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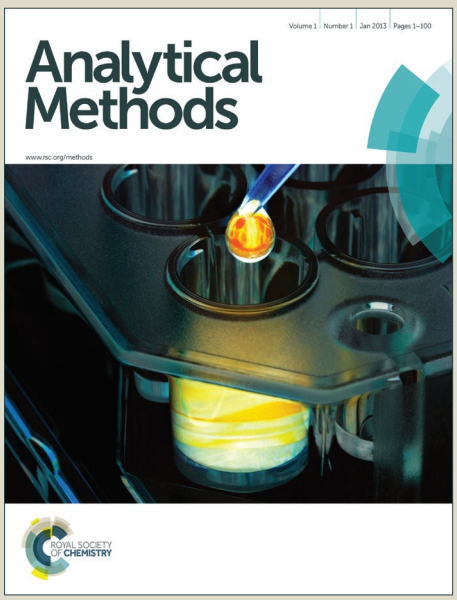

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Quartz Crystal Microbalance based biosensors for detecting highly metastatic breast cancer cells via their transferrin receptors

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Abstract: A quartz crystal microbalance (QCM) biosensor was developed to detect highly metastatic breast cancer cells by functionalizing gold sensor surface with the transferrin attachment. MDA-MB 231 breast cancer cells with high and MCF 7 cells with low metastatic potential and transferrin expression were used. Serum starved MDA-MB-231 cells were used as control cells. First, poly(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles were prepared by mini-emulsion polymerization of hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA). Nanoparticles were characterized with zeta sizer and then their suspension is dropped on the surface of QCM and dried QCM surface was modified further by activation with carbodiimide and transferrin attachment. The QCM biosensor was analyzed by using atomic force microscope (AFM), ellipsometer, Fourier transform infrared spectrophotometry (FTIR) and contact angle measurements. The cells were applied to derivatized QCM sensor to investigate the affinity and binding kinetics. The nanoparticles and transferrin were found to form a monolayer on the QCM surface. Binding kinetics was best fitted to Langmuir-Freundlich adsorption model. The QCM signal was correlated well with the number of transferrin receptors on cells. It is concluded that, QCM biosensor functioning via transferrin receptor interaction may be used to detect breast cancer cells with high metastatic potential.

Key words: QCM, nanoparticles, breast cancer, MDA-MB 231 cells, transferrin receptors, transferrin

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1. Introduction

Breast cancer is the most common cancer among women and the second common cancer overall [1]. Treatment of metastatic breast cancer is still a big challenge despite of developing strategies [2]. Early diagnosis and detection of metastasis saves many lives. Detection and characterization

of tumor cells are crucial in cancer treatment. Therefore, it is of prime importance to detect these cells in tumors as well as in the circulation [3]. Discrimination of breast cancer cells with high metastatic potential to those of low potential may shed light upon treatment regimes. Current breast cancer diagnosis largely depends on highly developed radiological methods such as mammography. However, this does not provide information on the metastatic power and the degree of malignancy of cancer cells. A variety of laboratory tests help reveal these characteristics. However, these methods are usually expensive, they require long and complicated processes and instruments. Therefore, development of simple, rapid and sensitive methods to be used in these molecular analyses has gained great importance [4,5]. Biosensors offer efficient and rapid methodology in diagnosis. The simple sample preparation, low sample volume, compact structure, rapid detection, high selectivity and sensitivity are some of their useful features [6-9]. A biosensor has two basic components: biological (receptor, enzyme, tissue, microorganisms, antibodies and a large variety of molecules including DNA) and physical (transducer). According to the transducer mechanism biosensors are grouped as optical, electrochemical, piezoelectric and thermometric [10]. Quartz crystal microbalance (QCM) is a piezoelectric transducer, which is known to be highly precise, stable oscillators that are capable of detecting nanogram mass changes [11,12]. QCM biosensors have been designed and fabricated to detect a large selection of molecules [13,14]. Transferrin receptors are expressed in all cells to internalize transferrin by receptor-mediated endocytosis in order to supply iron. Transferrin receptor is not only highly specific for transferrin but also has a great affinity towards this molecule. This high affinity is utilized in specific targeting of drugs [15,16]. As most cancer cells are in need of large amounts of iron, they express great number of transferrin receptors. Metastatic breast cancer cells also overexpress number of transferrin receptors as compared to their less metastatic counterparts [17-19]. Therefore, we have selected this ligand-receptor interaction with high affinity and selectivity to form a biosensor [20]. Nowadays, nanoparticles play an important role in improving sensor performance due to their large specific surface areas [21]. Combining the advantage of nanoparticle large surface with high receptor affinity may provide an efficient detection system. The other essential part of a biosensor is the signal transducer QCM that converts changes in mass into electrical signal. Under optimum conditions, it can measure a mass change of 0.1-1.0 ng/cm^2 [22,23]. The high sensitivity of QCM is combined with sensitivity and selectivity of receptor-ligand interaction in order to construct a

biosensor which would discriminate breast cancer cells with high metastatic power from those of low or no metastatic potential as an attempt to develop a simple, fast and efficient system to be used in breast cancer diagnosis.

