

Improvement of Biomolecule Immobilization on Polystyrene Surface by Increasing Surface Roughness

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Abstract

Fast growing used of QCM biosensor has been reported. It is well known that the QCM sensor responses both to mass or viscosity changes. In its operation as mass sensitive sensor, a QCM responses to the mass change per surface area. In the use of QCM biosensor, immobilization of the bio sensitive layer on top of the sensor surface is one of the importance factors. The immobilization of the biomolecule can be done directly on top of the sensor electrode or by using a coating material as supporting matrix. This experiment shows that the use of thick polystyrene coating with a rough surface resulted from air brush technique resulted in a significant improvement on the biomolecule immobilization compare to the polystyrene coating using spin coating method. As there is no chemical or physical modification on the polystyrene coating, the increasing numbers of the immobilized biomolecule merely a result of the surface geometry. The increasing number of immobilized biomolecule is caused by improving total surface area per perpendicular surface area of the sensor by introducing surface roughness. However, an appropriate care must be taken during the coating process to avoid over rough surface which in turn can heavily damp the QCM sensor.

Keywords: Air brush; Spin coating; Polystyrene; QCM

Introduction

Since the first report by Sauerbrey in 1957 on the effect of frequency change of crystal resonator caused by mass deposition on its surface, wide range application of QCM (Quartz Crystal Microbalance) for chemical sensor and biosensor has been reported [1]. In its application, the quartz crystal is operating in AT-cut mode. In most application, a thin sensitive layer including the support matrix is applied to the sensor surface to avoid any damping effect on the QCM, especially if the QCM sensor is used in an aqueous environment [2].

A pure mass loading effect on the QCM sensor follows the Sauerbrey equation, can be rewritten to calculate the mass change by measuring the frequency change following (1):

$$\Delta m = -\frac{\Delta f A \sqrt{\rho_Q \mu_Q}}{2f_0^2} \quad (1)$$

where Δf is frequency change of the QCM, f_0 is the initial frequency before mass loading, ρ_Q is mass density of the quartz (2.648 g/cm^3), μ_Q is quartz shear stiffness ($2.947 \times 10^{11} \text{ g/cm}^2\text{s}^2$), A is surface area and Δm is deposited mass on top of the sensor surface area. This equation is used to calculate the amount of mass deposited on top of the sensor surface by assuming that the mass behaves as a glassy material [3]. Compact polystyrene layers its modulus elasticity behaves as a glassy material and doesn't contribute mechanical loading [4].

In addition to the mass loading effect, frequency of the QCM sensor will change caused by liquid viscosity and density which is in contact to the sensor surface [5]. In the configuration where one side of the sensor is in contact with water, it is known that the frequency change is not only caused by the liquid loading effect and mass effect, but also affected by other physical and chemical interaction. Some applications for liquid viscosity measurement utilize this effect by introducing pattern on the QCM surface [6].

In biosensor application, most of QCM is used in aqueous environment, where one side is in contact with water based buffer solution where the bio-chemical reaction takes place. In many applications the sensor surface is made as a smooth surface [7-9]. This

approach is aimed to avoid any damping effect. Therefore, the bio-sensitive layer is mostly form a monolayer of biomolecule. The amount of sensitive layer and hence also the target molecule then only limited by one-by-one pair per surface area, as shown figuratively in Figure 1.

Suppose the surface of the coating layer of the QCM sensor for immobilization matrix is not flat, but wavy or rough, than it is expected there will be more place for the biomolecule to bind to the sensor surface. It results in a more biomolecule bind on the sensor surface. As the normal surface area of the sensor doesn't change, this can result in a higher mass per surface area of the sensor, see Figure 2. Thus theoretically, by increasing a surface roughness of the sensor will result in an increasing number of immobilized biomolecule on the sensor surface. However the surface roughness needs to be controlled to maintain the sensor oscillating.

Rougher surface results in more trapped water which in turn also shifted down the resonance frequency of the sensor. This frequency change does not affect the measurement result, as it will be taken as an initial frequency. However, in an extreme condition it can leads to a heavy mechanical damping to the sensor. During the experiment, we found that some extreme rough surface sensor coated using air brush does not oscillated when it surface in contact with water.

