Nucleosides analogues recognition by molecularly imprinted polymer-coated Love wave sensor

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Presented is a process for thin film molecularly imprinted polymer (MIP) coating based on commercial nucleotides adenosine monophosphate (AMP). The compatibility of the MIP film with acoustic propagation and sensor sensitivity for rebinding of AMP have been verified. Thin and porous layers of AMP-based MIP layers were successfully deposited on the sensor surface. Detection tests of AMP have been performed in aqueous media. The sensor response was recorded in terms of frequency and total insertion losses after both steps: AMP extraction from MIP then AMP rebinding. The sensor showed high sensitivity to 25 ppm AMP concentration. The effect of the extraction time on rebinding capacity of the MIP layer has been proven.

1. Introduction: The early diagnosis of cancer is the most critical factor for patient survival and the treatment of cancer. Rapid detection with an ultra-low detection limit of cancer markers is important for the early diagnosis of cancer. Specific protein markers for prostate, lung, breast and colon cancers are known. The detection of cancer markers can be done using a number of techniques including standard immunoassays using samples from blood, urine and biopsy. Based on the sensor's data along with correlated results from other techniques such as magnetic resonance imaging (which tells the tumour status such as the location, grade and stage of the tumour), the tumour can be properly treated. Detection of multiple markers using array-type sensors, which can quantitatively describe the status of cancer, and lab-on chip sensors allows point-of-care service as a non-invasive technique [1].

The great challenge for sensor systems to be accepted as a relevant diagnostic and therapeutic tool for cancer detection is the ability to determine the presence of relevant biomarkers or biomarker patterns comparable to or even better than the traditional analytical systems [2]. Our work focuses more particularly on colorectal biomarkers detection.

This Letter relates to a Love wave sensor coated with a molecularly imprinted polymer (MIP) sensitive layer, where the advantages of high sensitivity of the biosensor are combined with simple preparation, high selectivity and robustness of thin film MIP to detect the adenosine monophosphate nucleotide (AMP).

A biosensor is characterised by two main components: a recognitive or sensing element, which specifically interacts with a target analyte, and a transducing element, which converts the interaction into a quantifiable effect. Key requirements of an effective biosensor are specificity for its desired target and detection capabilities over the entire relevant concentration range. This necessitates that the recognition element be capable of selectively binding the analyte with high affinity and selectivity [3].

1.1. Transducing element: A Love wave sensor is based on the perturbation of a guided shear horizontal surface acoustic wave (SAW). This particular polarisation limits energy losses while the wave propagates along with a liquid environment, making this type of acoustic sensor the most suitable for applications in liquid. The Love wave acoustic energy is mostly confined in the guiding layer, leading to high surface density energy and therefore a high sensitivity to surface perturbations [4]. Another advantage of such sensors is their ability to record in real-time the affinity reaction allowing kinetic studies [3]. They are also versatile, reliable, reusable, small, inexpensive, can easily be designed for responding to various measurands, have a wide dynamic range and they are passive devices which can also be deployed as wireless units. Therefore acoustic wave devices present attractive alternatives to their counterpart technologies in their corresponding sensor applications [5].

Piezoelectric immunosensors are usually designed to detect cancer markers where the specific antibody is immobilised on the sensor chip. A range of biosensor platforms are reported in the literature for cancer disease diagnosis. One example is the novel SAW biosensor in complementary metal-oxide semiconductor technology that employs a streptavidin/biotin-based five-layer immunoassay for detecting a prominent breast cancer biomarker, mammaglobin (hMAM). A frequency sensitivity of 8.704 pg/Hz was obtained and the sensor showed good selectivity against bovine serum albumin [5]. Piezoelectric immunosensors for human ferritin [6] and human chorionic gonadotropin (hCG) [7] have also been proposed. Gronewold *et al*. [8] reported using a SAW sensor for the detection of individual point mutations in cancer-related gene DNA fragments from single injections.

Despite the successes of systems based on natural recognition elements, their inherent disadvantages which include poor chemical, physical and long-term stability; batch-to-batch variability; skilled-labour intensive; as well as relatively high cost have led researchers to investigate alternative synthetic receptor systems which can overcome these weaknesses. One such technique that has gained significant interest recently is MIPs.

1.2. Sensing element: Molecular imprinting is a promising field in which a polymer network is formed with specific recognition for a desired template molecule. Briefly, functional monomers are chosen which exhibit chemical structures designed to interact with the desired template molecule via covalent or non-covalent chemistry. The monomers are then polymerised in the presence of the desired template; the template is subsequently removed; and the product is a polymer with binding sites specific to the template molecule of interest. Our template is a commercial nucleotide called AMP. It is similar to the nucleoside molecule (cancer biomarker) we aim to detect later.