2. Materials and methods

2.1. Cells

MDA-MB 231 (ATTC- HTB-26) human breast cancer cells with high metastatic power were cultured in dMEM, high glucose supplemented with 10% FBS, 2mM L-glutamine and 1% antibiotic-antimycotic solution in carbon dioxide (5%) incubator at 37^{o}C. To test the binding efficiency, MCF 7(ATTC-HTB-22) breast cancer cells with low metastatic power and low number of transferrin receptors was grown under similar conditions. Additionally, MDA MB 231 cells preserved in 10 mM PBS, pH: 7.4 for 24 hours in order to inactivate transferrin receptors were also tested as control. Cells were washed in PBS and count using a hemocytometer. Samples containing different number of cells were applied to QCM surface in PBS.

2.2. Preparation and characterization of nanoparticles

Poly(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles were prepared by using a two phase mini-emulsion polymerization method [24]. For polymerization, two different aqueous phases were prepared. The first one was prepared by dissolving 93.7 mg polyvinyl alcohol (PVA), 14.4 mg SDS and 11.7 mg sodium bicarbonate in 5 mL deionized water. The second phase was prepared by dissolving 50 mg PVA and 50 mg SDS in 100 mL deionized water. The monomer phase was consisted of 0.45 mL HEMA and 1.05 mL EGDMA (ethylene glycol dimethacrylate). The monomer phase was added to the first phase to obtain a mini-emulsion; then the mixture was homogenized at 25.000 rpm. This mini-emulsion was slowly added to the second aqueous phase, which was being stirred in a sealed-cylindrical polymerization reactor. The reactor was stirred at 600 rpm. The polymerization mixture was heated to 40°C. Then the initiators, 57.3 mg sodium bisulfide and 63 mg ammonium persulphate, were added to the mixture. Polymerization reaction was completed in 24 hours at 40°C. Nanoparticles were washed with water/ethyl alcohol mixture in order to remove unreacted monomers by centrifugation at 14.000 rpm. The size distribution of nanoparticles was determined by using a zeta-sizer (nanoS, Malvern Instruments, London, UK). Light scattering was done at incidence angle of 90 and 25 degrees. For data analysis, density and

refraction index of deionized water were used as 0.88 mPa.s and 1.33, respectively. Light scattering signal was calculated as nanoparticle number/second.

2.3. Preparation of QCM sensor chips

In order to attach nanoparticles to the QCM sensor, a 7 mL aliquot of 4.2% nanoparticle dispersion was dropped on the gold surface of the QCM sensor chips. MAXTEK 5 MHZ Cr/Au polished quartz crystals chip was supplied from USA by INFICON. The AT-cut quartz is chosen for its superior mechanical and piezoelectric properties. Then, the sensor was dried in the oven at 37°C for 6 h. Finally, nanoparticle adsorbed QCM sensor surface was washed with deionized water and ethyl alcohol and dried in vacuum incubator under 200 mmHg pressure at 40°C. Transferrin was attached to the QCM biosensor surface through coated PHEMA nanoparticles by using carbodiimide. For attachment, 50 mg carbodiimide and 5 mg transferrin were dissolved in 10 mL of 0.1 M phosphate buffer (pH: 7.4). QCM chip was dipped into this solution. Then the solution was stirred in an incubator at 100 rpm for 24 h. The chip was then washed with 0.5 M sodium chloride and phosphate buffer.

2.4. Characterization of QCM sensor chips

The nanoparticles and transferrin coated QCM surface was analyzed by FTIR-ATR, ellipsometer, contact angle measurement and atomic force microscopy.

For FTIR-ATR analysis, QCM sensor was put into the sample holder of FTIR-ATR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA) and total light reflection from surface was measured in the wavenumber range of 400-4000 cm^{-1} at 2 cm^{-1} resolution.

The contact angle of the surface was determined with a KRUSS DSA100 (Hamburg, Germany) instrument. Measurements were made by Sessile drop method. Ten photos were obtained from different areas of QCM chips. The calculated values for the QCM surfaces were the average of 10 measurements.

Ellipsometer measurements were performed by using an auto-nulling imaging ellipsometer (Nanofil EP3, Germany). All the measurements have been carried out at a wavelength of 532 nm with an angle of incidence of 62°. In the layer thickness analysis, a four-zone auto-nulling

procedure integrating over a sample area of approximately 50 µm x 50 µm followed by a fitting algorithm has been performed. The measurements were carried out at six different points of QCM sensor surface and the results were given as average value.

AFM observations were carried out using AFM (Nanomagnetics Instruments, Oxford, UK) in tapping mode. QCM chip was installed on a sample holder. Applied experimental parameters were oscillation frequency (341.30 Hz), vibration amplitude (1 V_{RMS}) and free vibration amplitude (2 V_{RMS}). 5µm x 5µm sample area was showed with a 128 x 128 pixels resolution.