In this work, surface modification of the QCM is done by using polystyrene through spin coating and air brush methods. By using the methods, the surface roughness of the sensor significantly difference due to the different process of the polystyrene layer formation. The

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difference surface roughness is expected to result in a different amount of biomolecule immobilization. In this experiment, Bovine Serum Albumin (BSA) is used as a sample biomolecule. It is already known that antibody, BSA, enzyme can bound to the polystyrene surface [10] through physical adsorption, therefore a polystyrene coating is used in this experiment as coating material for QCM sensor.

Materials and Methods

In this experiment, we used a commercially crystal resonator with silver electrode from Greatmicro Electronic. The crystal is commonly used as a crystal resonator in electronic devices. Polystyrene layer is made from a polystyrene plate (Nunc Maxisorp) which is commonly used as a plate well for ELISA. Chloroform from Sigma Aldrich is used to solve the polystyrene. The equipment used is oscillator circuit, frequency counter, storage oscilloscope, sprays coater made from air brush gun, mini spin coater, and SEM. The BSA and Phosphate Buffer Saline (PBS) were bought from Sigma Aldrich.

Prior to the coating procedure, polystyrene is solved in chloroform with a concentration of 1% mass of polystyrene to chloroform. The concentration is selected after some optimization by measuring the mechanical load on the QCM sensor by observing the electrical signal of the oscillator. The polystyrene is then coated on both side of the resonator by spin coating or by air brush method.

Following coating procedure, the electrical signal of the oscillator is measured using oscilloscope and frequency counter. Coated crystal surface is observed using optical microscope and SEM (Fei Inspec S50). Thin gold coating is applied to the polystyrene prior to the SEM measurement.

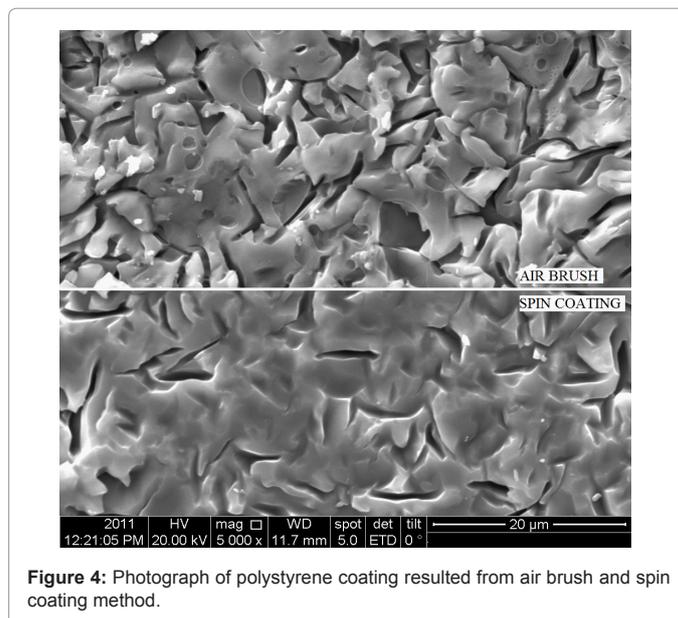
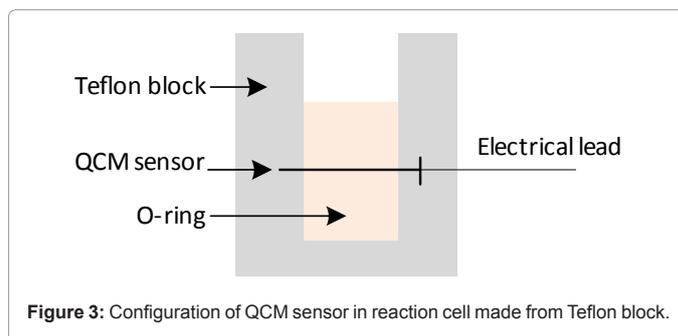
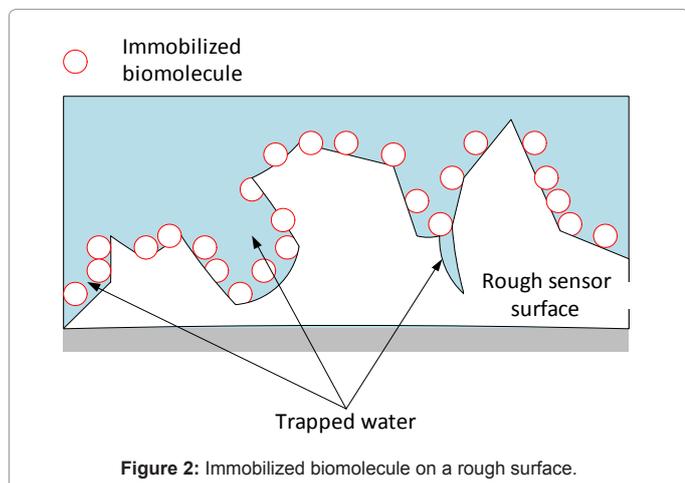
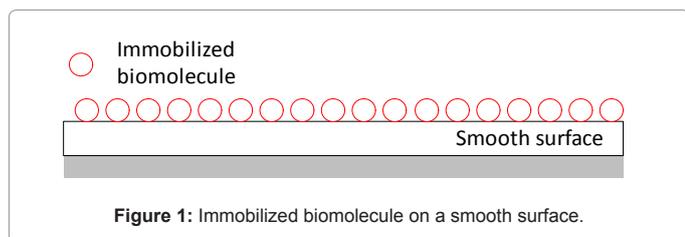
Immobilization of BSA is done on a real time basis by measuring the frequency change of the sensor during the immobilization using frequency counter. BSA concentration in the range of 0.15mg/