We chose an MIP surface imprinting strategy, where the imprinted binding sites are located at or very near the surface of the polymer. This is achieved by synthesising a thin polymer film using similar approaches to those in bulk imprinting. This method

facilitates diffusion of the large macromolecule into and out of the network, thereby minimising template size concerns. In addition, surface-imprinted MIPs tend to be more physically robust because of the presence of support and allow for easier integration with sensor platforms. However, the trade-off is a decrease in specificity as only a portion of the protein is imprinted, thus later recognised. Many excellent studies have been published using this approach [9].

2. Materials and apparatus

2.1. Materials: AMP, acrylamide (AA), ethylene glycol dimethacrylate (EGDMA), 2-(dimethylamino)ethyl methacrylate (DMAEM), 3-(trimethoxysilyl) propyl methacrylate (silane), acetic acid, hydroxylamine, toluene and methanol were purchased from Sigma Aldrich (France) and used as received. Azobisisobutyronitrile (AIBN), dimethylsulfoxide (DMSO) and deionised water (resistance $\geq 18 \times 106\ \Omega$) were also used.

2.2. Apparatus: The Love wave sensor used in this study consists of a dual delay line deposited on an temperature complensated (AT) cut quartz substrate (Euler angles: 0°, 121.5° and 90°) used as the piezoelectric material. Interdigital transducers (IDTs) were made by sputtering 70 nm of gold on a 30 nm titanium layer to achieve good surface adhesion. Each IDT is composed of 44 splitted pairs of electrodes with a $40\,\lambda$m periodicity, which defines the wavelength λ. Each electrode is 5 m wide with an aperture W of $40\,\lambda$, whereas the centre to centre path length between electroacoustic transducers (L_{cc}) is equal to $209\,\lambda$. A $4\,\lambda$m plasma enhanced chemical vapour deposited SiO_2 layer is used as the guiding layer. These characteristics lead to a 117 MHz synchronous frequency f_0. This dual delay line setup allows differential measurements with a reference, improving the robustness of the platform when the environmental conditions vary during measurements [4] (Fig. 1).

3. MIP technological process

3.1. Piranha cleaning: The Love wave substrates were cleaned by piranha solution [1:1 (v/v) concentrated sulfuric acid/30% hydrogen peroxide] (caution: piranha solution is extremely corrosive and can react violently with organic compounds; gloves, goggles and face shields should be used for protection). This very strong piranha mixture suppresses the organic and metallic impurities and forms an oxide layer at the sensor surface. The substrates were then rinsed thoroughly with pure water and dried by a stream of nitrogen [10].

3.2. Surface activation: The sensors were rinsed with toluene and purged over night in a silane/toluene [2%/1 (v:v)] mixture before putting them in a stove at 200°C. The silane promotes covalent attachment of the MIP layer to the sensor surface [11].

3.3. MIP solution preparation: The MIP-AMP solution preparation is an adaptation of a bulk MIP process of ICOA Orleans, some MIP constituents are provided by this Institute [12]. Then, 50 mg of template AMP were added to 102.3 mg of functional monomer AA and stirred together with 24.2 µl of DMAEM and 1.49 ml of EGDMA, during 5 min. The mixture was dissolved in 1.1 ml of DMSO and stirred for 1 h. The mixture was then purged with nitrogen for 3 min to remove oxygen. Finally, 16 mg of AIBN were added to the solution and the flask was sealed with parafilm before mixing it for 1 h. Note that we operated a changing in the solution viscosity compared with the bulk solution so that this process is more suitable for thin film coating. The obtained solution is light and heat sensitive so it is better to keep it in a stained flask.

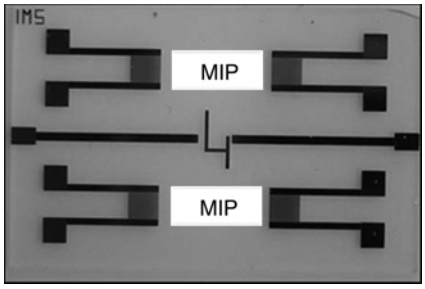

Figure 1 *MIP-coated Love wave sensor*

3.4. Thin film MIP coating: Approximately 10 µl of MIP solution was coated on the sensors using the spin-coating method. The spin-coater parameters are crucial for control of the MIP film thickness and homogeneity. To prevent deposition of polymeric material onto IDTs, they were protected using adhesive tape (Kapton) which was removed immediately after spin coating.

