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Photonic sensor to detect rapid changes in CRP levels

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Abstract

One of the most important biomarkers used to determine inflammation is C-reactive protein (CRP). Its level, when it is within the range that does not define inflammation, informs about the risk of cardiovascular events. If the norm is exceeded and inflammation is detected in the body, CRP level can increase 1000 times within a few hours. The type of infection can also be determined based on the level of elevated CRP. All this makes CRP a very important element of diagnostics.

A sensor based on low coherence interference is presented. Preliminary studies have shown that its sensitivity is 5.65 μg/L and the measurement time is short, < 10 minutes. The entire system is built of commercially available components, which allow production cost minimalization. In addition, the user-friendly operation allows it to be operated by unqualified people. Due to these features, our solution is a promising alternative to commercially used ELISA, which needs trained personnel to perform time-consuming measurement procedures.

Keywords: biomarkers, optical fiber sensor, CRP detection

1 INTRODUCTION

Inflammation is a non-specific response of the immune system to actual or potential infection [1] It is a defense mechanism necessary to maintain health because its purpose is to protect against the spread of injury and then restore the normal structural and functional state of damaged tissues [2]. Inflammation can be caused by various factors, i.e. contact with pathogens - bacteria and viruses - or non-infectious factors such as damaged cells, chemical irritants [3]. It triggers a chemical cascade of tens of molecules, which causes physical symptoms such as fever, high blood sugar, and pain. All of these chemical and physical symptoms are desirable unless they develop into chronic inflammation [4].

Two functionally related biomarkers are used to detect inflammation. One of them is the cytokine interleukin 6 (IL-6), which stimulates the production of the second important protein, which is the acute phase C-reactive protein (CRP)[5]. CRP protein has two varieties

[6]. The pro-inflammatory pCRP protein, which is secreted during inflammation. There is also the mCRP protein, which is involved in the restoration of damaged tissues even in the absence of inflammation [7,8]. Its role can be considered anti-inflammatory. In the literature, the vast majority of researchers analyse the pCRP protein as a factor of inflammation. It is a very good marker of the state of the body because its level changes very quickly when immunological dangers are detected. Up to a 1000-fold increase in CRP levels during infection is possible, and this increase occurs within a few hours [7]. Maximum production of CRP is achieved after 24-30 hours after inflammation offset [9]. The CRP level in a healthy adult human should not exceed 3 mg/L. The value above 5 mg/L is considered alarming, and above 10 mg/L as a sign of inflammation [10]. The distinctions result from taking into account individual differences and the presence of inflammations with low CRP levels. If inflammation occurs, the type of infection can be determined from the CRP level [11–13]. If the CRP level does not exceed 40 mg/L, it is most likely a virus infection [14]. Bacterial infection increases the amount of CRP protein to a level above 60 mg/L [15,16] or 100 mg/L [17]. The CRP protein can also be used to assess the risk of cardiovascular events [18]. In this case, a level below <1 mg/L is a low risk, a level between 1 mg/L and 3 mg/L is a moderate risk, and a level above 3 mg/L is a sign of a high probability of a cardiovascular event [19]. CRP is a very universal indicator, but still not fully comprehended. Much research is being done on its correlation with other diseases such as Alzheimer's [20], depression [19,21], various types of cancer [22,23], diabetes [24] and chronic dialysis [25]. Studies are also carried out to correlate the level of CRP in a specific disease with the appropriate treatment [26,27].

Usually, immunoturbidimetric and immunonephelometric tests are used for CRP measurements. The standard method is the high-sensitivity enzyme-linked immunosorbent assay (ELISA) [28]. Its sensitivity is on the level of pg/L [29] but is sensitive to the presence of non-specific proteins. ELISA is also a time-consuming method, requiring complex detection steps and professional personnel [28]. CRP is a very important biological marker that is used in many aspects of the detection and treatment of inflammation and its genesis. For this reason, there is a need to create new sensors that will allow for accurate, easy, fast, safe, and highly sensitive measurement of the CRP level. In response to these needs, this article proposes a fiber optic sensor to detect the CRP level.

