal cavity of CD has a hydrophobic interior and a hydrophilic exterior [63]. The cavity size of β -CD fits tightly with the sterol group of cholesterol molecule [64], making β -CD a highly efficient cholesterol receptor in many biomedical uses [65].

Figure 1. Schematic illustration of the proposed highly-integrated microfiber-based biosensor. Top inset: The conjunction between macrocyclic β -CD and AuNP surface. Bottom Inset: The molecular structure of cholesterol.

3.2 Synthesis and Characterization of AuNPs

The β -CD-capped AuNPs are synthesized in an eco-friendly way as described in the Experimental Section, where β -CD functions as both reducing and stabilizing agent. The AuNPs solution is purified by going through centrifugation and redispersion in DI water for next-step characterization and optical biosensing. Figure 2a shows the SEM image of the as-synthesized β -CD-capped AuNPs. The particle size distribution is homogeneous and the AuNPs tend to contiguously attach to each other after the solvent fully evaporates. From the SEM image, we can roughly estimate the average diameter of β -CD-capped AuNPs is ~ 19 - 20 nm. A clear picture of AuNPs morphology can be provided by TEM and high-resolution TEM (HRTEM). The TEM image in Figure 2b indicates that the AuNPs have almost uniform spherical structure and the particle diameters mostly range from ~ 18 nm to ~ 21 nm. We can conclude the good crystallinity of the β -CD-capped AuNPs from the distinct lattice fringes under HRTEM (Figure 2c). The (1 1 1) plane can be clearly observed. To obtain the precise distribution of AuNPs sizes, we characterize the β -CD-capped AuNPs solutions by dynamic light scattering (DLS). As shown in Figure 2d, the AuNPs diameters fall within a narrow range. The majority of AuNPs diameters range from 14 nm to 21 nm, which matches with the observations from SEM and TEM. The comparison between the particle size distributions obtained from DLS and TEM is shown in Figure S1. Due to the limited nanoparticle samples in TEM characterization, the TEM based size distribution is narrower.

Figure 2. (a) SEM of the as-synthesized β -CD-capped AuNPs. (b) Low-magnification TEM of β -CD-capped AuNPs. The average diameter of AuNPs is ~ 20 nm. (c) HRTEM of a single β -CD-capped AuNP. (d) DLS measurements of the size distribution of as-synthesized β -CD-capped AuNPs.

Next, we investigate the relation between the quality of as-synthesized AuNPs and the reaction time. During the heating process, solution color gradually evolves from nearly colorless to light purple and then stabilizes at light red (see the color evolvment in the Inset of Figure 3a). We measure the absorption of the β -CD-capped AuNPs solution at different reaction time. As shown in Figure 3a, when the reaction time is 20 mins, at which the solution color is light purple, a weak and broad absorption peak appears at 535 nm. The weak peak is due to the low concentration of formed AuNPs in the solution. After 30-min of the reaction time, the solution color remains light red, and sharp absorption peaks with resonant wavelength at 521 nm are observed, based on which we can estimate the nanoparticle sizes stabilize at ~ 20 nm [66]. DLS measurements are carried out for the β -CD-capped AuNPs solutions with different reaction time. As shown in Figure 3b, at 20 mins, the AuNPs sizes have a broad distribution around 83 nm. This is in accordance with the absorption curve in Figure 3a that the larger particle sizes lead to a longer LSPR wavelength. The broad particle size distribution also explains the corresponding broad absorption spectrum in Figure 3a (the black curve) [66]. Similar to the solution color and the absorption spectra, the DLS result also stabilizes after the reaction time of 30 mins. As shown in Figure 3b, the AuNPs sizes stabilize around ~ 18 nm from the reaction time of 30 mins to 60 mins with much narrower size distribution. It can be concluded from Figure 3a and 3b that the reaction time of 30 mins can deliver β -CD-capped AuNPs with good plasmonic property and monodispersity. Therefore, we finalize our synthesis time to be 30 mins. After centrifugation (see more details in the Experimental Section), we

obtain a purified and more condensed AuNPs solution with ruby-red color (Inset of Figure 3a). The peak absorption remains at 521 nm yet with a much sharper plasmonic resonance (the dashed red curve in Figure 3a). The linewidth of resonance band is as narrow as 47 nm, which is competitive with conventionally synthesized AuNPs.

