

# The use of Quartz Crystal Microbalance in Cell Differentiation detection

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## ABSTRACT

We try to investigate whether the hematopoietic cell differentiation can be detected by Quartz Crystal Microbalance. Because certain hematopoietic transcription factors play a critical role in cell differentiation and lineage determination, they can be alternate markers of cell differentiation [2]. This study creates a new application and a novel methodology to detect the cell differentiation in Hematopoiesis by the Quartz Crystal Microbalance with acoustic wave sensor [3]. This method differs from the traditional ways such as detecting surface cell differentiation marker using fluorescence or luminescence, such as ELISA and flow cytometry. In our method, the variation of weight can be sensed by QCM sensor after the target molecules bind to the chip. Hence, we immobilized a DNA with specific sequence that could bind to transcription factor or specific protein for tracing the differentiation stage of hematopoietic cells.

In the present study, we used the DNA molecules containing a 5'-thiol anchoring group which can form covalent bond with nano-gold [1]. After the DNA binds with transcription factor or determining protein, the weight change can be sensed by QCM [4]. To prove this, the binding complex structure can be observed by Field emission scanning electron microscopy (FE-SEM), Field emission X-ray photoelectron spectroscopy (FE-XPS), immuno-fluorescence (IF) microscope or QCM devise. In our presentation, the Quartz crystal oscillation circuit, amplifies signal circuit, and the measurement set up are shown. Our results demonstrate that the modified structures can be detected by Fluorescence or optical microscope and we also confirm the formation of their covalent bond. In addition, we also show that the frequency changes of QCM (data not shown) are due to weight increase which is resulted from the attachment of determining protein or transcription factor onto DNA. The data also indicate that using gold colloid to anchor DNA-SH could increase sensing areas. Taken together, our study provides an alternate way to look insight into how the transcription factors influence hematopoiesis in a relative simple and convenient manner.

**Keywords:** Hemopoiesis/hematopoiesis; Transcription factor; Differentiation; Surface acoustic wave; QCM (quartz crystal microbalance); Promotor

## 1. INTRODUCTION

Hemopoiesis refers to the formation and development of mature blood cells involving proliferation, commitment and differentiation from early progenitors [4]. It's able to recruit mature blood cells in normal "steady state" cellular turnover and to control of the capability of the immune system. The intricate regulation of this hemopoiesis is, therefore, essential to the proper control of innate and acquired immune responses. All of complex network of hemopoietic organs from which early progenitors arise or intermediate cells of eight hemopoietic lineages [4]. Maturation of hemopoietic progenitors leads to the production of several potent effector cells with diverse roles in antimicrobial host defenses, including phagocytosis, production of bioreactive pathogen-killing molecules, interactions between innate and acquired immune systems, and homeostasis. In previous research, hemopoiesis is regulated extrinsically (through hemopoietic growth factors, other cytokine signals, and interactions with stromal cells or other extracellular matrix components), intrinsically (as outlined in stochastic models), or both [4]. Overall, the regulation of hemopoiesis is the result from signal transduction pathways converge at the level of gene expression where positive and negative modulators of transcription interact and delineate the pattern of genes expressed by the cell and its overall hemopoietic response. As such, transcription factors represent a nodal point of hemopoietic control.

Transcription factors are sequence-specific DNA binding protein with a variety of functions that include: (a) folding of the DNA molecule into distinct domains; (b) the initiation of DNA replication; and (c) control gene transcription [5]. Therefore, to understand the process of hemopoiesis control, it is important to identify and characterize the transcription factors that specifically activate important genes for the development of cells along the various hemopoietic lineage. Our research is currently investigating various transcription factors that lead to up-regulation of hemopoiesis and the downstream events

that lead to modulation of gene expression in developing hemopoietic cells.