ml to 0.8mg/ml is immobilized on top of sensor surface. For each concentration three difference measurements has been done.

Frequency measurement was done by observing the frequency change of the QCM in contact with air, and after contact with PBS. PBS volume of 70uL was added on top of the QCM surface in the compartment reaction. After the QCM reaches it stable resonance, an addition of 30uL BSA in PBS for each concentration is added to the PBS to reach the final concentration of 0.15mg/mL to 0.8mg/mL. The frequency of the QCM is observed continuously. For the frequency measurement, the QCM sensor is putted in a reaction cell made from Teflon block. The sensor is clamped in the middle of the cell by using a silicone-ring. Figure 3 shows the configuration of the sensor in the reaction cell.

Results and Discussion

After careful coating procedure, a photograph of the sensor with polystyrene coating is taken using scanning electron microscope. The photograph is taken after the electrical signal measurement using oscilloscope and frequency counter. During the coating process, coating thickness which is indicated by the frequency shift of the QCM sensor is controlled by changing polystyrene concentration in the chloroform. It is measured that higher polystyrene concentration result in a thicker coating. But there is no difference signal output between uncoated and coated resonator. It means that the coating doesn't introduce any damping load to the resonator.



It is observed that the surface of the sensor resulted from air brush technique is more groovy and porous compare to the surface resulted from spin coating. Whilst the surface of the sensor coating resulted from spin coating shows a more compact surface. Figure 4 shows the comparison between the polystyrene coatings resulted from air brush and spin coating taken using SEM (5000 magnification, 20kV).

In Figure 4 it can be observed that the polystyrene coating resulted from spin coating shows a more compact layer compare to the air brush one. The polystyrene coating from airbrush shows a groovy surface. It is also seen that the surface is looks not tightly merged from part to part.