The surface area of the PHEMA nanoparticles was calculated by using the following equation (Eq 1):

$$N = \frac{6.10^{10}.S}{\pi \rho d^3} \quad (1)$$

Here, N is the number of nanoparticles per milliliter; S is the % of solids; ρ is the density of bulk polymer (g/mL); d is the nanoparticle diameter (nm). The number of nanoparticles in mL suspension was determined from mass-volume graph of nanoparticles. Specific surface area of the PHEMA nanoparticles was calculated by multiplying N and surface area of 1 nanoparticle.

2.5. Cell binding studies

MDA MB 231 cell samples were prepared at seven different cell densities ranging between 500-125000 cells/mL for determining the kinetics of binding. First, QCM surface was washed with deionized water. Then, the transferrin functionalized QCM sensor was equilibrated with PBS and resonance frequency (f_o) was determined. QCM sensor was then washed with PBS for 3 min. Five milliliter of each cell sample were applied to the sensor at a speed of 1.0 mL/min. Shift in the resonance frequency was monitored and when equilibrium was reached in around 20 minutes, 1.0 M NaCl in PBS buffer was circulated for desorption. Before applying each sample, washing and equilibration was performed. For cell binding studies, QCM system (RQCM, INFICON Acquires Maxtek Inc., New York, USA) was used. Data was evaluated with RQCM (Maxtek) software system. In this system, Sauerbrey equation [25] is used to convert frequency to mass by using geometrical and physical characteristics of the quartz crystal. Linear

relationship between the changes in the resonance frequency of a quartz crystal and the mass of a thin rigid film added to its surface is shown in Eq.2.

$$\Delta f = -\frac{2{f_0}^2}{\rho_q v_q}\frac{\Delta m}{A} \quad (2)$$

Where f_0 is the resonance frequency, A is the active electrode area, ρ_q is the density (2.65 g/cm^3), is the shear modulus and v_q ($3.34.10^5$ cm.s^{-1}) is the shear wave velocity of the quartz crystal.

For the analysis of competitive binding of cells with high, low metastatic capacity and inactivated cells were each applied to the QCM sensor at a density of 75000 cells/mL in PBS. Furthermore, to test their affinity for transferrin, same density of cells were applied to QCM sensors with surfaces coated with transferrin and with only PHEMA.

3. Results and Discussion

3.1. Preparation and characterization of QCM sensors

The size distribution of the PHEMA nanoparticles, prepared by using EGDMA as a crosslinker, was measured with zeta-sizer. As shown in Figure 1, the average size of nanoparticles was detected as 58 nm and polydispersity was 0.229. The percentage of nanoparticles at the average size was 44%. This low nanometric scale is thought to be suitable for increasing the sensitivity of QCM chip to be used as a sensor. Nanoparticles and nanorods which has special properties, such as specific area, fine particle size, and the quantum confinement, is believed to be a good candidate for biosensing or chemical sensing with high sensitivity [19,20]. Nanoparticles can produce larger specific surface area and therefore may result in high protein adsorption, which are used as ligand in cell adsorption studies. Therefore, it may be useful to synthesize nanoparticles with large surface area and use them as suitable carriers for the adsorption of human breast cancer cells. The sensitivity of the signal response is proportional to the surface-area-to-volume ratio [26]. 58 nm-sized spheres were used because they have the largest surface-area-to-volume ratio while still having visible peak absorption. Poly(hydroxyethyl methacrylate) (PHEMA) nanoparticles with an average size of 58 nm in diameter were produced by mini

emulsion polymerization method in which SDS was used as a surfactant. Specific surface area of the PHEMA nanoparticles was found to be 1899 m^2/g. These particles were attached to gold surface of QCM chip and the surface was monitored by FTIR-ATR. The FTIR-ATR spectrum demonstrates the presence of nanoparticles on the chip surface. The band at 1730 cm^{-1} belongs to carbonyl of the ester group of HEMA while the wide band at 3000-3700 cm^{-1} belongs to the hydroxyl group of HEMA. Furthermore, the C=C double bonds belonging to the HEMA monomers which gives a band at 1633 cm^{-1} was not detected in PHEMA FTIR-ATR spectrum shows that polymerization was completed **(Figure S1.A).**

Following the attachment of transferrin by carbodiimide the surface was analyzed by FTIR-ATR and the spectrum obtained is shown in **Figure S1.B**. The band at 1107 cm^{-1} belongs C-O-C bond stretching, which is formed as a result of transferrin binding to PHEMA. The –C=O bond stretching band of HEMA at 1730 cm^{-1} have shifted to 1654 cm^{-1} as a result of transferrin binding. The intensity of the –OH band of HEMA at 3000-3700 cm^{-1} has increased in parallel to joining more –OH and –NH groups from transferrin. The amide I and II bands, which belong to transferrin molecule were found at 1640 cm^{-1} and 1577 cm^{-1}, respectively. The wideness of these bands clearly demonstrates the presence of transferrin, which houses a great number of them. Figure 2 shows schematic illustration of transferrin modification of PHEMA nanoparticles by carbodiimide activation and QCM measurements.

