



Functionalized optical fiber ball-shaped biosensor for label-free, low-limit detection of IL-8 protein

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Abstract: Detection of biomarkers for tracking disease progression is becoming increasingly important in biomedicine. Using saliva as a diagnostic sample appears to be a safe, cost-effective, and non-invasive approach. Salivary interleukin-8 levels demonstrate specific changes associated with diseases such as obstructive pulmonary disease, squamous cell carcinoma, oral cancer, and breast cancer. Traditional protein detection methods, such as enzyme-linked immunosorbent assay (ELISA), mass spectrometry, and Western blot are often expensive, complex, and time-consuming. In this study, an optical fiber-based biosensor was developed to detect salivary IL-8 protein in a label-free manner. The biosensor was able to achieve an ultra-low limit detection of 0.91 fM. Moreover, the tested concentration range was wide: from 273 aM to 59 fM. As a proof-of-concept for detecting the protein in real clinical samples, the detection was carried out in artificial saliva. It was possible to achieve high sensitivity for the target protein and minimal signal alterations for the control proteins.

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1. Introduction

While blood products, including serum and plasma, are of common use in clinical testing several studies consider saliva as a potential alternative fluid for biomarker discovery and diagnostic purposes. A study comparing 2290 salivary proteins with 2698 proteins found in plasma, showed that 58% of plasma immunoglobulins and 40% of proteins classified as biomarkers for various cancers and systemic diseases are also found in saliva [1]. A strong association between the levels of overlapping immunoglobulins in saliva and plasma hints at the possibility of leakage from plasma, although the exact process remains unclear. The biomolecules may move from blood to saliva through passive intracellular diffusion, active transport, or paracellular pathways involving extracellular ultrafiltration within salivary glands or via gingival crevices [2,3]. When comparing the effectiveness of saliva and blood as sources for biomarker detection, it is crucial to consider that blood is a more complex medium, presenting numerous challenges during analysis. Factors such as the wide variation in substance concentrations (spanning nine orders of magnitude) can significantly reduce the chances of detecting lower-concentration targets. Furthermore, the components of blood have varying half-lives, ranging from seconds to months, which means peptide detection in the blood may not accurately represent the organism's current state. On the other hand, the oral cavity's physiology ensures a constant flow of secreted fluid, continually flushing and refreshing the mouth's fluid content. As a result, the composition of saliva closely mirrors the metabolic activity of the secretory elements producing the fluid at any given time, providing a valuable source for monitoring both oral and systemic health [4].

Saliva, as a body fluid for disease diagnosis, has several advantages, including non-invasiveness, reduced risk of infection compared to blood, and ease of collection despite the age (e.g. infants) and health conditions (e.g. disability, anxiety). The long-term stability of the oral fluid and the presence of a wide range of biomarkers in saliva can facilitate the monitoring of the health condition [5,6]. In order to use saliva in clinical settings, there is a need in building accurate and convenient point-of-care devices that use it as a biological sample [7].

Interleukin 8 (IL-8) or CXCL8 (C-X-C Motif Chemokine Ligand 8) is a soluble, low molecular weight (6-8 kDa) protein that acts as a chemoattractant for guiding the recruitment of immune cells such as basophils, neutrophils, and T cells to the site of inflammation. IL-8 primarily influences neutrophils by controlling their movement toward the site of infection. Like other CXC chemokines, IL-8 plays a role in the recruitment of immune cells such as monocytes [8]. IL-8 is found in very low amounts or even is not detected in normal tissue. Its expression substantially increases in response to the release of pro-inflammatory cytokines (TNF, IL-1) or reactive oxygen species, hypoxic conditions, and other external stress. In response to stimuli, cells with toll-like receptors such as dendritic cells, macrophages, fibroblasts, and adipocytes can secrete IL-8 [9].

Alterations in the level of salivary IL-8 are associated with the progression of specific types of chronic diseases and the efficiency of radiotherapy in cancer patients, validating its use as a diagnostic biomarker. The data obtained from a meta-analysis of 11 studies highlight a significant increase in the level of salivary IL-8 in oral cancer patients compared to the control group [10]. While for healthy patients it varied from 52.1 to 1580.7 pg/mL, for the oral cancer group it ranged between 283.7 and 4082.8 pg/mL, suggesting the potential of salivary IL-8 to be used as a biomarker for oral cancer. Particularly, salivary IL-8 has been extensively studied in the framework of oral squamous cell carcinoma (OSCC), where studies validate its use for the non-invasive early diagnosis of OSCC [11,12]. The combined tracking of IL-8 in saliva and IL-6 in serum can further increase the accuracy of OSCC diagnosis with 99% sensitivity for the biomarkers [13]. The concentration of IL-8 in normal conditions is significantly lower when compared to pathological conditions, corresponding to 30 pM in healthy patients, and 86 pM in patients with oropharyngeal squamous cell carcinoma [14]. Salivary IL-8 concentration was also shown to be associated with the response to radiotherapy (RT) in patients with head and neck cancer, where higher levels of IL-8 before treatment correlated with a worse response to RT, and vice versa [15]. Statistically significant increase in the levels of IL-8 in saliva of patients with oral potentially malignant disorders such as oral lichen planus has also been shown to be concordant with concentrations in serum [16]. A study on the salivary biomarkers discovered that salivary cytokine concentrations fluctuate across various stages of breast cancer when compared to a control group. The proteomic analysis of salivary proteins in secondary breast cancer patients verified notable changes in IL-8 levels throughout the progression of the disease [17]. Moreover, the differentiation potential of IL-8 was established for distinguishing healthy smokers from those with chronic obstructive pulmonary disease (COPD). The levels of IL-8 and MMP-9 in saliva, which are related to lung function in individuals with COPD, have the potential to be used for monitoring disease progression [18].