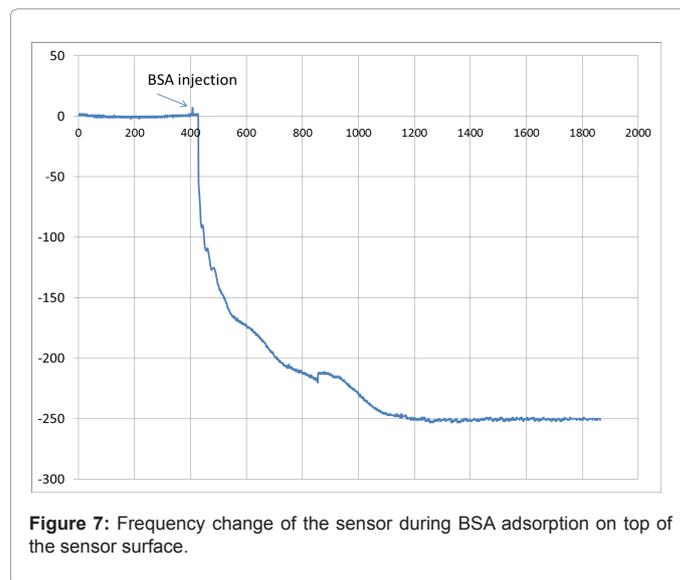
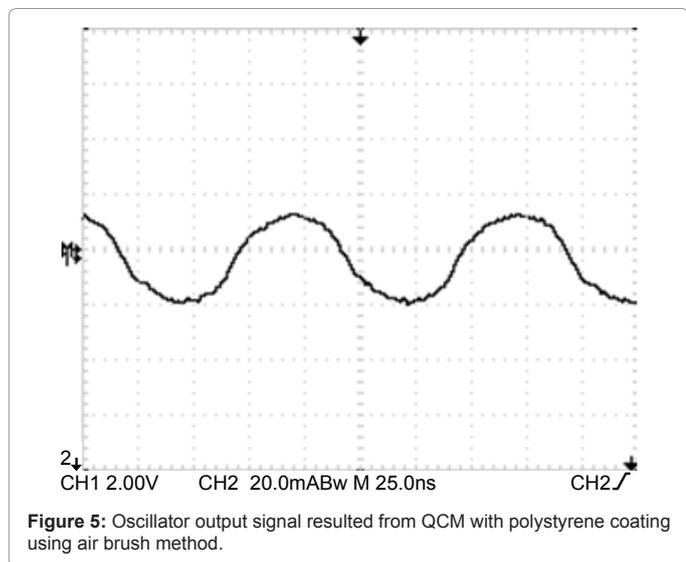
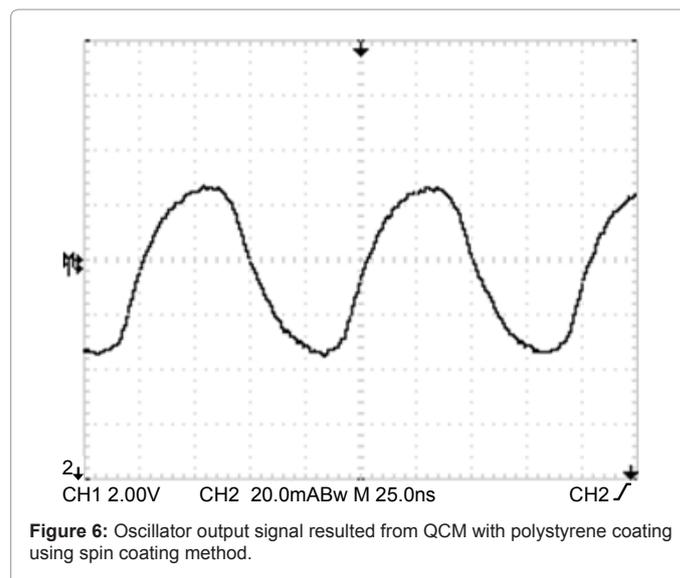
The difference surface roughness and topography is resulted from difference layer formation process. By using a spin coating method, the polystyrene layer is formed from a polystyrene solution which is stretched out during the resonator rotation in the spin coating. As the chloroform evaporated the rest is a polystyrene layer. It can be seen in the Figure 4 that there is still some crack on the polystyrene surface. It is also observed that the surface is not perfectly smooth. Soft gradation on the image indicated that the surface roughness of the polystyrene change gradually. In contrast, it can be observed that there is abrupt change on the polystyrene surface resulted from air brush method.