< Figure 1 >

< Figure 2 >

The hydrophobicity of the surface was analyzed by contact angle measurements. As shown in Table 1, the contact angles for uncoated, nanoparticle coated, nanoparticle and transferring coated surface contact angles were 84.0°, 78.3° and 73.6° respectively. The decrease in contact angle shows that by coating with PHEMA nanoparticles and transferrin, the surface becomes more hydrophilic. This can be explained by the polar nature of PHEMA due to its –OH groups and this was increased by the addition of transferrin with charged amino acids.

Table 1. Contact angle values of QCM surfaces.

Surface	Contact Angle (^{o})
Uncoated surface	84.0 ± 1.2
PHEMA nanoparticles coated surface	78.3 ± 2.5
PHEMA and Transferrin coated surface	73.6 ± 3.2

The PHEMA nanoparticles attached QCM chip surface was further analyzed by ellipsometer to measure the mean surface layer deepness **(Figure S2)**. QCM sensor surface with and without transferrin was analyzed. Deepness of surface with transferrin was 64.2 ± 3.1 nm and without transferrin it was 59.1 ± 2.04 nm. These measurements are in agreement with the data obtained by zeta sizer. The QCM chip surfaces were also analyzed by AFM **(Figure 3)**. As shown in Table 2 the surface thickness was again in accordance with the previous data. These results show that the QCM surface was homogenously coated as a monolayer with PHEMA nanoparticles and transferrin.

Table 2. AFM characterization data of QCM chip.

Surface	Thickness (nm)
Uncoated surface	10.0
PHEMA nanoparticles coated surface	41.1
Transferrin coated surface	59.9

< Figure 3 >

3.2. Real time monitoring of QCM biosensor response

In order to detect the highly metastatic MDA-MB- 231 human breast cancer cells, they were applied to QCM biosensor, which is functionalized with transferrin. To evaluate the sensitivity of the system, cell suspensions containing 500-125000 cells/mL were used and the correlation between the QCM signal and the cell number was determined. The detection limit was found to be 500 cells per mL (S/N = 3). The sensorgram obtained by these measurements is given in

Figure 4a. As may be depicted from the figure, the real time change in QCM response was parallel to the increase in cell number. In other words, Δf was correlated with the number of cells or rather their transferrin receptors. **Figure 4b** shows the relationship between mass shift and the number of cells. As the figure demonstrates, QCM sensor displays linearity for a wide concentration in the range of 500 cells/mL-125000 cells/mL. The results show that the developed system may be used in detecting the MDA-MB-231 cells quantitatively. One of the critical parameters of biosensors is the limit of detection (LOD). Only a few references have reported the detection of cancer cells via QCM. The designed biosensors have different limit of detection values such as the Ma's report in which a QCM biosensor for detection of human lung carcinoma cells was designed (LOD=100 cell/mL) **[27]**. Zhang have designed a chitosan-based QCM biosensor for detection of breast cancer cells (LOD= 430 cell/mL) **[28]**, Shan et al. have developed an aptamer based QCM biosensor for the detection of leukemia cells (LOD=1160 cell/mL) **[29]**. A label-free and high-sensitive sensing technology for tumor cell recognition and detection was developed based on a novel 2×3 model of leaky surface acoustic wave (LSAW) aptasensor array by Chang et al. [30]. The detection limit as low as 32 cells mL^{-1} was achieved for MCF-7 cells. The LSAW aptasensor also exhibited excellent specificity and stability. The system included mercapto modified aptamer sequence and random sequence. The mercapto modified aptamer was firstly immobilized on detection sensor, and random sequence was immobilized on reference sensor. So manipulation procedures for preparing aptamer-based sensor are relatively time-consuming. Synthetic polymers designed for the cell studies must comply with many requirements, the most important of them being a very good biocompatibility with recipient's tissue, mechanical stability, resistance to degradation and hydrolysis **[31].** PHEMA nanoparticles with excellent biocompatibility properties are suitable for clinical applications and were chosen as a matrix for this study. Our biosensor showed wide linear range and acceptably low limit of detection (500 cell per/mL). Compared with the other methods based on QCM, proposed QCM cytosensor has a relatively wider linear range and lower detection limit **[32,33]**.

To determine the kinetic and equilibrium isotherm parameters of the QCM biosensor, the binding data was analyzed using a pseudo-first-order kinetic analysis and four different equilibrium isotherm models **(Figure 4c) [34]**.

< Figure 4 >

Equilibrium Kinetic Analysis $d\Delta m/dt = k_a C\Delta m_{max} - (k_a C + k_d)\Delta m$ **(3)**

Scatchard $\Delta m_{ex}/C = K_A\Delta m_{max} - K_A\Delta m_{eq}$ **(4)**

Langmuir $\Delta m = \{\Delta m_{max[}C]/K_D + [C]\}$ **(5)**

Freundlich $\Delta m = \Delta m_{max}[C]^{1/n}$ **(6)**

Langmuir−Freundlich $\mathbf{\Delta m} = \{\Delta m_{max}[C]^{1/n}/K_D + [C]^{1/n}\}$ **(7)**

Δm defines amount of increased mass on unit area of a QCM biosensor (number of cell/cm^2); C is the cell concentration (number of cell/μL); 1/n is Freundlich exponent; k_a (number of cell/μL) and k_d (1/s) are forward and reverse kinetic rate constants; K_A (number of cell/μL) and K_D (μl/number of cell) are forward and reverse equilibrium constant; subscripts ex, max, and eq indicate experimental, maximum and equilibrium, respectively. When applying the equilibrium kinetic analysis, we have calculated slopes of curves and plotted concentration vs. slope curve to determine k_a and k_d, respectively **[35]**.

