

# Sensor Array Based on Molecularly Imprinted Polymers for Simultaneous Detection of Lipoproteins <sup>†</sup>

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**Abstract:** Herein we report a sensor array based on quartz crystal microbalance (QCM) to simultaneously detect two biomarkers, namely low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Selective recognition takes place through molecularly imprinted polymers (MIP) with both MIPs and corresponding non-imprinted polymer (NIP) as reference electrode. Sensor array performs highly appreciably in term of selectivity to LDL and HDL (defined through its cholesterol LDL-C, and HDL-C) when operated in 10 mM PBS. The sensor response time is less than 15 min. Furthermore, coefficients of variation indicating precision of our sensor array are at 2%–8%.

**Keywords:** lipoproteins sensor array; low-density lipoprotein; high-density lipoprotein; molecularly imprinted polymer; quartz crystal microbalance

## 1. Introduction

Molecular imprinting constitutes a procedure synthesizing polymers containing specific recognition sites that are complementary to the template concerning shape and positioning of functional groups, namely molecularly imprinted polymers (MIPs). They are established as synthetic receptors with binding ability comparable to natural receptors, but provide longer stability in harsher conditions including high temperature, non-physiological pH, and organic solvents, low manufacturing cost, and straightforward synthesis [1]. Current MIPs are very attractive biomimetic receptor materials. Coupled with suitable transducers they lead to appreciable biological assays. To date protein imprinting approaches demonstrate broad applicability in the area of biomedical analysis, including detecting human serum albumin (HSA) [2], human papillomavirus derived E7 protein [3], and insulin [4].

Within this paper, we present MIP for complex aggregates of lipids and protein, namely lipoprotein. Two classes of lipoproteins, namely low-density lipoprotein (LDL), and high-density lipoprotein (HDL), are critical biomarkers in clinical analysis for assessing coronary heart disease (CHD) and monitoring its treatment [5]. Serum concentrations of LDL and HDL particles in clinical assessment are usually determined in term of the amount of cholesterol within lipoprotein particles, namely LDL-C and HDL-C, respectively. Therefore, our all sensor array signals are calibrated toward LDL-C and HDL-C, resulting data comparable to recently clinical relevance ranges which are shown in Table 1.

**Table 1.** Clinical concentration ranges of lipoproteins.

| Risk Level of CHD | LDL-C (mg/dL) | HDL-C (mg/dL) |
|-------------------|---------------|---------------|
| Low risk          | <129          | ≥60           |
| High risk         | 130–159       | <40           |
| Very high risk    | >159          | -             |

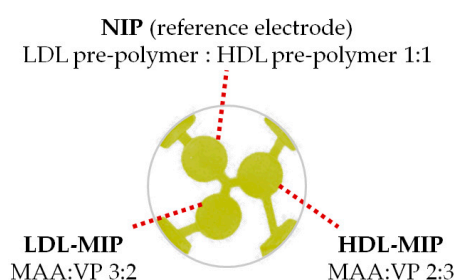
In clinical laboratory, cholesterol contents in each lipoprotein class are analyzed using a homogenous enzymatic colorimetric assay. However, this system requires sample pre-treatment for isolating desired lipoprotein from other lipoprotein classes before the cholesterol was measured [6]. Therefore, novel MIPs of LDL [7] and HDL [8] were synthesized as biomimetic selective layers to detect LDL and HDL, respectively. Our MIPs studies are more challenging, than those for “simple” proteins, because lipoproteins surfaces reveal large numbers of functional groups of three constituents comprising amphipathic phospholipids, free cholesterol, and apolipoproteins, which are all available to interact with functional monomers. In terms of surface chemistry, LDL and HDL surface layers are both composed of similar types of the three components mentioned, but they contain different ratios of each component and also slightly differ in diameter ( $28.9 \pm 9.2$  nm for LDL,  $21.5 \pm 6.5$  nm for HDL).

Recently, independent sensing of LDL and HDL with MIP-based quartz crystal microbalance (QCM) sensor was successfully developed for measuring amount of LDL and HDL in sera and achieved rapid detection speed (within 10 min) per each sample. Herein, we report preliminary results for the design of LDL and HDL sensor array which can assess both lipoproteins simultaneously.