Given the prospects of detecting salivary IL-8 in the context of diagnosing diseases and predicting the response to radiotherapy, there is a need for tools with a highly sensitive and robust response. Conventional techniques used to detect IL-8 biomarkers include enzyme-linked immunosorbent assay (ELISA), mass spectrometry, western blot, and flow cytometry. However, these techniques require complex instrumentation that is expensive and requires trained personnel for their operation. The main drawbacks of ELISA kits and sandwich assays are the long turnaround time, which might reach up to 24 hours, the limitation in the analyzed volume of the sample, the need in labelled reagents and the detection of only one analyte at a time. To achieve more rapid and point-of-care testing, different types of biosensors have been developed. While several fluorescence-based optical sensors and electrochemical biosensors have emerged, there

are challenges in translating these developments for clinical use. The study on the fluorescence probe-based method achieved a 4 fM limit of detection for measuring IL-8 in saliva, but the optimization of the sensor for measurements is labor-intensive procedure with a time-consuming analysis [19]. Other different tools developed for the detection of salivary IL-8 protein are listed in Table 1.

To address the limitations of existing detection approaches, this study emphasizes the implementation of an optical fiber-based biosensor that enables rapid and highly sensitive detection of the target analyte. Optical fiber-based biosensors employ photons as signal carriers, circumventing complications associated with electrical interference typically observed in electrochemical biosensors. The insensitivity of fiber optic biosensors to electromagnetic disturbances, coupled with numerous benefits such as economical raw material costs, compact dimensions, and minimal detection thresholds, render them superior for sensing applications compared to alternative sensor modalities. Furthermore, these optical fiber sensors demonstrate exceptional performance metrics in regard to limits of detection, response time, and selectivity, and are capable of simultaneously detecting multiple analytes through the construction of multiplexed assays [20]. Biosensing elements of the sensor, which can be enzymes, cells, antibodies, aptamers are responsible for binding to the analyte in the medium. Transducers connect changes in the concentration of the analyte to the intensity of the light, transforming the signal received from the bioreceptor into the electrical signal that transmits the information of the quantity or the presence of the analyte. The electronic component of the biosensor integrates an amplifier and processor, which collectively manage the detection, transmission, and documentation of signals in the form of photons [21].

Optical fiber sensor frequently used in biosensing are grating based sensors such as tilted fiber Bragg gratings (TFBG), etched FBG (EFBG), long-period grating (LPG), plasmonic FBG, interferometric sensors and lossy mode resonance-based sensors [22–26]. Together with the advantages they offer, they also have some limitations. Grating-based optical fiber sensors need an inscription to obtain a periodic modulation of the refractive index (RI) inside the core which is usually done by special instruments [27] adding up to the price of the sensing probe. LPG and TFBG operate in a transmission mode making them a less attractive biosensing unit. To work in reflection, the fiber needs to be cleaved and a broadband mirror has to be fabricated there which adds an additional manufacturing step [28]. For LPG, the fiber has to be cut precisely after grating so that interference fringes are not formed [29]. Tapers, on the other hand are fragile; and they together with plasmonic sensors have a low fabrication yield [30]. BR sensors used in this study have several advantages: it is a low-cost platform fabricated using a telecommunication-grade fiber; it is a label-free sensing unit; it has a potential to be used in situ and multiplexed; and it previously showed high sensitivity towards analyte of interest when used as a biosensing transducer [31]. The first ball resonator (BR) sensor was introduced by Shaimerdenova *et al* in 2020 [22] when they fabricated it using telecommunication-grade fiber with a CO₂ laser forming a sphere on the tip. It behaved as a weak interferometer with a return loss below -50 dB. When interrogated with an optical backscatter reflectometer, it showed sensitivity to the RI change of the surrounding media; their simple fabrication method together with its sensitivity makes it a good platform in biosensing.

As the role of IL-8 has been progressively investigated, a particular emphasis should be posed to the detection of IL-8 with particular emphasis to the low concentrations when the detection occurs in saliva which might have low abundance of target protein and high dilution rates [32]. Clinical data obtained from the study of patients with OSCC reveals the levels of salivary IL-8 protein to be in the range of 70.86–652.83 pg/ml for a group of oral precancer patients with oral submucous fibrosis and 308.09–3123.53 pg/ml for OSCC patients [33]. Considering the complex and multistep progression of diseases characterized with varying levels of biomarkers and individual differences in the concentration of biomarkers among patients, detection tools with a high sensitivity covering a wide range of detection provide a significant clinical utility.