2 MATERIALS AND METHODS

2.1 Measurement setup

The schema of the measuring system is shown in Figure 1. During measurements, a broadband light source emitting light with a central wavelength of 1310 nm (SLD-1310-18-W, FiberLabs Inc., Fujimi) was used. The light propagated through the fiber optic coupler (G657A, CELLCO, Kobylanka, Poland) into the biofunctionalized end-face of the optical fiber. Then, the wave reflected from the end-face of the fiber propagated to the detector, which was a spectrum analyzer (Ando AQ6319, Yokohama, Japan).

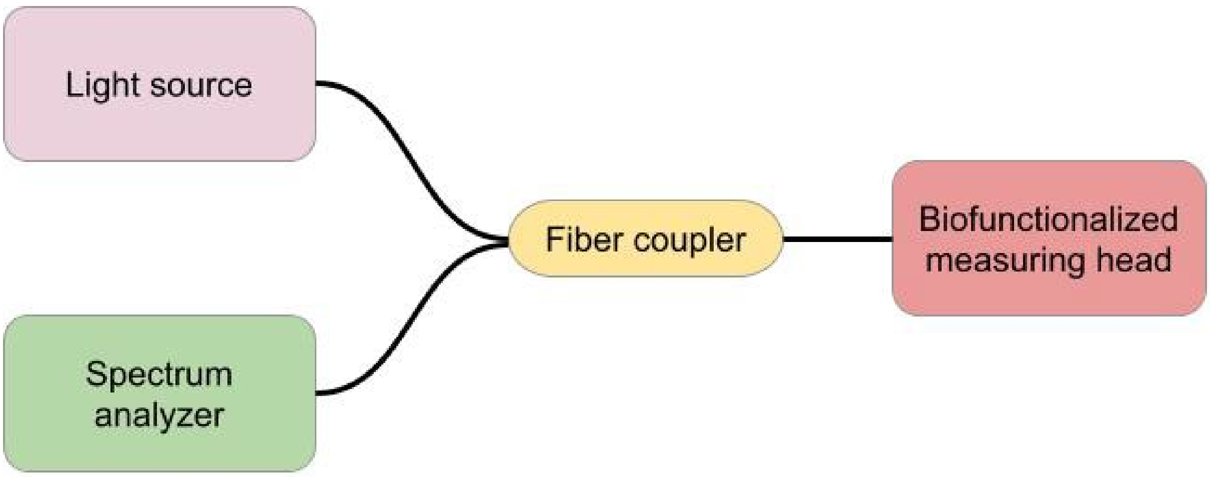

Figure 1 Schema of the measuring system.

2.2 Measuring head biofunctionalization

Optical fiber biofunctionalization was performed as follows. The molecular sieves (3 Å, Pol-Aura, Poland) were activated in an oven (10 hours, 350°C) and cooled, then transferred to the 250 mL glass bottle with tight sealing and filled with dimethyl sulfoxide (molecular biology grade, Merck, Germany) or acetone (molecular biology grade, Merck, Germany) (1:1 (v/v)). Finally, left for 72 hours to dehydrate the solvents. The sensor was cleaned extensively by immersing it in H_2O_2/NaOH (WarChem, Poland; ACS reagent, Merck, Germany, respectively) water solution (10%, 25 mM respectively at 80°C) for 30 min. After the cleaning procedure, the optical fiber was immersed in ultrapure water and finally in anhydrous acetone solution to remove excess moisture (3 times in different solutions, each incubation lasted 10 min in an anhydrous environment). Cleaned optical fiber was immersed in a freshly prepared 1% 3- Aminopropyltriethoxysilane (99% purity, Merck, Germany) solution in anhydrous acetone for 12 hours in an anhydrous environment - to cover the optical fiber in amino groups. After 12 hours the optical fiber was immersed in anhydrous DMSO (3 times, 5 min of incubation) to remove excess APTES solution. Cleaned optical fiber with amino groups on the surface was then immersed in a freshly prepared 10 mM stock solution of NHS-LC-Biotin (95% purity, Merck, Germany) in anhydrous DMSO and left for 24 hours in an anhydrous environment. Finally, after incubation, the sensor was immersed in the Streptavidin (1 mM solution, J&K Scientific, Poland) for 10 hours at 15 °C. Not immobilized protein was removed by washing in 1xPBS (Phosphate buffered saline tablets; Merck, Germany) solution. Finally, the sensor was immersed in biotinylated antiCRP IgG (1 µg/µL, Merck, Germany). After the antiCRP IgG attachment, a biological layer was formed at the end-face of the optical fiber, as shown in Figure 2.