To confirm the one-step synthesis, FTIR spectra of β -CD and β -CD-capped AuNPs shown in Figure 3c indicate that the macrocyclic structure of β -CD is well preserved on AuNPs surface after the synthesis process. The FTIR spectra of β -CD and β -CD-capped AuNPs have very similar profiles except that the band corresponding to hydroxyl group becomes much narrower after the formation of AuNPs, which indicates that the hydroxyl groups of β -CD molecules mainly participate in reducing Au^{3+} ions to metallic Au^0 . To obtain a precise characterization of the transformation of β -CD featured groups during AuNPs synthesis, we carry out XPS measurements for pristine β -CD (Figure S3) and as-synthesized β -CD-capped AuNPs (Figure 3d and 3e). Compared with pristine β -CD, a new peak appears at 289.1 eV in the C 1s XPS spectrum of β -CD-capped AuNPs (Figure 3d), which is correlated with the O–C=O bond [67]. Meanwhile, we observe an obvious shrink of the peak area of C–OH bond. In addition, there is a new peak appears at 531.0 eV in the O 1s spectrum (Figure 3e), corresponding to the C=O bond [68]. Hence, we can deduce that the reacted hydroxyl groups of β -CD molecules are oxidized to carboxyl groups. The newly formed carboxyl groups strongly interact with the AuNPs surface via O–Au bond (Top inset of Figure 1) and effectively stabilizes the AuNPs. Figure 3f shows the NMR spectrum of β -CD-capped AuNPs in D_2O , the six chemical shifts are essentially the same as the β -CD molecules (Figure S4), which further verifies that the macrocyclic structure of β -CD is well preserved on AuNPs surface after the synthesis process. However, the integral ratios of the 5, 6-H peak area to other chemical shifts on the β -CD-capped AuNPs are significantly reduced compared with the β -CD molecules (see more details in S3), further demonstrating that the hydroxyl groups are oxidized to carboxyl groups.

Figure 3. (a) The evolvement of AuNPs solution absorption during the synthesis process. Inset: The variation of solution color along with the synthesis time and the ruby-red color of the purified AuNPs solution. (b) The DLS measurements of AuNPs size distributions at different synthesis time. (c) The FTIR spectra of pristine β -CD and β -CD-capped AuNPs. (d) C 1s XPS spectrum and (e) O 1s XPS spectrum of β -CD-capped AuNPs. (f) The ^1H NMR spectrum (400 MHz, D_2O) of the β -CD-capped AuNPs. Inset: Schematic β -CD structure associated with the corresponding chemical shifts.

3.3 Selective Detection of Cholesterol

Then, the microfiber is fabricated by tapering a standard single-mode silica optical fiber (YOFC FullBand Ultra fiber) using the heat-and-pull method [69]. The thinnest portion of

microfiber has a diameter of $\sim 4\ \mu\text{m}$ (Figure 4a) and a length of $\sim 8\ \text{mm}$. Electrostatic deposition of polyelectrolyte is a facile yet efficient surface functionalization technique [70]. Since the β -CD-capped AuNPs are negatively charged (see more details in S1), we functionalize the microfiber surface with a layer of positively charged poly(allylamine) (PAA) to adsorb AuNPs via electrostatic interaction. More details of microfiber surface functionalization can be found in the Experimental Section. Figure 4b shows the surface morphology of microfiber immobilized with β -CD-capped AuNPs. AuNPs evenly spread on the fiber surface other than packing together as shown in Figure 2a, indicating the homogeneous distribution of the positively charged PAA. As the AuNPs are gradually immobilized on the microfiber surface, an attenuation band centered at 530.7 nm appears on the transmission spectrum and gradually deepens. The black curve in Figure 4c is the stabilized LSPR peak after the redundant AuNPs are removed by rinsing. The reason for the redshift of resonance peak from 521.5 nm as shown in Figure 3a to 530.7 nm in Figure 4c is due to the high refractive index of silica microfiber, since the LSPR resonant wavelength is proportional to $\sqrt{2n_m^2 + 1}$ at optical frequencies, where n_m is the refractive index of ambient dielectric material [13]. The decorated AuNPs on microfiber are effectively immobilized and the LSPR peak on transmission spectrum can be stabilized for several days (Figure S6). The β -CD-capped AuNPs decorated microfiber is sequentially immersed in 200 μL aqueous cholesterol solutions with concentrations ranging from 5 aM to 0.5 μM . We carry out three independent experiments of cholesterol concentration detection by using three different β -CD-capped AuNPs decorated microfibers (Figure S5). Each time we can observe a distinguishable deepening of the attenuation band that is much larger than the standard deviation of the blank when the cholesterol concentration is as low as 5 aM (the red curve in Figure 4c). Such a low LOD is benefited from the highly efficient interaction between β -CDs and cholesterol molecules. The formation of host-guest inclusion complexation at AuNPs surface increases the local refractive index as well as the scattering of evanescent field, leading to a higher transmission loss. As the cholesterol concentration increases, the resonance peak keeps descending and then tends to ease when the concentration is higher than 5 nM (Figure 4c). Meanwhile, the resonant wavelength undergoes a redshift from 530.7 nm to 531.4 nm, verifying the increase of surrounding refractive index. As the adsorbed cholesterol molecules only perturb the evanescent field within 1-2 nm distance from AuNPs surface, the overall effective refractive index of the whole penetration depth of surface plasmon is not largely increased. Hence the resonant wavelength shift is not obvious. Taking the relative variation of transmission minimum as the sensing indicator, our demonstrated biosensor provides a linear response within a wide range of log-scale cholesterol concentration from 5 aM to 5 nM (Figure 4d), thereby spanning 9 orders of magnitude of the measuring concentration. The response tends to saturate when the cholesterol concentration is larger than 5 nM. Limited by the poor water solubility of cholesterol molecules [71], only concentrations less than 5 μM can be prepared and tested. It is notable that the cholesterol in human serum is at mM level, which is nearly 10^{15} times higher than the LOD of our proposed sensor. This is a huge advantage in the case of testing real clinical samples. The high background contents in human serum including cells, proteins, lipids, electrolytes, etc. could introduce uncertainty of the background refractive index of the serum sample or non-specific binding to the sensing area that results in measurement inaccuracy especially for the sensitive optical platforms. Hence the dilution of clinical samples is an effective and widely adopted method to eliminate the signal perturbation induced by uncertain background contents [72]. In the meantime, however, the concentration of target molecule also get diluted and could become undetectable. Therefore, the ultra-low LOD of our proposed biosensor can still guarantee the accurate detection of target cholesterol molecules when the serum sample is diluted to a great extent to minimize the signal disturbance.