Table 1. Sensing platforms for the detection of salivary IL-8 protein^a

Table/Assay	Concentration range	LoD	Main advantages	Main drawbacks	Ref.
ELISA	0.0128–5000 pg/ml	2.38 pg/ml (average for 10 kits)	Established technology	Time-consuming; Not label-free; One analyte detection per analysis Requires sample processing	Table S1
Electrochemical magnetosensor	87.9–5000 pg/mL	72.4 pg/mL	Multiplex detection of IL-8 protein and IL-8 mRNA	Complex fabrication; Susceptibility to interference	[34]
Nanoparticle-based electrochemical immunosensor	500 fg/ml to 4 ng/ml	72.73 pg/mL	Stable for up to 3 months; Fast detection (9 min)	Complex fabrication; High cost	[35]
Fluorescent optical sensor	NA	4fM	Cost-effective; Reduced optical noise	Labor intensive; Fluorophore induced binding hindrance	[19]
Surface plasmon resonance	1.1–22.7 fg/ml	1650 pg/ml	Fast detection (13 min); Improved signal to noise ratio	Limited throughput for multiplexing; complex fabrication	[14]
Chip based electrochemical sensor	NA	50 pg/mL	Rapid analysis; POC device	Complex fabrication; Use conjugates for electrodes	[36]
Optical microfluidic biosensor	80–120 pg/ml	90 pg/mL	Detect up to eight analytes in parallel; Detection in spiked saliva	Low stability; Complex fabrication	[37]
Fiber-optic microsphere-based antibody array	30 pg/mL - 10.5 µg/mL	0.95 fg/ml	Absorbance-based Simple and flexible fabrication; simultaneous detection of 10 salivary proteins including IL-8	Fluorescence readout; Long assay time (2.5 h)	[38]
Optical fiber ball biosensor	2.28 fg/ml - 835.6 ng/ml	7.6 fg/ml	Small size; Femtomolar detection; Simple fabrication; Sensor can be reused (after regeneration) Potential for <i>in-situ</i> application	High cost of the analyser Not tested on clinical samples Fragility	This work

^aELISA – enzyme-based immunosorbent assay; LoD – limit of detection; NA – not available; POC – point-of-care

The present study is focused on the detection of IL-8 in the artificial saliva samples as salivary diagnostics holds promise in terms of safe and non-invasive collection of samples from the patients. In this work, we report the development of a fiber optic biosensors for measurement of IL-8 protein. A sensor sensitive to RI change was fabricated and functionalized with ligands specific to the target protein. The surface of the sensors was analysed by atomic force microscopy and the biosensor was used to measure a wide concentration of IL-8 protein in artificial saliva. Specificity of the biosensors was also studied using no ligand sensor and control proteins.

2. Materials and methods

2.1. Fabrication of fiber optic spherical tip

Fiber optic spherical tip sensors were fabricated from the standard single-mode fibers using a CO₂ laser splicer (Fujikura LZM-100). The fabrication process is depicted in Figure S1. To form a ball at the end of the fiber, two fibers were aligned, spliced and the resulting structure was subjected to high laser power by setting the parameters on the splicer device. Through power calibration, the parameter for the absolute power was determined to be 314 bit that was used for the fabrication of the ball at the tip of fiber. The tip of the sensor was imaged using the Zeiss AxioZoom V16 macroscope with a PlanNeoFluar Z 1x/0.25 FWD 56 mm objective at 112x digital zoom. Sensors with ball diameters of 524 μm , 551 μm , 556 μm and ellipticity, respectively, 0.174, 0.159, 0.188 were used in the experiment. The fabrication process took a short time (~ 1 min) and the obtained sensors were further selected based on their reflectivity and sensitivity to RI changes.

2.2. Calibration of the fiber optic sensors

To check the sensitivity of the sensors to the RI changes of the media, calibration of the sensors using sucrose solutions of different concentrations was performed. The experimental design of this work was adapted from the study on the detection of CD44 protein [31]. The sensor tip was placed in a vial and was connected to the optical backscattering reflectometer (OBR 4600, Luna Innovations, US) to interrogate sensor measurements. BR sensor has low reflectivity in the interface between fiber surface and surrounding medium, and have imperfection of the shape of the spherical tip and its spectra is characterized by shallow fringes; as a result each fabricated sensor has its own unique spectral feature [22]. The reflection data is acquired based on the parameters set for the interrogator: resolution bandwidth of 0.257 GHz, electrical gain 0 dB, and scan range corresponding to 527 nm to 1613 nm. Following the checking of sensor response in air and water, measurements were continued in sucrose solutions of increasing concentrations by starting from 6 ml of 10% sucrose solution and adding stepwise 400 μl of 40% sucrose solutions to reach a total of 7 measurements. Verified RI values for the sucrose solutions correspond to an increase from 1.34761 to 1.35696 which was measured using an Abbemat refractometer (Anton Paar, Austria). As selection criteria, changes in the amplitude peaks were tracked during the measurement, and obtained data from calibration was analyzed using MATLAB software to check the reflectivity, sensitivity and evaluate the coefficient of determination (R^2). To remove the background noise signals from the spectral data, 7th order Chebyshev low-pass filter with characteristics of 0.0084 normalized frequency cut-off was used to filter the data. Sensors with a high reflectivity and (R^2) value of at least 90-95% were considered to be sufficiently responsive to be used for further functionalization procedures.