Here, we use better analytical technology has been demanded for accurate and rapid determination of trace amounts of chemical compounds, such as marker proteins for disease or endocrine disruptors like dioxin, which might be contained in blood, food and the environment. Quartz crystals are highly precise and stable oscillators and have therefore been widely used in electric circuits as frequency standard clocks in computers, communication systems and frequency measurement systems. It is crucial for the quartz crystal microbalance (QCM) because they convert mass deposited on the electrodes quantitatively with changes in oscillation frequency. And it's also used in the immunosensor technique, as thickness meters, as chemical sensors and as a mass sensor. In the below equation [6], we have studied a theoretical equation of change in oscillation frequency due to an increase in mass deposited on the electrodes:

$$\Delta F_N = -N \frac{2F_1^2}{A\sqrt{\mu\rho}} \Delta m \quad (1)$$

In this equation N is the order of the overtone (N=1,3,5,7.....),  $\Delta F_N$  is the change in oscillation frequency of a quartz crystal of Nth mode, and  $\Delta m$  is change in mass on the crystal electrodes.  $F_1$  is the fundamental frequency of the quartz crystal, A is the electrode area of the quartz,  $\mu$  is the elastic modulus of the quartz and  $\rho$  is the density of the quartz. The mass sensitivity per unit area of electrode for each quartz crystal was calculated using the above equation. Therefore, we try to use this system for sensing which transcription factors active during hemopoiesis, and it will be used to indicate the increasing surface mass density during the interaction between a specific binding sequences on the DNA and transcription factors.

## 2. EXPERIMENTAL PROCEDURES

Here, we first used the the substrates in the present experiments were p-type (100) oriented silicon wafers with deposition 300 Å chromium and 2000 Å gold for immobilize 1,6-Hexanedithiol and gold nanoparticles which were increased immobilization area to specific-sequence DNA. The Si wafers were cleaned in a dilute H<sub>2</sub>SO<sub>4</sub> solution, and rinsed in de-ionized water prior to loading into the CVD system. The 30 nm Cr films and 200nm Au films were deposited by PECVD. Several wafers were further received in-suit plasma treatments after deposition of Cr films and Au films. In order to determinate the capability of compact films, we was examined by  $\alpha$ -step. The plates were clean by hot Piranha solution (concentrated H<sub>2</sub>SO<sub>4</sub>: 30% H<sub>2</sub>O<sub>2</sub>=7:3) 30mins, and then wash with DI-water two times. The plates was air-dried and pre-treated with 1,6-Hexanedithiol 0.5% (v/v) in ethanol 30mins at 27°C, and coating with colloidal Au solution

100ul (0.01% HAuCl<sub>4</sub> in 0.01% tannic acid with 0.04% trisodium citrate, 0.26% mM potassium carbonate buffer) at room temperature for 1 hr. And then we add 20 ug/mL HS-DNA (0.1 M NaCl, PBS buffer, pH = 7.0) for 1 hr, and do hybridization experiments at 40°C for 1 hr, and their oscillation frequency read and record by QCMs system (data not show). For immobilization analysis, the Field emission scanning electron microscopy (FE-SEM), Field emission X-ray photoelectron spectroscopy (FE-XPS) and immuno-fluorescence (IF) microscope were examined.

## 3. RESULT AND DISCUSSION

### 3.1 QCM and oscillation circuit systems

All of the systems were set up from the Fig.1 and Fig.2. In Fig.1, the oscillation circuit of the QCM is to 10 MHz fundamental frequency and in Fig.2, the measurement of all experiments were performed prior to stable frequency. All frequency were record by a computer with labview 7.01 and the universal counter (Hewlett-Packard, Model 53131A). We found that the frequency would be effected by the fabrication in the QCM system, and designed, therefore, a series of various fabrication devies (data not shown) to test which one is the best effectively and low diffuse energy.

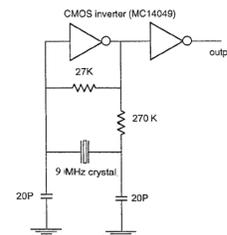


Fig.1 Quartz crystal oscillation circuit.