The adsorption models can be used to define recognition ability, interaction selectivity and surface homogeneity of the biosensors. For testing the experimental data Langmuir, Freundlich and Langmuir–Freundlich isotherm models were evaluated (Table 3). Langmuir-Freundlich was the best-fitted isotherm model among the applied models with a high regression coefficient (R^2: 0.99). Langmuir model is generally used to explain monolayered binding isotherms on a homogeneous surface and the binding sites have the same adsorption affinity, which is energetically equal without any interaction between the adsorbed molecules **[36]**. Freundlich model is used to show multilayer binding of analyte molecules. Langmuir- Freundlich model can be applied to a system that is not fitted to both systems, provides heterogeneity information of adsorption behavior over wide concentration regions. These results show that the binding of cells on to QCM sensor is heterogeneous due to complexity of interactions between cell-cell and nanoparticles-cell.

Table 3. Calculated values of the isotherm models.

Langmuir	**Freundlich**	**Langmuir-Freundlich**
$\triangle m_{max}$ 0.14	$\triangle m_{max}$ 1.4	$\triangle m_{max}$ 0.12
K_A, cell/µL 0.8	1/n 1.23	1/n 0.1272
K_D, µL/cell 1.21	R^2 0.95	K_A, cell/µL 1.4
R^2 0.986		K_D, 1µL/cell 0.7
		R^2 0.99

3.3. Selectivity of QCM biosensor

In order to show the selectivity of the transferrin functionalized PHEMA nanoparticle attached QCM biosensor, competitive adsorption of MDA-MB-231, MCF-7 and starved MDA-MB-231 human breast cancer cell lines were investigated (75000 cell/mL). As seen in the **Figure 5,** transferrin functionalized QCM biosensor was more selective for MDA-MB 231 cells as compared to MCF-7 cells and starved MDA-MD-231 cells. Selectivity coefficient (k) and relative selectivity coefficients (k′) values are given in Table 4. Transferrin functionalized QCM biosensor was 1.85 and 6.32 times more selective for highly metastatic MDA-MB-231 than MCF-7 and starved MDA-MB-231 cells, respectively. These results indicate that transferrin functionalized QCM biosensor has higher adsorption affinity for highly metastatic MDA-MB-231, due to higher transferrin receptor expression. To show the specificity of the biosensor, PHEMA nanoparticle attached QCM biosensor (without transferrin) was used and the responses of this non-functionalized QCM system to MDA-MB-231, MCF-7 and starved MDA-MB-231 cell lines were determined as 0.03, 0.0325 and 0.013, respectively. These results demonstrated the transferrin had successfully bound to chip surface and the interaction of cells was occurs through the transferrin molecules.

< Figure 5 >

Table 4. Selectivity coefficient (k) and relative selectivity coefficient (k′) values.

Cell Sample	PHEMA with Transferrin		PHEMA without Transferrin		
	Δm	k	Δm	k	k′
MDA-MB-231	0.316	-	0.03	-	-
MCF-7	0.17	1.85	0.033	0.92	2.01
Starved MDA-MB-231	0.05	6.32	0.013	2.3	2.74

4. Conclusion

A QCM sensor was developed to detect human breast cancer cells with high metastatic power. PHEMA nanoparticles were attached to sensor surface to increase the surface area and followed by transferrin attachment to functionalize the QCM surface. The analysis of nanoparticles and the functionalized QCM sensor have revealed that an efficient sensing platform is formed. This QCM sensor have shown good sensitivity and selectivity for the real time detection of metastatic breast cancer cells via their over expressed transferrin receptors. The results revealed the QCM sensor had an excellent capability in discriminating MCF-7 breast cancer cells from other two kinds of cells. Binding data was best fit to Langmuir-Freundlich adsorption model. The system offers label-free detection, low volume sample application, real-time monitoring, quantitative measurement and kinetic evaluation. Manipulation procedures for preparing QCM sensor are relatively not time-consuming and require labeled molecule.This transferrin receptor targeting QCM biosensor may also enable the fabrication of a low cost and rapid detection system, which can be used for the screening of metastatic breast cancer cells in serum or biopsy samples.

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Figure Legend

Figure 1. The size and size distribution of PHEMA nanoparticles.

Figure 2. Schematic illustration of (a) transferrin modification of PHEMA nanoparticle attached

QCM sensor (b) QCM measurements.

Figure 3. 3D and surface AFM images of uncoated (a), PHEMA nanoparticle (b) transferrin coated (c) QCM surfaces.