The formation of the polystyrene layer from the air brush method is formed by drop-let of chloroform containing polystyrene. The layer is formed by stacking chloroform-polystyrene droplet. It can be happen that some dropped chloroform with polystyrene already evaporated before the next droplet coming. Thus it can be assuming that the surface layer is porous and rough. The rough surface is indicated by sharp edge polystyrene layers.

Theoretically, rougher surface results in wider surface area of the sensor. This in turn can result in more immobilized biomolecule per surface area in a perpendicular direction of the surface. However, the formation which may form a porous layer can also leads to a mechanical damping to the sensor as the coating layer no more perfectly rigid. High mechanical damping on the sensor can make the sensor fails to oscillate in contact with water. Comparison between the oscillator signal output between two coating method is presented in Figure 5 and 6. It can be seen from both figure, that the polystyrene coating using air brush resulted in a lower output signal compare to the spin coating one.

This result suggests that surface modification of the QCM sensor using polystyrene need to be done carefully. Although, the polystyrene is considered as glassy material, the porous structure of the coating layer may contributing into a lower value of Young modulus, which result in a higher mechanical damping on the QCM. Higher mechanical damping of the sensor is indicated by a lower electrical voltage output of the oscillator. Compact polystyrene layer is needed in order to maintain a lower mechanical damping of the QCM sensor. Unfortunately, a compact but rough surface is not easily obtained by air brush or spin coating.

Adsorption of the BSA on top of sensor surface coated with polystyrene can be seen in Figure 7. Prior to injection of BSA, 70uL of PBS has been put to the sensor surface. After the QCM sensor oscillation reaches its stable frequency, 30uL BSA solution in PBS is added on top of the sensor. From Figure 7, it can be seen that the BSA is shortly immobilized on top of the sensor surface after its injection. The maximum frequency change is reached 15-20 minute BSA injection. This maximum frequency change value is then taken.



Difference BSA concentration has been injected on top of the sensor surface. This results in difference maximum frequency change of the sensor caused by immobilized BSA. Figure 8 shows a frequency change resulted from the BSA immobilization on top of the QCM sensor coated with polystyrene. Variation on the frequency change of the sensor caused by the BSA immobilization is observed. However the deviation is not significant compare to the absolute frequency change. From both graph it can be seen that the frequency change of the QCM sensor using air brush method results in a much higher frequency change compare to the spin coating one.

This higher frequency change can be resulted from more BSA immobilized on the sensor surface caused by the surface roughness (Figure 4). It also means that the surface mass density of the immobilized BSA resulted from the BSA immobilization on polystyrene surface through air brush is bigger compare to the spin coating one. However, a more systematic investigation is still needed, for example by using surface structural patterning.

By using equation (1), adsorbed BSA on top of the sensor electrode can be calculated based on the initial frequency of the sensor

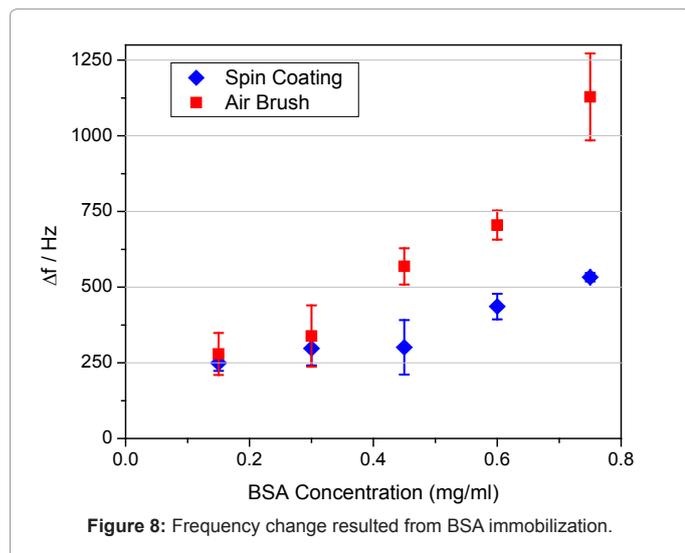


Figure 8: Frequency change resulted from BSA immobilization.