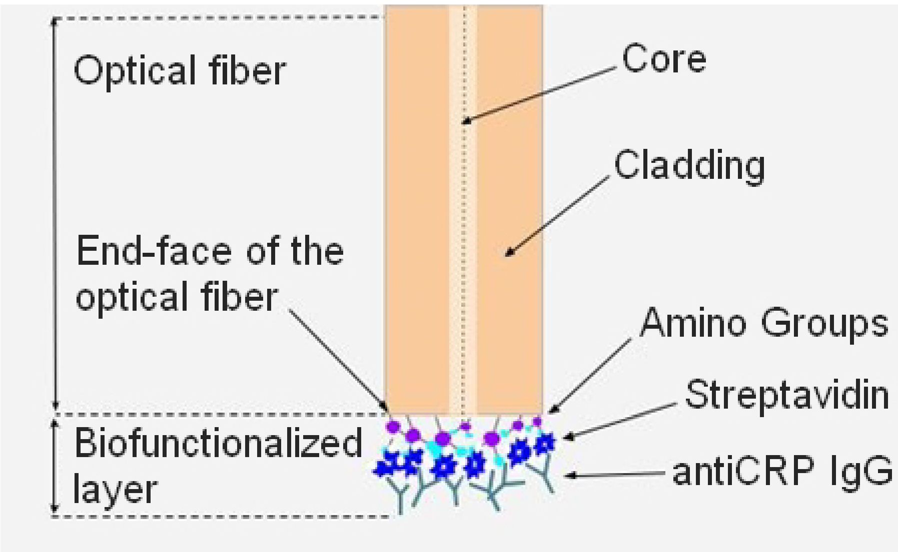

Figure 2 Elements of the biofunctionalized fiber head.

3 RESULTS AND DISCUSSION

CRP detection consisted in carrying out a series of measurements of optical spectra during the attachment of the protein to the antigens covering the measuring head. For this purpose, the biofunctionalized measuring head was placed in a test tube filled with the solution. The tube was filled with a solution of human CRP (1 µg/µL, Merck, Germany) in 1xPBS with a concentration of 5.65 µg/L. The occurring chemical interactions (hydrogen bonds, water bridges) between the CRP protein and its antigens caused changes in the observed optical signal. The test tube was held in a device that kept the temperature constant, which was 25°C. After inserting the measuring head into the solution, the recording of optical spectra with the analyzer was started. The optical spectra were recorded for 10 minutes, about every 6 seconds. To detect CRP, the change in signal intensity over time had to be observed. Data analysis began with the analysis of a single optical spectrum. The received signal is noisy, hence it was necessary to denoise it prior to its analysis. The FFT (Fast Fourier Transformation) algorithm was used. FFT filtration consists in transforming the sampled signal, i.e. a sequence of real numbers, into a sequence of complex numbers. In this way, a frequency spectrum is obtained containing information about the signal components. Unwanted components, e.g. those responsible for noise or not carrying important information, are removed. The inverse transformation is performed and the result is a signal with selected components.