2.3. Functionalization of the fiber surface with anti-IL-8 antibody

The protocol for the functionalization procedure was adapted from the work focused on the application of fiber-optic BR for the detection of CD44 protein [31]. The schematic representation of the functionalization steps is illustrated in Fig. 1. To remove any impurities from the sensor's

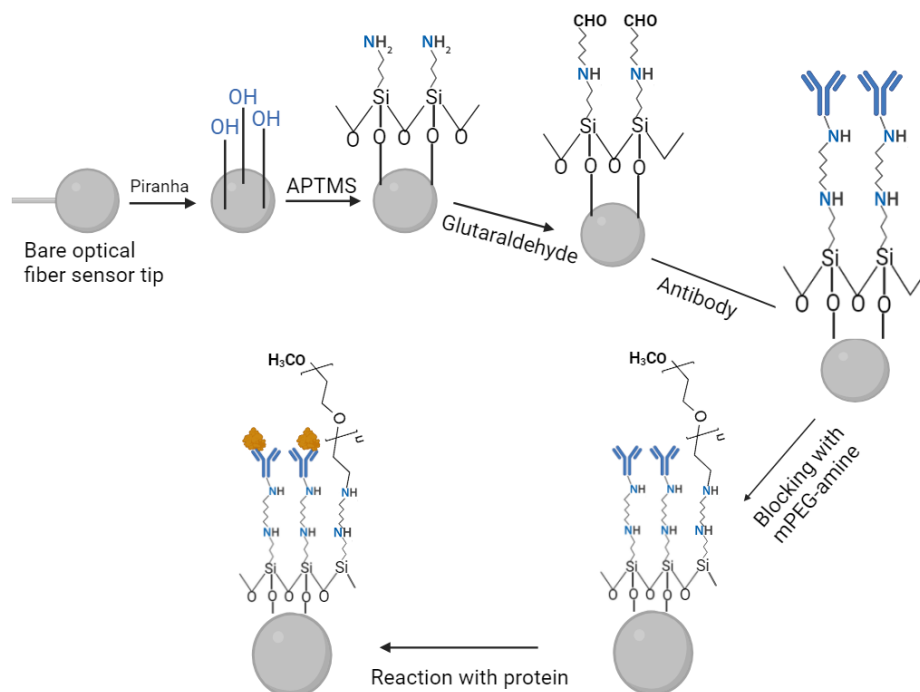


Fig. 1. Steps in the functionalization of the ball resonator at the tip of the optical fiber sensor for IL-8 protein measurement.

surface and increase hydroxide groups for further silanization, sensors were immersed in the Piranha solution for 15 min. Solution was prepared by using the sulfuric acid and hydrogen peroxide solutions in a 4:1 ratio. Following washing with deionized (DI) water, nitrogen gas was used to dry the surface of sensors. For the silanization step, 1% (3-Aminopropyl)trimethoxysilane (APTMS) solution in methanol was prepared and sensors were treated for 20 minutes. Following cleaning with methanol, sensors were heat treated for 1 hour in the oven at 110 °C and rinsed with water before incubating sensors in the 25% glutaraldehyde (GA) solution in phosphate buffer saline (PBS) buffer for 1 hour. For the subsequent attachment of the anti-IL-8 antibody (Abcam, Cat.# ab18672), antibody solution (4 µg/ml) in a fresh PBS was prepared and sensor tips were incubated for one hour on a shaker. After the antibody treatment, sensors were incubated for another 30 min in the 1% poly(ethylene glycol) methyl ether amine (mPEG-amine) solution in PBS to block non-specific binding. Sensor tips were washed with PBS between each step, including after GA and antibody, and after full functionalization biosensors were stored at 2–4 °C in PBS before further use. For the negative control sensor, which has no immobilized antibody on the surface, exactly the same conditions were maintained: negative control sensors were incubated in PBS only before being blocked. For surface morphology analysis, sensors were functionalized following the same protocol as the main sensors for target protein measurement.

2.4. Measurement of the target and control proteins

To perform protein measurements with the functionalized sensors, serial dilution of recombinant human IL-8 protein (Abcam, Cat.# ab259397), with concentrations ranging from 273 aM to 100 nM (1:6 dilution) in artificial saliva (LCTech GmbH) was prepared. By connecting the sensor to the OBR device and placing the tip of the fiber in the vial with 300 µl of IL-8 protein solution, measurements were performed starting from the artificial saliva as a blank solution.