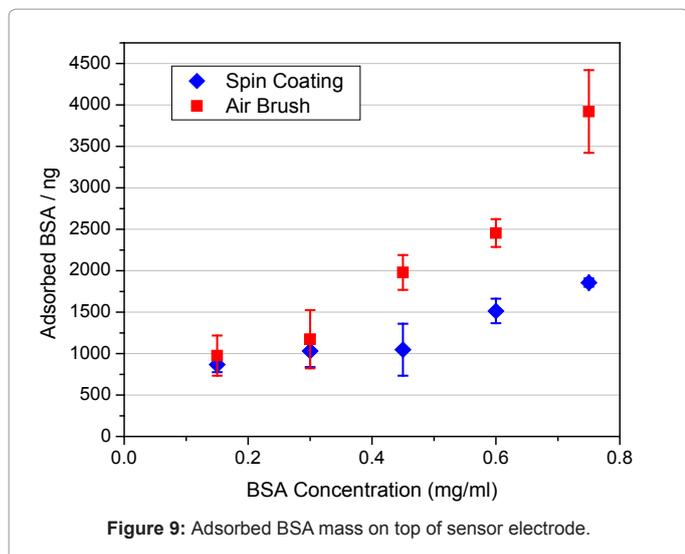


Figure 9: Adsorbed BSA mass on top of sensor electrode.

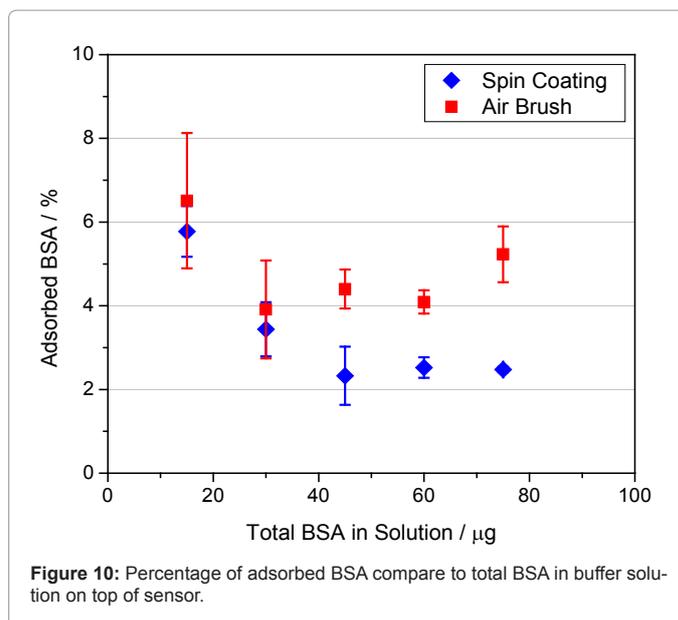


Figure 10: Percentage of adsorbed BSA compare to total BSA in buffer solution on top of sensor.

before immobilization and other sensor parameters. Figure 9 shows relationship between BSA adsorbed on top of the sensor to added BSA concentration. It can be seen that higher BSA concentration result in a higher number of adsorbed BSA on top of the sensor surface.

The percentage of the adsorbed BSA on top of the sensor surface compare to total BSA in the buffer solution is presented in Figure 10. It can be observed that the adsorbed BSA on top of the sensor surface is almost constant. The average percentage of adsorbed BSA on top of polystyrene surface resulted from spin coating and air brush method is $(3.3 \pm 1.4) \%$ and $(4.8 \pm 1.1) \%$ respectively. This number indicated that the adsorbed BSA on top of polystyrene resulted from air brush and spin coating is difference. The difference is big when high concentration BSA is given.

Conclusions

This paper shows that air brush and spin coating method for coating polystyrene on the QCM sensor result in a significant difference on the surface properties. The air brush method result in a more rough and porous coating compare to the spin coating one. Rougher surface and more porous surface increases mechanical load to the QCM sensor results in a lower signal output on the oscillator system. The immobilization of the BSA on top of the QCM sensor coated with polystyrene using air brush results in higher frequency change. This higher frequency change is caused by an increase of mass density per surface area of the immobilized BSA. The percentage of the adsorbed BSA on the polystyrene surface resulted from air brush is also higher compare to the spin coating one.

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