3.5. Polymerisation: The coated sensors were then polymerised under 265 nm UV light for 1 h in a polymerisation box where a nitrogen gas flows permanently.

4. MIP films characterisation: Film thickness estimation was carried out using optical profilometry, as shown in Fig. 2, whereas the scanning electron microscopy (SEM) in Fig 3 revealed the film surface morphology and the pores sizes.

As expected, the higher spinning speed resulted in the formation of thinner films because of more effective spreading of the polymeric material across the surface of the substrate.

The obtained films were homogeneous and limited to the wave propagation area of the sensor. We can see the pores sizes that range from 1 to 5 µm.

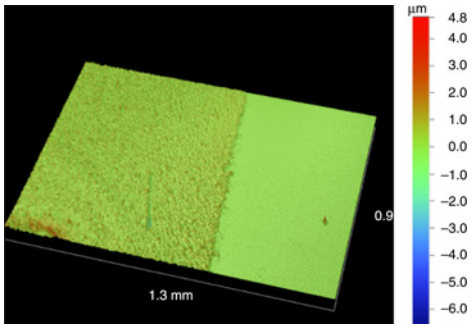

Figure 2 *MIP film picture obtained using optical profilometry*
Layer thickness is estimated to be 1 µm

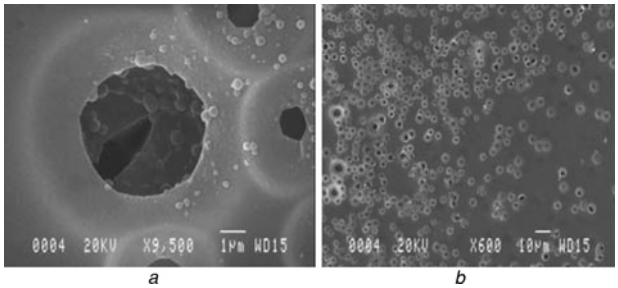

Figure 3 *MIP film pictures obtained using SEM*
a Film showed a porous morphology with pores sizes ranging from 1 to 5 µm
b Zoom of a pore containing AMP nanoparticle

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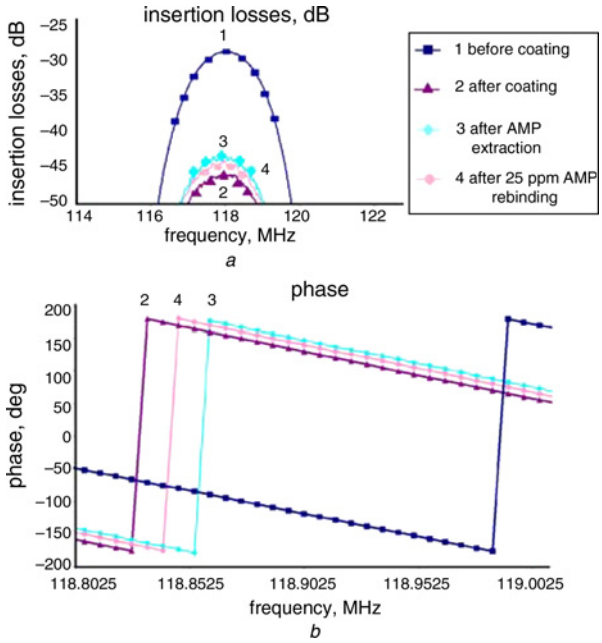

Figure 4 *Electrical characterisation of the sensor*
Response was recorded in terms of
a Phase
b Insertion losses using a network analyser after washing with buffer solution and drying with nitrogen flow

5. Electrical characterisation: The typical performance characteristics of interest for SAW devices are the insertion loss, centre frequency and their respective behaviour against various measurands. As a secondary performance metric, phase responses are also used. To obtain these performance metrics, a wide spectrum, high-accuracy radiofrequency network analyser is required. In addition to this requirement, the overall testing setup should accommodate direct electrical access to the devices in hand [5].

The sensor response was recorded in terms of frequency shift and insertion losses using a network analyser (Anritsu MS4623B) in both steps: before coating, after coating, after AMP extraction from and AMP rebinding by the MIP film (see Fig. 4).