To verify the reliability of our proposed biosensor in practical applications, we carry out an interference study for some of the commonly existed compounds in human serum, including glutamic acid, cysteine, ascorbic acid, dopamine and HSA [44,55,56]. For each tested cholesterol concentration, the interfering substances are diluted to their corresponding concentrations based on the realistic proportions in human serum [44]. For example, when 50 aM cholesterol is tested, glutamic acid, cysteine, ascorbic acid, dopamine and HSA solutions with concentrations of 1.5 aM, 3.1 aM, 1.3 aM, 3.75×10^{-23} M and 0.44 pg/L are sequentially introduced, respectively. Figure 4e presents the real-time monitoring of the average transmission intensity of 530-535 nm when we detect 50 aM – 0.5 fM cholesterol while introducing the interfering substances. It shows that each time when the cholesterol is added, the average transmission intensity quickly descends by ~1%, which matches with the descending trend of the transmission minimum shown in Figure 4d. Introducing the interfering substances induces slight but tolerable fluctuations on the transmission intensity. Each addition of the interfering substance leads to slow and small-scale variation of transmission intensity. After the intensity is stabilized, the redundant substances are flushed away by rinsing with DI water (the blue arrows in Figure 4e). The rinse almost brings the intensity back to the level before the interfering substances are added. The negligible impact of interfering substances on the biosensing performance is due to the much weaker host-guest interaction between the interfering compounds and β -CDs compared with the cholesterol- β -CD inclusion complexation and the relatively low concentrations of interfering substances in human serum. As the interaction between β -CDs and guest molecules is mainly driven by hydrophobic attraction, the hydrophobic groups in guest molecules play an essential role in forming the host-guest complexation. A glutamic acid molecule contains two hydrophobic $-(CH_2)$ groups and one hydrophilic $-(COOH)$ group. The interaction between glutamic acids and β -CDs are based on the association between the $-(CH_2)$ group and the interior cavity of β -CD [73]. However, due to the mismatch between sizes of $-(CH_2)$ group and β -CD cavity, although the inclusion complexation of glutamic acid and β -CD can be formed, it would be much weaker compared with the tightly fitted cholesterol. Similarly, a cysteine molecule has only one hydrophobic $-(CH_2)$ group, the interaction between cysteine and β -CD would be even weaker than glutamic acid. The lactone ring in ascorbic acid and the benzene ring in dopamine can also be housed by the CD cavity [74–76]. However, their sizes fit tightly with α -CD cavity, while fit loosely with β -CD cavity [77]. Moreover, the concentrations of ascorbic acid and dopamine in human serum are much lower than that of cholesterol. Especially for dopamine, although the host-guest interaction with CD has been adopted for dopamine sensing [74], its concentration is lower by 10^6 in human serum, which has negligible interference to realistic cholesterol detection. For HSA, the large molecular structure of HSA can hardly form host-guest interaction with the relatively small β -CD cavity. Also, the electrostatic repulsion between the negatively charged HSA molecules and negatively charged β -CD-capped AuNPs can further reduce the affinity of HSA to AuNPs thereby the optical signal is barely affected [78].