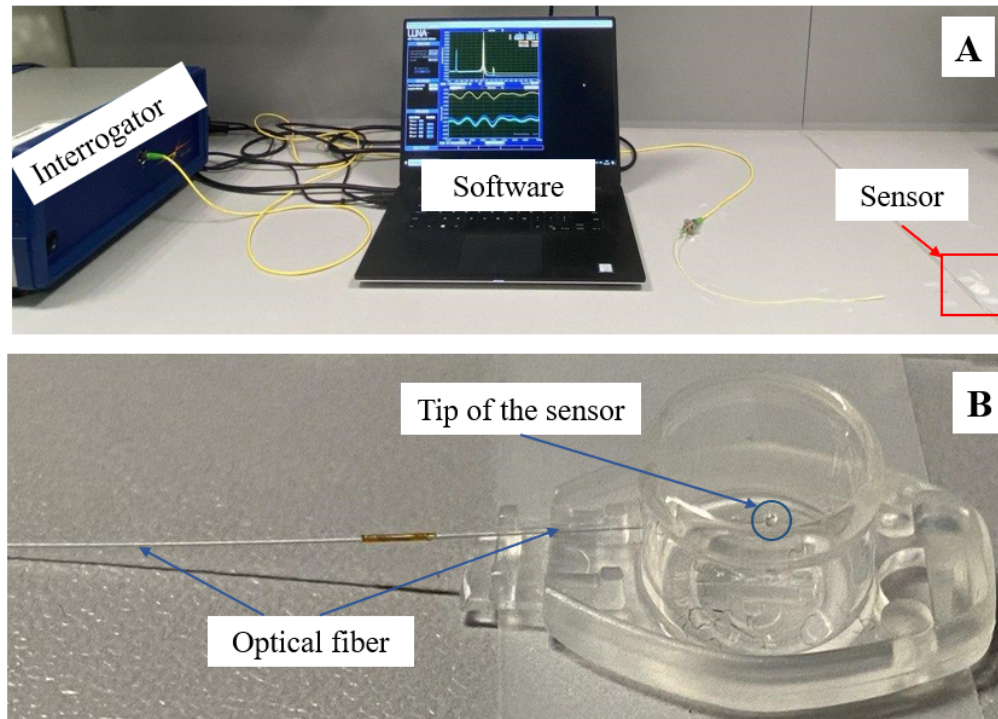


Fig. 2. The experimental setup shows a fiber optic ball resonator sensor that was used to detect IL-8 protein. A. A general setup with the sensor connected to an interrogator and a computer for data collection and analysis. B. A magnified view of the fiber optic ball resonator sensor (circled) inside the vial for protein measurement.

The position of the sensor within the vial and overall setup can be seen in Fig. 2. For each protein concentration, 10 measurements were performed with an interval of 1 min. By using data on the response of the sensor at the lowest IL-8 concentration as a blank value and the maximum standard deviation the following equation was used for calculation of limit of detection (LoD); $LoD = f^{-1}(y_{blank} + 3\sigma_{max})$. To ensure the replicability of obtained results, detection of IL-8 with functionalized sensors has been demonstrated by repeating measurements with three sensors. Collected data on the sensor response to different protein concentrations was analyzed using MATLAB software.

To check the selectivity of the functionalized sensor, measurements with control proteins were conducted maintaining the same conditions. As controls, carcinoembryonic antigen (CEA), and lysozyme proteins were used. The specificity of the biosensor has been further verified by comparing the amplitude change of two sensors during measurements of the target protein: sensor functionalized with IL-8 antibodies and a negative control sensor (with no antibodies).

2.5. Surface morphology analysis

The height and roughness of the surface coating were evaluated using a JPK Nanowizard 4XP AFM (Bruker Instruments Germany) coupled with an inverted ZEISS Axio Observer 7 microscope (ZEISS Instruments Germany) to analyze the surface morphology of the ball surface following each step of the functionalization process. The scanning parameters of 0.5nN setpoint, z-speed of 40 m/s, and scan rate of 1 Hz were applied to a RESPA20 probe (Bruker, Germany) with a nominal spring constant $K = 0.9$ N/m, resonant frequency $f = 20$ kHz, and radius $r = 8$ nm.

A scanning area of $10\mu\text{m} \times 10\mu\text{m}$ was used to probe the broad view of the ball in order to observe the homogeneity of the surface prior to acquiring numerous $1\mu\text{m} \times 1\mu\text{m}$ images with a resolution of 5 nm/pixel in the X-Y plane. All images were acquired using QI Biomolecules in liquid mode in PBS at room temperature. Images were visualized using Gwyddion software. For each layer, three different samples were obtained, each with at least 10 different $1\mu\text{m} \times 1\mu\text{m}$ regions for statistical analysis. GraphPad Prism 9 was used to conduct statistical tests to accurately quantify the dispersion in surface height and root-mean-square (RMS) roughness, as well as the statistical significance of the results between various phases.

3. Results and discussion

3.1. Fabrication and calibration of ball resonators (BR)

To detect IL-8 proteins in saliva, BR were fabricated and interrogated with the OBR device to check their sensitivity to the changes in the RI. The interrogation of the fabricated ball resonators helps to evaluate the sensitivity and quality of the response produced by the fabricated sensors to the RI changes of the media. The two-sided profilometry of the spheres on the tip of single-mode fibers is shown in Figure S2. Figure S3 shows the microscopic image of a BR sensor. The performance of sensors was evaluated based on the reflection spectra and changes in amplitude. Given the high responsiveness of the sensor to the changes in RI and a desirable decrease in the return power as evidenced by Figure S4, these sensors were selected for further functionalization steps and were used during measurements with IL-8 and control proteins. Figure S5 demonstrates a consistent decrease for all three sensors in the amplitude changes with increasing RI values. A linear trend was observed for three sensors with a coefficient of determination $R^2 > 0.99$. Fabricated BR act as weak interferometers that are responsive to external physical stimuli. To achieve the detection of a specific analyte, sensing units were further functionalized to develop a biosensor specific to the IL-8 protein.

3.2. Surface morphology of the functionalized sensors

The surface morphology of the sensor was imaged using AFM for all functionalization steps, and 3D images illustrating the variations on the surface are shown in Fig. 3(a-f). Figure 3(g-h) clearly demonstrated that the sensor surface treated with Piranha solution has the lowest height and RMS roughness. APTMS treatment resulted in a smoother surface with increasing height and roughness. Heat treatment promoted cross-linking of the APTMS polymers on the sensor's surface, effectively increasing the height and roughness similar to other reported work [31]. After GA treatment, there were more peaks leading to surface irregularities, with a slight decrease in height compared to the sensor surface following heat treatment. This decrease in surface height and roughness is caused by the surface being conjugated with the linker for antibody (GA), which fills in the valleys and reduces the height difference between the peaks and troughs on the surface. The data in Fig. 3(g-h) showed that antibody immobilization had no significant effect on height or roughness. The overall gradual increase in the thickness of the layers is consistent with the literature data showing an increase in the height of the surface layer following APTMS and antibody attachment compared to the bare sensor [39]. Small difference in roughness between APTMS-GA and antibody layer could be due to a full coverage of the surface by APTMS similar to the work by To *et al* [40]. The addition of mPEG-amine as a blocking agent to prevent non-specific binding to the immobilized antibodies resulted in a much smoother surface. Variations in the height and roughness of the ball surface can be used to track the immobilization of each functionalization step and confirm layer attachment.