Figure 4. Kinetic (a,b) and isotherm (c) studies with QCM sensor.

Figure 5. Selectivity (a) and specificity (b) of QCM sensor.

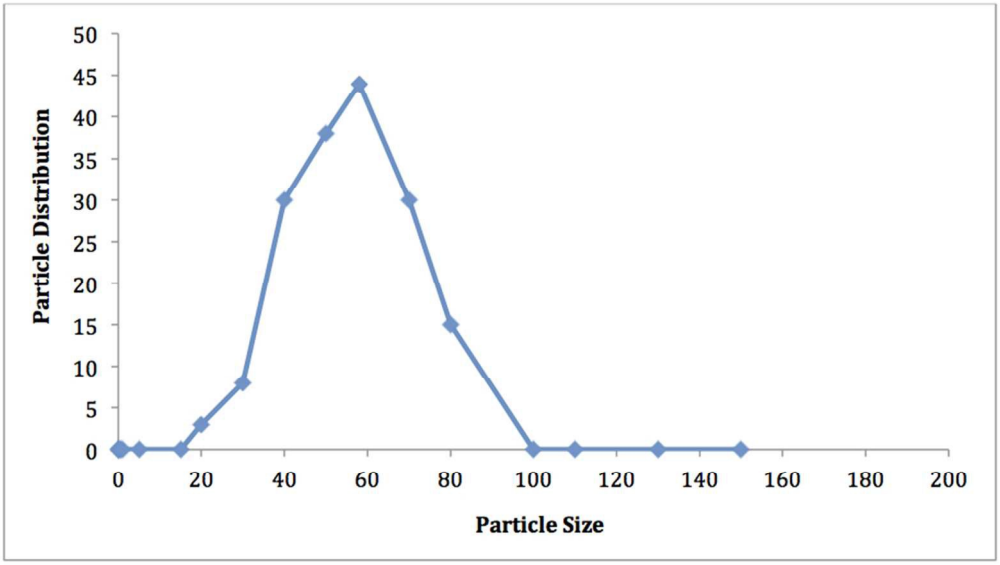
Table Legend

Table 1. Contact angle values of QCM surfaces.

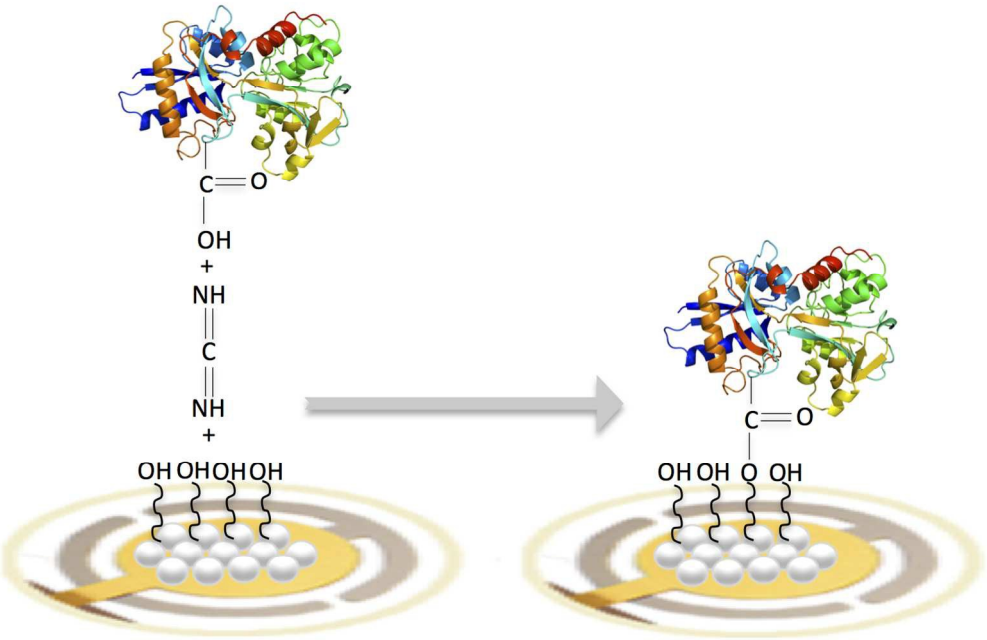
Table 2. AFM characterization data of QCM chip.

Table 3. Calculated values of the isotherm models.