To evaluate the practical applicability of our proposed biosensor in analysis of real human serum, we carry out recovery experiments for detecting cholesterol in human serum samples spiked with different cholesterol concentrations. As shown in Figure 4d, the standard deviations of cholesterol determination are more promising at aM – fM levels and the cholesterol in human serum is at mM level, we dilute the human serum sample by a factor of 10^{14} for a more precise determination of cholesterol concentration. No pretreatment other than the dilution of human serum sample with DI water is conducted. Three human serum samples spiked with different standard cholesterol concentrations (0, 50 and 100 aM) are prepared and examined by our proposed biosensors. The transmission spectra of β -CD-capped AuNPs decorated microfibers when measuring the three human serum samples in 4 independent

recovery experiments are shown in Figure S7. Table 1 summarizes the results of recovery experiments. The mean cholesterol concentration we found in the unspiked human serum sample is 4.23 mM. As a comparison, the mean cholesterol level in the same human serum sample detected by a commercial blood cholesterol monitor (BeneCheckTM) in 4 independent measurements is 4.35 mM, which indicates our proposed method is reliable and appropriate for the monitoring of cholesterol in human serum. The recoveries of the spiked serum samples are within a satisfactory range (105.2-112.2%). Although the standard deviations and recoveries of our proposed method are not the most promising among the state-of-the-art cholesterol sensors, we demonstrate the recovery experiments as a proof-of-concept that the β -CD capped AuNPs microfiber is applicable in the analysis of real clinical samples. The accuracy of cholesterol determination in human serum can be further improved by a more sophisticated control of the structural parameters of fabricated microfibers. As the waist diameter and the taper length of microfiber have large impacts on the transmission spectrum [79], the plasmonic behaviors of microfiber based LSPR biosensor can be considerably affected by even slight variation of structural parameters. The reduction of the structural difference among different microfibers can effectively improve the standard deviations as well as the preciseness of the fitting function in Figure 4d thereby achieve better recoveries. A more precise control of structural parameters can be realized by using a more stable flame and higher-precision translation stages during the heat-and-pull fabrication process [79,80].

Table 1. Recovery results of detecting cholesterol in human serum samples.

Sample	Added (aM)	Found* (aM)	Recovery (%)
Human serum (male)	0.0	42.3 $\pm$ 2.8	-
	50.0	94.9 $\pm$ 6.7	105.2
	100.0	154.5 $\pm$ 16.5	112.2

* The values are mean of 4 independent experiments $\pm$ standard deviation

Figure 4. (a) SEM image of the thinnest portion of microfiber. (b) Distribution of the immobilized β -CD-capped AuNPs on microfiber surface. (c) Variation of the transmission spectrum as the cholesterol concentration increases. (d) Relative variation of the transmission minimum against log-scale cholesterol concentration. Error bars indicate the standard deviations of three independent experiments. (e) Interference study with the existence of common substances in human blood serum. The concentrations of interfering substances are added based on the realistic proportions in human blood serum.

4. Conclusions

In conclusion, we investigate the highly integrated LSPR biosensing potential of β -CD-capped AuNPs synthesized in a facial and eco-friendly way where β -CD functions as reducing and stabilizing agents as well as selective bio-interface. The synthesis of β -CD-capped AuNPs is a

one-step process that realizes not only the formation of uniform nanoparticles but also the functionalization of biorecognition layer. Benefited from the very thin thickness of β -CD layer which is even comparable to 2D materials and the highly efficient molecular recognition capability, the β -CD-capped AuNPs based fiber-optic biosensor achieves ultralow LOD as well as specific recognition of cholesterol molecules. Given that CD can house a variety of guest molecules, such as adamantane, lithocholic acid, ferrocene, adenine and guanine of DNA, etc., our proposed highly integrated and biocompatible plasmonic sensing platform can be versatile in various biosensing scenarios.

Author Contributions

‡ Nancy Meng Ying Zhang and Miao Qi contributed equally.

Conflict of Interest

The authors declare no competing interests.

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Appendix A. Supporting Information

Supporting information associated with this article can be found in the online version.

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