## 2. Materials and Methods

Tri gold-electrode patterns of 10 MHz QCM were spin-coated with oligomer mixtures containing methacrylic acid (MAA) and N-vinylpyrrolidone (VP) cross-linked by ethylene glycol dimethacrylate (EGDMA), followed by imprinting with LDL and HDL which led to LDL-MIP and HDL-MIP, respectively. The third electrodes served as reference electrodes, respectively, containing non-imprinted polymer (NIP) as shown in Figure 1. For sensor measurements, the baseline signals were obtained via 10 mM PBS until reaching the equilibrium state. The sensor was then exposed to standard solutions containing the same concentration at 200 mg/dL of individual LDL-C, HDL-C and mixed LDL-C and HDL-C, respectively.

For precision test, the sensor data of mixed- standards containing 200 and 100 mg/dL of LDL-C and HDL-C, respectively, were recorded three times.

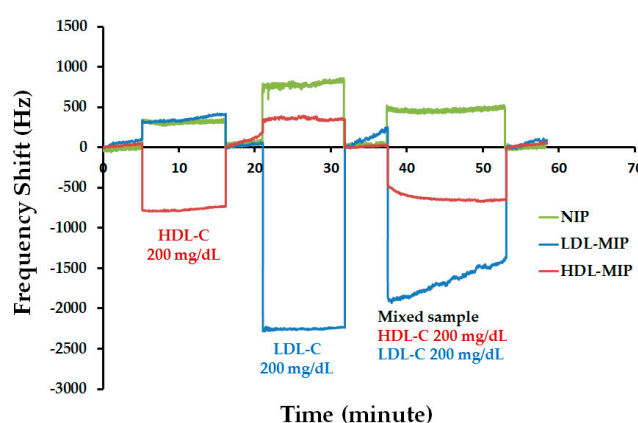


**Figure 1.** A scheme diagram for lipoproteins sensor array based on three-electrode QCM.

## 3. Results and Discussion

Figure 2 shows sensor outcomes for tri-electrode QCM coated with HDL-MIP, LDL-MIP, and NIP toward a standard HDL-C, LDL-C, and mixed-HDL-C and LDL-C solutions in 10 mM PBS at similar concentrations of 200 mg/dL, respectively. After adding 200 mg/dL HDL-C, HDL-MIP-coated

electrode leads to decreasing frequency signals of  $-783$  Hz that correspond to mass loading. In contrast to this, LDL-MIP and NIP yield positive frequency shifts of 317, and 359 Hz, respectively. Actually, non-Sauerbrey behavior of increasing frequency has been observed in biospecies, such as bacteria [9], yeasts [10], including lipoproteins [7,8], due to weakly bound and retain some mobility on flat surfaces. After flushing the system with mixed 10% aqueous solution of acetic acid and 0.1% sodium dodecyl sulfate solution, followed by deionized water, the analytes are removed out and the frequency increases to baseline revealing fully reversibility. In parallel, 200 mg/dL LDL-C loading, reveals negative frequency shift only on the LDL-MIP side of  $-2291$  Hz. HDL-MIP and NIP (again) showed positive frequency signal of 153, and 759 Hz, respectively. In following, mixed-HDL-C and LDL-C solution added, sensor reveals negative signal of the both electrodes of HDL-MIP and LDL-MIP at  $-678$ , and  $-1943$  Hz, respectively, NIP also still show positive signal at 424 Hz. Obviously, at the same concentration of each standard, the sensor response of LDL-MIP toward LDL-C ( $-2291$  Hz) yields 3 times higher signal than the effect of HDL-MIP to HDL-C ( $-783$  Hz). This stronger signal might due to higher molecular weight (MW) of LDL ( $2.30 \times 10^6$  Da) than HDL ( $0.36 \times 10^6$  Da) [11], resulting increased mass loading on the sensor. Response times are around 10–15 min per experiment.



**Figure 2.** QCM responses toward standard HDL-C, LDL-C and mixed-LDL-C and HDL-C in 10 mM PBS.

In terms of precision, coefficients of variation (CVs) of sensor array measurement at mixed concentrations of 200 mg/dL LDL-C and 100 mg/dL HDL-C were 2% and 8%, respectively, as shown in Table 2.

**Table 2.** Repeatability test.

| Mixed-Standard Concentration | Mean of Frequency Shift (Hz) ( $n = 3$ ) | Standard Deviation | % CV |
|------------------------------|--|--------------------|------|
| 200 mg/dL LDL-C              | 1619                                     | 39                 | 2    |
| 100 mg/dL HDL-C              | 471                                      | 37                 | 8    |

#### 4. Conclusions

All results indicate successful imprinting with a clearly selective capability of each polymer layer to each lipoprotein class. This setup thus constitutes the first step for a sensor array. Next steps will comprise fully evaluating sensor array that can indeed quantify both LDL-C and HDL-C in serum simultaneously.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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