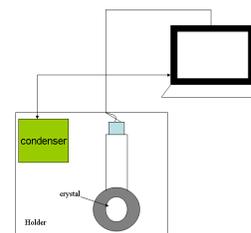
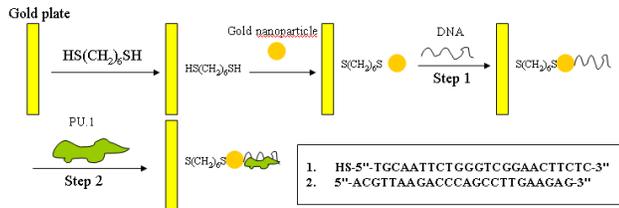


Fig.2 The measurement is set-up for all experiments.

### 3.2 Analysis for covalent bond formation and immobilize on the substrate

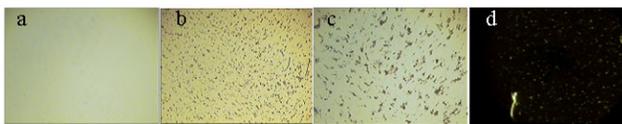
Fig.3 showed the results that the schematic illustration of the sensing and immobilizing procedures and all of immobilization experiments were performed at room temperature. In this schematic illustration, we used 1,6-hexanedithiol as a crosslinker to recruit and immobilize specific-sequence DNA but in order to increase sensing

areas, we made use of gold nanoparticles (diameter:9~12nm) to expand areas. And then the specific-sequence DNA having 5' thiol group which was modified and formed to covalent bond. On the other hand, it was the more probes for increasing the more sensitivity of fabrication devices.



**Fig.3 Schematic illustration of the sensing and immobilizing process.**

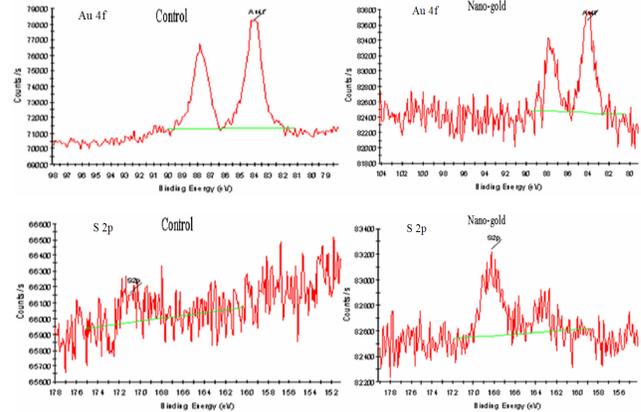
For confirm the covalent bond formation, we first used light and immuno-fluorescent microscope to observe morphology when the gold nanoparticles were coated on the substrate plate with modifying 1,6-hexanedithiol. Fig.4 showed the results of the gold nanoparticles on the modifying substrate plate at room temperature in air. Fig.4a was the control plate without anything on it, and we found some nanoparticles on modifying plate in Fig.4b, magnification, x100. For advance observation, we used light and fluorescent microscope to observe, magnification, x400, in Fig.4c and 4d. Although we investigated that it is never observed by general light microscope for the diameter was 9~12nm nanoparticle, it was electrostatic induction between the nanoparticles each other so that we could observe from light microscope. This data demonstrated that the only nanoparticles were on the substrate plate not other dust, and they were presence to dependent on the concentration of 1,6-hexanedithiol (data not shown).



**Fig.4 Modified surface was observed by light and fluorescent microscope.**

In contrast, the similar results were consistent with the Fig.5 and showed that it was the covalent bond formation from FE-XPS. The modifying substrate plate with gold nanoparticles increased energy right shift in the same period (top panel). In addition, the thiol group was detected with increasing the concentration of 1,6-hexanedithiol (bottom row) and at the same time, it's the increasing 26% e.v in the right column. Consistent with the previous data observed on the plates, minor expression of ultimate analysis on other elements excepted for S and Au elements. Collectively, these data show that crosslinkers contributed

substantially to the expand sensing areas and the sensitivity and efficiency of the fabrication devices.



**Fig.5 Analyzed covalent bond (Au-S) by FE-XPS.**

Although a number of elements had not been shown significant expression, it was possible that some elements didn't exist on the period of the area or it was relatively low amount on total area. Thus, if we want to develop the sensor devices in the future, the presence of the crosslinkers or probes per the unit area are efficiently established and contribute to increase the sensitivity of the fabrication.

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