The next step in the signal analysis was to plot the changes in the intensity during the attachment of the CRP protein to the functionalized measuring head. The plotted curve, based on the studied changes in intensity, has exponential form between 200 and 250 seconds. The exponential characteristic of homopolyvalent antibody-antigen interaction is dependent upon: (I) – antigen concentration, (II) – surface immobilized antibody density and (III) – their orientation on the surface of the sensor head. Those characteristics regarding the interaction of pentameric CRP with immobilized antiCRP antibody was investigated by S.Lin et al. [30]. Their results indicate the exponential characteristic of antigen/antibody interaction in different antigen concentration ranges and different binding stoichiometry forms (Ag1Ab1; Ag1Ab2, Ag2Ab1). To make it easier to observe, the resulting curve was averaged. The MAF (Moving Average Filter) algorithm was used, which calculates the mean value of a sample from a set of samples of the specified length. This length is referred to as the window. For the analysis of the measured signals, a rectangular window with a length of 8 samples was used. Time studies were carried out to investigate whether the CRP proteins had attached to the functionalized measuring head. They lasted 10 minutes and the result is shown in Figure 3.

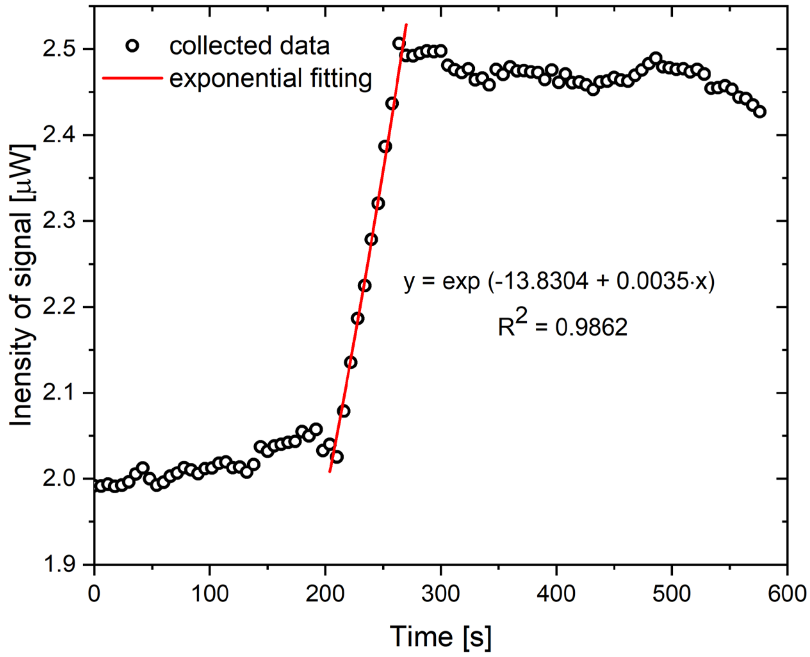

Figure 3 Change in signal intensity during antibody and CRP recombination. The plot presents collected data and the fitted exponential curve .

In the initial phase, which lasted about 200 seconds, the signal intensity value ranges from 1.98 µW to 2.05 µW. After this time, the rapid increase of the signal intensity appears due to progressing sensor saturation. It reaches a value above 2.45 µW and fluctuates around it. The obtained characteristics are consistent with the theory. This means that the proposed sensor enables the detection of CRP concentration of 5.65 µg/L.

Detecting the CRP level is an extremely important element of diagnostics, which means that research on dedicated sensors is extensive. Among others, the optical methods are successfully used due to their unique properties. One of the solutions proposed in the literature is the use of an optical cavity biosensor [31]. This system detects the local change in refractive index caused by the adsorption of biomolecules on the receptor molecules. The method is based on differential detection, which ensures high sensitivity. The system consists of cheap parts and components as well as assures relatively simple operation. The test sample is CRP standardized solution in an amount of 15 µL. The entire measurement takes less than