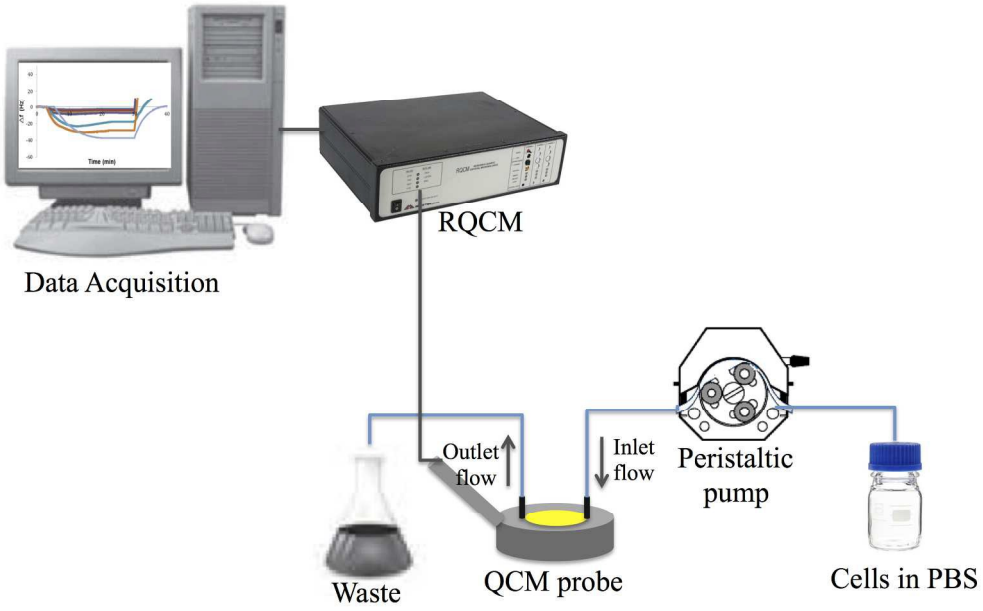
Table 4. Selectivity coefficient (k) and relative selectivity coefficient (k′) values.



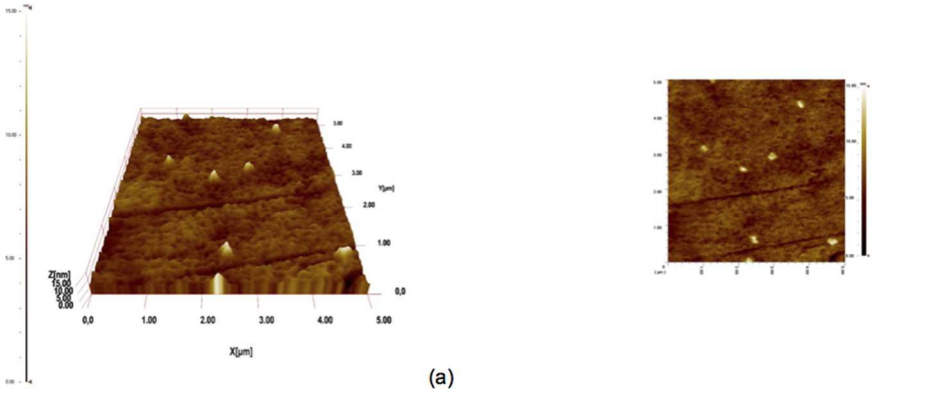
The size and size distribution of PHEMA nanoparticles.
154x88mm (150 x 150 DPI)



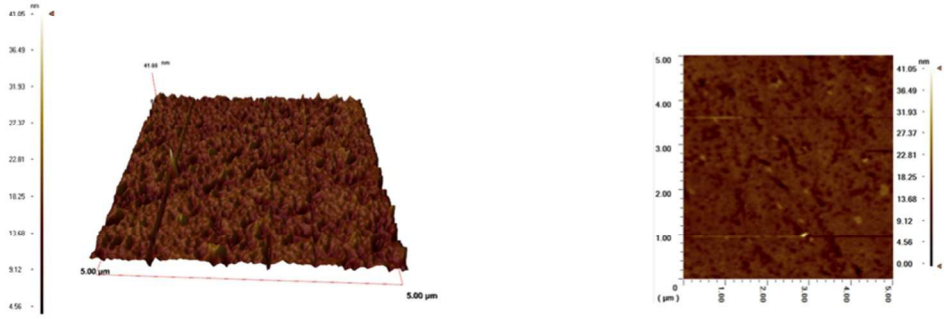
130x86mm (300 x 300 DPI)



219x135mm (300 x 300 DPI)



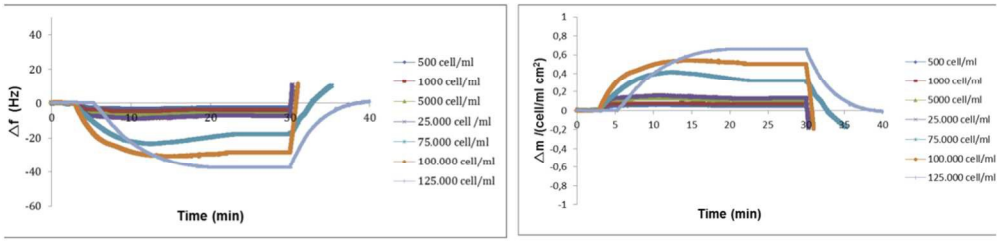
(a)