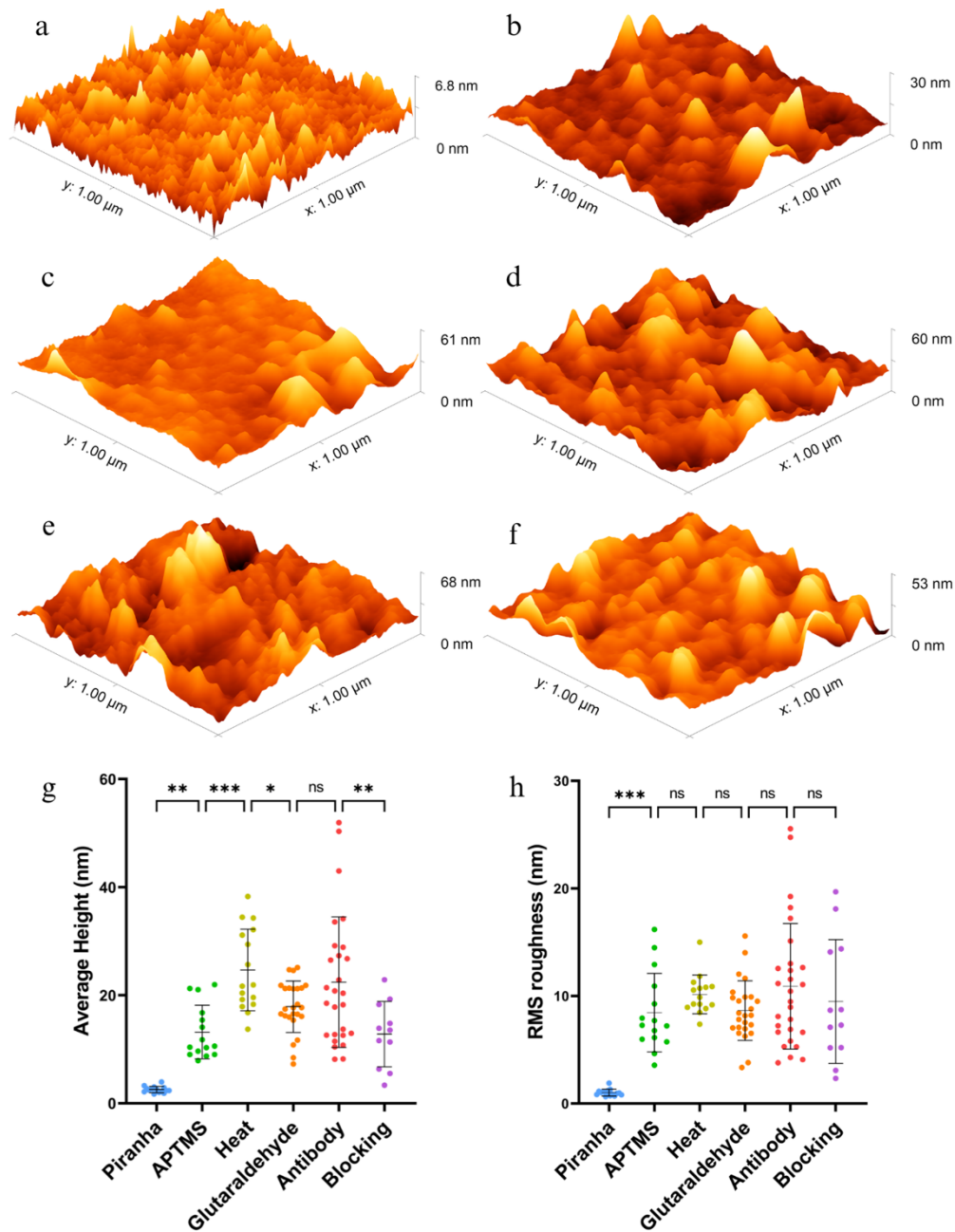


Fig. 3. Analysis of surface morphology for bare and functionalized sensors. Representative 3D images of a $1 \mu\text{m}^2$ scanned area on the electrode surface for: (a) treatment with piranha, (b) treatment with APTMS, (c) additional treatment with heat, (d) addition of glutaraldehyde, (e) treatment with antibody, and (f) blocking with mPEG-amine. Comparison of the height (g) and RMS-roughness (h) for all functionalization steps ($N \geq 310$).