30 minutes, the achieved limit of detection is 43.3 mg/L [31]. Another design of the optical sensor that can be used to determine the CRP level is a biosensor based on an long-period grating (LPG) made of double cladding fiber type W with graphene oxide [32]. The operating point was adjusted to the mode transition region by etching the fiber outer cladding, which increased the sensitivity while maintaining the visibility of the spectral features of the grating. The measurement time was 20/30 minutes and the detection limit achieved in serum was 0.15 µg/L. The clad etched fiber Bragg grating (FBG) sensors with graphene oxide were also developed [33]. The sensor has shown high sensitivity in the presence of interfering factors and wide linear range of operation. Detection limit was equal to 10 µg/L, the measurement time lasted about 10 min, and the relatively high sample volume of 200 µg/L was used. A different approach to create a sensor that determines the level of CRP utilizes a plastic optical fiber and a surface plasmon resonance (SPR) [34]. The sensor was integrated with a thermally stabilized microfluidic system. The detection was in human serum, and the lowest value detected was 9 µg/L. The measurement lasted 15 minutes [34]. Another SPR-based fiber-optic biosensor working in a label-free manner was developed using dopamine as a cross-linking agent [35]. A multi-mode plastic clad fiber was used. The sensor shows satisfactory sensitivity and linear response. P. Zubiate et al. developed a fiber-optic sensor for detection of CRP utilizing Lossy Mode Resonance (LMR) [36]. The interaction of the protein with the aptamer results in the resonance wavelength shift. The constructed device achieved low limit of detection (62.5 µg/L), fast response time (61 s) and satisfactory sensitivity, as well as specificity. The measurement time was 700 s. The Table 1 shows a comparison of the solution proposed in this study with other selected methods described in the literature.

Table 1 Comparison of CRP detection methods using optical fibers

Number of method	Detection method	Sample	Sample volume	Detection limit	Measurement time	Ref
1	Proposed solution	CRP standardized solution	10 µL	5.65 µg/L	<10 min	N/A
2	Optical cavity sensor	CRP standardized solution	15 µL	43.3 µg/L	<30 min	[31]
3	LPG in double cladding fiber coated with graphene oxide	Human serum	40 µL	0.15 µg/L	20/30 min	[32]
4	clad etched FBG with graphene oxide	CRP standardized solution	200 µL	10 µg/L	10 min	[33]
5	SPR-based plastic optical fiber sensor	Human serum	20 µL	9 µg/L	15 min	[34]
6	SPR-based fiber-optic sensor	CRP standardized solution	no data	10 µg/L	60 min	[35]
7	LMR-based fiber-optic sensor	CRP standardized solution	no data	62.5 µg/L	11 min	[36]

Among presented techniques, the sensor described in this study assures the smallest sample volume needed to perform a measurement. The requirement of the minimal sample usage is advisable as it is associated with a smaller amount of blood taken for testing. In terms of measuring time, the presented sensor is the fastest solution: achieving the measurement result is possible in less than 10 minutes. The achieved detection limit of 5.65 µg/L is the second lowest result among presented methods, placing it behind a sensor with more complicated manufacture procedure. A significant sample volume reduction, the shortest measurement time and relatively simple construction give a possibility of the wider use of the developed optical sensor outside the professional diagnosis laboratories. The obtained results of the preliminary studies performed in CRP standardized solution assure a strong basis for further research, involving measurements in real biological samples.

4 CONCLUSION

Compared to other sensors for a CRP detection, the proposed sensor requires a small amount of sample (10 µL), assures the shortest measurement time (<10 min), and its sensitivity is sufficient for medical measurements. The simplicity of its design and the possibility of constructing it from commercial elements are other advantages. As the sensitivity is acceptable for performing fast medical measurements, slightly worse measurement parameters compared to professional laboratory instruments requiring trained personnel to carry a long-time procedure are acceptable. Moreover, the proposed solution can be further miniaturized. The measurement head could be modified to a microsphere for additional real-time control of the sensors integrity in real-life applications [37]. The presented preliminary results are promising and constitute a strong base for further research, which will focus on establishing the full measuring range and determining sensor’s parameters in real biological samples more precisely.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.S.; methodology, M.S.; preparation of biofunctionalized fiber head, P.W.; measurements, P.W.; measurement data processing and analysis, M.K. and M.S.; writing—original draft preparation, P.W., M.K.; writing—review and editing, M.K, R.V. and M.S. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Please, contact Małgorzata Szczerska at malszcze@pg.edu.pl

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