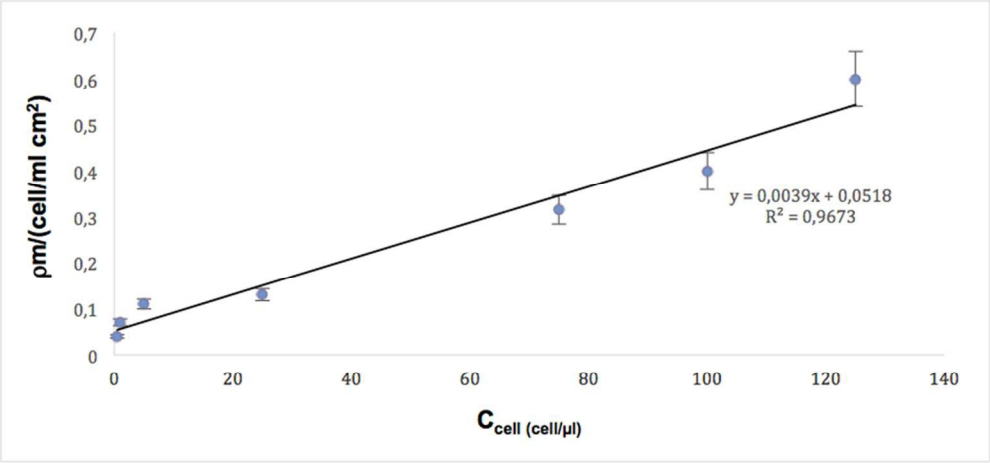
(b)

(c)

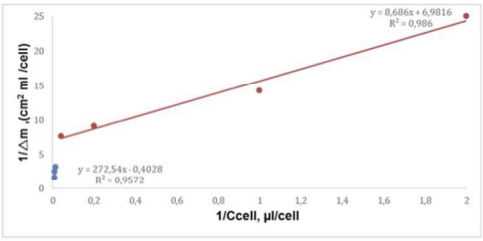
161x204mm (150 x 150 DPI)



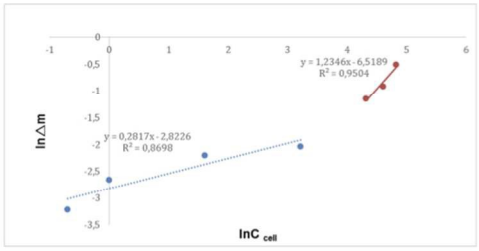
209x51mm (150 x 150 DPI)



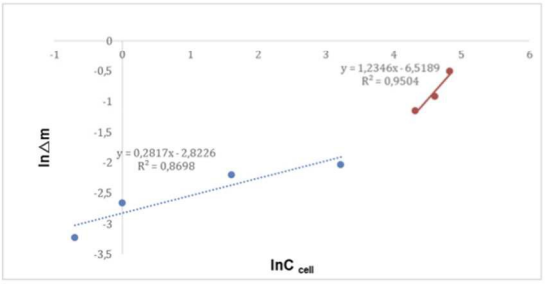
145x68mm (150 x 150 DPI)



Langmuir

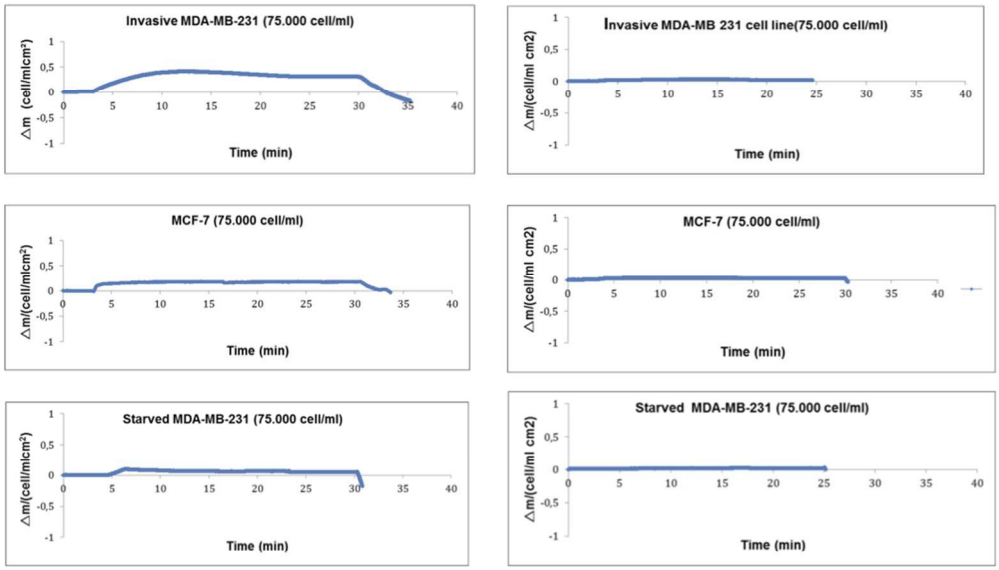


Freundlich



Langmuir- Freundlich

179x120mm (150 x 150 DPI)



(a) (b)

180x116mm (150 x 150 DPI)

The high sensitivity of QCM is combined with sensitivity and selectivity of receptor-ligand interaction in order to construct a biosensor which would discriminate breast cancer cells with high metastatic power to those of low or no metastatic potential as an attempt to develop a simple, fast and efficient system to be used in breast cancer diagnosis