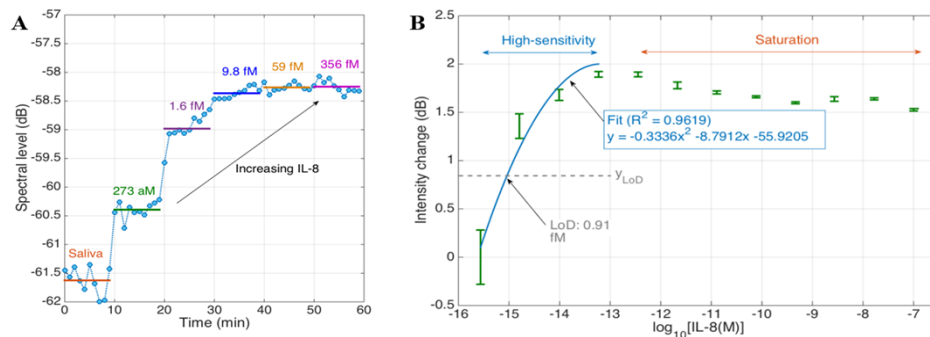


Fig. 4. The performance of the biofunctionalized ball resonator (BR) biosensor during IL-8 detection. A) Sensorgram displaying the spectral level with increasing concentrations of IL-8 in artificial saliva; data for the sensor with diameter 556 μm is presented. B) Response of the BR sensor across a wide range of IL-8 concentrations (273 aM to 100 nM). Error bars = measured data (average value $\pm$ standard deviation, over 6 consecutive measurements). In the high-sensitivity region (273 aM – 59 fM), data are compared to a log-quadratic fit ($R^2 > 96\%$); the limit of detection is to 0.91 fM.

3.3. Detection of IL-8 by the functionalized optical fiber biosensor

BR sensors which showed sensitivity to RI change were functionalized with ligands specific to IL-8 protein, and the functionalized sensors were used to detect the IL-8 in diluted artificial saliva solutions, measuring each concentration for 10 minutes as shown in Fig. 4(A). Changes in the intensity of the signal for the sensor in response to different concentrations of protein are given in Fig. 4(B). A rise of amplitude as the protein concentration increases can be observed with a flattening of the signal towards the last three protein concentrations (Fig. 4(A)). When a wider analyte concentration was tested, the results also demonstrated that at higher protein concentration saturation occurred (Fig. 4(B)). The region where the sensor had the highest sensitivity for IL-8 was used for the estimation of the LoD. The response of the sensor at the lowest protein concentration (y_{blank}) and the maximum variation in the standard deviation (σ_{max}) for the data used for the calculation of LoD, which was calculated to be 0.91 fM. Highly sensitive response corresponded to the lowest concentrations of IL-8 and changes in the spectral intensity for the given region demonstrated in Fig. 5 indicate the capacity of the sensor to detect variations in the protein concentrations at ultra-low levels.

Figure 6(a-c) shows three IL-8 biosensors used for the target protein detection, which displayed a consistent response in intensity change with high differentiation power for the region of low IL-8 concentrations. The response of all sensors began to saturate at high protein concentrations. The acquired data provides strong evidence for the replicability of our observations for the intensity change during protein measurements.

A possible explanation for the observed trend in the sensors' response could be due to the high binding affinity of the antibody-protein pair. The high response shown by the BR biosensor, that is in analogy with some previously reported detection of proteins [31], can prove that the binding process is repeatable and detectable at concentrations that are inferior to the range of use of ELISA, showing a high response at the lowest concentration and over 3 orders of magnitude. In converse, the sensor offers a saturated response over this range, suggesting that saliva samples can be diluted to match the target detection range, which results in the saturation of the binding sites. In this case, the potential mechanism behind this observation could be connected to the slow-release kinetics of the protein from the antibody compared to that of binding. As the sensor is placed in the serially diluted solutions of protein, starting measurements from lowest to highest

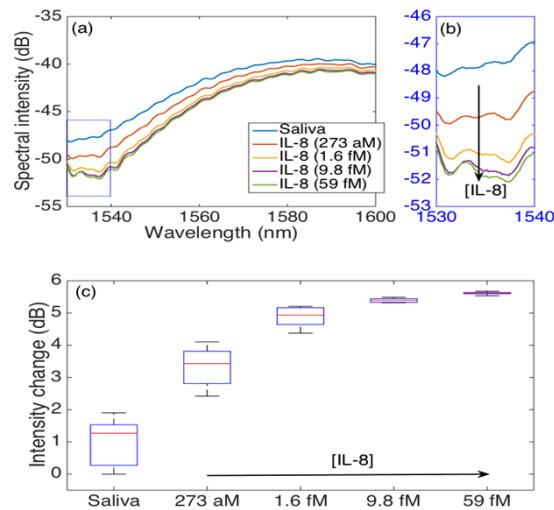


Fig. 5. Detection of IL-8 at ultralow concentrations. (a) S-polarization spectrum of the functionalized BR sensor (size 551 μm) exposed to the reference artificial saliva and to IL-8 concentrations ranging from 273 aM to 59 fM. (b) Inset on the spectral portion around 1534 nm demonstrating the highest detection sensitivity. (c) Boxplot displaying the intensity change from the reference condition for each low-concentration IL-8 condition; red line = median, box = 25/75th percentile, bars = minimum/maximum values, acquired over 10 minutes with 1-minute sampling time.

leads to the occupation of antibody binding sites by the protein, which prevents the sensor from detecting the changes in concentration for the subsequent measurements. The small variation in the response collected for the third sensor displayed in Fig. 6(c) can imply that sensor was still able to detect some changes for the high protein concentrations, which requires further experiments to optimize antibody concentration used during the functionalization procedure to achieve better differentiating power. Despite the given variations in size and spectral characteristics of each biosensor, functionalization procedure allows to achieve a comparable sensitivity for all samples, with a high response at the lowest concentrations, a log-quadratic pattern up to 0.1 pM followed by the saturation pattern as shown in Fig. 6(d). The different sensitivity of each sensor was reflected in the slight divergence between the intensity values, without affecting the overall trend for IL-8 detection.