Film thickness is an important parameter that should be controlled for optimising SAW sensors. It can be seen that, after coating, the sensor response in terms of frequency and insertion losses, can be modified by controlling the characteristics and thickness of the sensitive polymer film. Thus, the polymer film thickness should be evaluated and adjusted to establish the optimal film thickness value to meet the sensitivity requirements.

6. AMP detection tests

6.1. Removal of AMP template: The extraction of AMP was realised by immerging the sensor in methanol and ammonium solution over night in a flask covered with aluminium.

6.2. AMP template rebinding: The AMP rebinding by the MIP film was realised by soaking the sensor in a buffer solution (acetic acid/hydroxylamine 1 mM pH 7) containing AMP.

Three sensors with the same coating thickness (1 µm) were subjected to a 3 hour time extraction and then immerged in a 25 ppm AMP buffer solution and frequency shifts were recorded after 30 minutes, 1 and 2 hours.

AMP buffer solution and frequency shifts were recorded after 30 minutes, 1 and 2 hours. Before each measure, the sensors were only rinsed with the buffer solution and not with the AMP extraction solution. Then, they were dried with nitrogen.

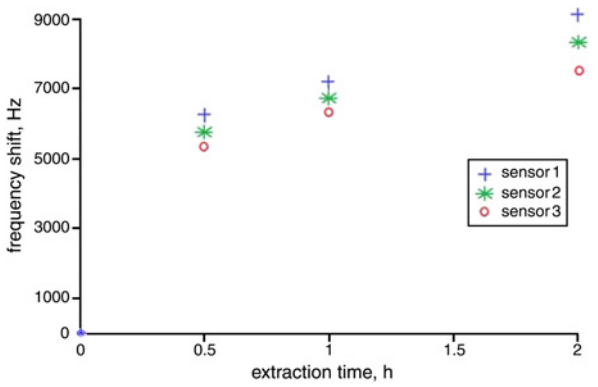

Figure 5 *Frequency shifts after multiple AMP rebinding steps for three sensors with the same MIP film thickness (1 µm)*
Shifts are expressed here in absolute value and the AMP concentration is 25 ppm

7. Results and discussion: Electrical characterisation is performed after both steps of AMP extraction from and AMP rebinding by the MIP film. Considering the mass loading effect, the frequency shifts recorded in Fig. 3 are coherent with the MIP principle. In other terms, the Love wave sensor is sensitive to the extraction of AMP from the MIP film with a frequency shift estimated to kilohertz and to the AMP rebinding by the MIP film with a frequency shift of kilohertz.

These preliminary results showed the feasibility of nucleotides detection with MIP on a Love wave sensor, with a frequency shift of kilohertz for 6 ppm of AMP (rebinding step: 137 Hz. ppm-1).

Fig. 5 shows the frequency shifts after 30 minutes, 1 and 2 hours for three sensors coated with a MIP film thickness of 1 µm. We can see that the three curves are approximately the same for the sensors that were subjected to the same spin-coating parameters (speed: 2000 rpm, acceleration: 4000 rpm/min, time: 10 s), polymerisation conditions (265 nm, N_2 flow, UV lamp power 62.3 µW/cm^2), extraction time (3 h) and AMP concentration (25 ppm).

8. Conclusion: A new thin film coating process has been developed in this work. Thin films of AMP based MIP could be successfully deposited on the propagation path of the Love wave sensor. The obtained MIP film thicknesses are controlled by solution viscosity, spin-coating parameters and polymerisation conditions.

A characterisation was performed using optical profilometry and the film thicknesses range from several hundred nanometres to 2.6 µm. On the other hand, the SEM images reveal a porous surface morphology with pores sizes ranging from 1 to 5 µm.

An electrical characterisation was realised and the sensor response was recorded in terms of frequency and insertion losses in both steps: before coating, after coating, after AMP extraction from and AMP rebinding by the MIP film. The measured frequency shifts were coherent with the MIP principle and the obtained films show high sensitivity to 25 ppm AMP concentration, which is higher than 45 Hz for 60 ppm albumin concentration reported in [13].

Reproducibility tests have been performed for sensors that were subjected to the same experimental conditions and thus with the same coating thicknesses (1 µm). The curves representing the recorded frequency shifts after 30 minutes, 1 and 2 hours were similar for the three sensors.

The simplicity of the obtained sensor makes it an attractive candidate as a nucleoside detector for colorectal cancer diagnosis. We plan in future works to integrate a new MIP coating based on nucleosides instead of nucleotides. We also envisage associating

a microfluidic system to the Love wave sensor for real-time detection of nucleosides.

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