3.4. IL-8 biosensor is selective for the target protein

To ensure the selective detection of target of interest by the functionalized sensor, it was important to evaluate its response against other proteins that are commonly found in saliva or increased due to pathological conditions. Changes in the intensity parameter for the IL-8 detection and control proteins are shown in Fig. 7. Sensors used for the measurements of CEA and lysozyme were functionalized in the same way as the sensor used for the IL-8 detection. These proteins were chosen as controls because the levels of CEA protein have been found to be elevated in the saliva of patients with oral cancer, while the lysozyme is one of the abundant antimicrobial proteins in saliva [41]. Obtained results show that the responses of the IL-8 biosensor to the CEA and lysozyme control proteins were significantly lower compared to the target IL-8 protein. The difference in the measured intensity change confirms the specificity of the IL-8 biosensor to the target protein.

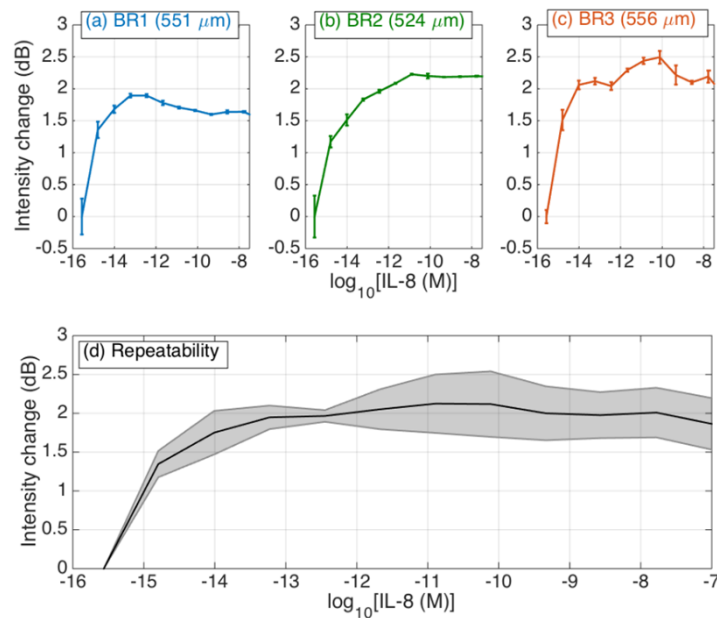


Fig. 6. Repeatability of the IL-8 detection, evaluated over 3 different sensors having diameters 551, 524, and 556 μm undergoing the same functionalization process and detection over the same concentration range. (a-c) Intensity decrease observed from the reference value observed for each of the three sensors (error bar = value $\pm$ standard deviation). (d) Repeatability trace combining the response of all three sensors (solid line = mean response; shaded region = $\pm$ standard deviation).

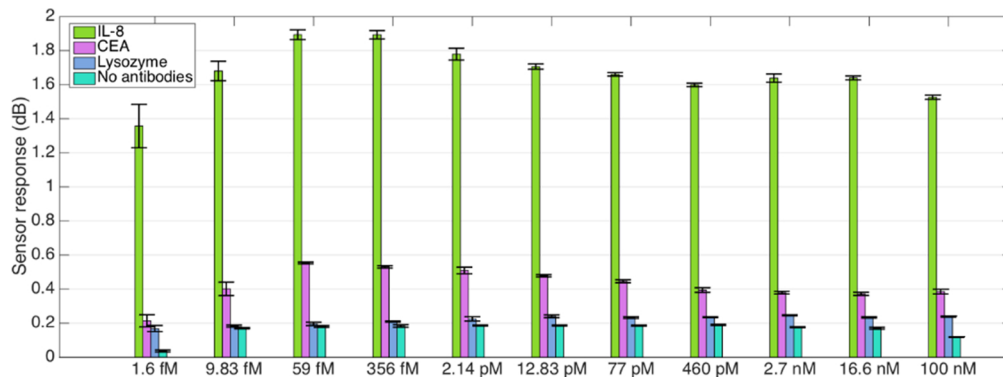


Fig. 7. Specificity analysis for the IL-8 ball resonator. The chart displays the sensor response, measured from the lowest concentration (273 aM), compared with other two sensors undergoing the same functionalization and detecting non-specific controls (CEA and lysozyme), and a probe functionalized with no antibodies

4. Conclusion

This work provided the proof that optical fiber biosensors could be used to detect IL-8 proteins in artificial saliva solution at extremely low levels. A sensor functionalized with anti-IL-8 antibodies enabled us to develop a highly selective and sensitive sensor for the detection of IL-8 protein in saliva, allowing for the rapid and non-invasive identification of salivary biomarker biosensor for

the selective detection of IL-8 protein. The development of multiplex systems to detect incredibly low concentrations of salivary biomarkers is possible given the femtomolar detection limit of the biosensors. This would allow for a non-invasive diagnosis of diseases. In comparison to currently available IL-8 detection tools, obtained results demonstrate the functionalized sensor's high sensitivity and selectivity. It is also simple to fabricate, offers a reproducible response, and enables rapid detection.

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Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

Supplemental document. See [Supplement 1](#) for supporting content.

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