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Quartz crystal microbalance for the cardiac markers/antibodies binding kinetic measurements in the plasma samples

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ABSTRACT

The quartz crystal microbalance (QCM) was exploited for cardiac markers detection and kinetic studies of immunochemical reaction of cardiac troponin I (cTnI) and human heart fatty acid binding protein (H-FABP) with the corresponding monoclonal antibodies in undiluted plasma (serum) and standard solutions. The QCM technique allowed to dynamically monitor the kinetic differences in specific interactions and nonspecific sorption, without multiple labeling procedures and separation steps. The affinity binding process was characterized by the association (k_a) and the dissociation (k_d) kinetic constants and the equilibrium association (K) constant, all of which were obtained from experimental data.

1. Introduction

Precise and rapid diagnosis and immediate therapeutic agents' uptake are essential for successful disease treatment. Detection of rapidly appearing blood biochemical markers specific for cardiac injury would provide more appropriate clinical diagnostics and therapy for patients with suspected acute myocardial infarction (AMI). The most widespread AMI biomarkers are troponin I, troponin T, creatine kinase-MB, myoglobin, and human heart fatty acid binding protein. AMI biomarkers detection is part of the typical clinical routine. Recently, great efforts have been made worldwide to develop and improve immunoassays for the detection of cardiac markers with the aim of designing affordable devices [1–8]. The main principles of these immunosensors are based on employing (i) sandwich immunoassays with labeled secondary antibodies, (ii) nanoparticles of different nature or (iii) immunochromatographic approaches. Table 1 summarizes the analytical performance (detection method, sample volume, sensitivity, assay time) of the recent by published papers dealing with detection of troponin and human heart fatty acid binding protein.

It should be noted that when antibodies are immobilized on the solid surface of sensor by chemical coupling reactions or by cross-linking reagents, their binding activity is usually less than that of unmodified native antibodies [16]. Compared to other methods, the method proposed herein is more simple and cost-effective: it allows to exclude the amplification steps and to do without the cover layer of labeled antibody; also, it is rather fast – the stable response is obtainable within 2 min.

Earlier we have developed a QCM immunosensor for the cardiac myoglobin detection in the plasma samples based on direct monitoring of the immunochemical reaction between the cardiac myoglobin and monoclonal antibodies immobilized directly onto a gold surface of quartz crystal electrode due to Au–S bond formation by drop casting [17]. The presently proposed approach is based on differences in the kinetics of specific interactions vs. nonspecific sorption and it permits to rule out the separation steps. The measuring of specific binding events was performed in a short time (30–120 s) after the addition of plasma samples. Immunosensor has a detection limit of 34.2 ng mL^{-1} of cardiac myoglobin (2 nM), whose cut-off value is 100 ng mL^{-1}. This work is an extension of our previous study [17] and it is now focused on the detection of cardiac troponin I and human heart fatty acid binding protein in undiluted plasma (serum) and standard solutions, which their respective cut-off concentration values of 20.4 pg mL^{-1} for cTnI and 1 ng mL^{-1} for H-FABP [2,3].

2. Material and methods

2.1. Chemicals and proteins

To prepare 'piranha' solution, sulfuric acid and 30% hydrogen peroxide were purchased from Fisher Chemicals (United Kingdom) and ChemMed (Russia), respectively. Ethanol was from ChemMed (Russia). The following reagents were purchased from USBiological (USA): mouse anti-human troponin I, cardiac, 16–20 (anti-cTnI, 4.5 mg mL^{-1}, T8665-17C) and cardiac human troponin I, calibrator kit, bioassay (cTnI, 0 ng mL^{-1}, 0.54 ng mL^{-1}, 2.54 ng mL^{-1}, lyophilized powders, T8665-15R). Mouse anti-human fatty acid binding protein (anti-H-FABP, stock solutions 0.1 mg mL^{-1}, HM2016) and

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Table 1
Summary of detection methods for troponin I, troponin T and human heart fatty acid binding protein.

Detected cardiac markers and reference	Detection method	Sample (volume)	Assay time	Concentration range and detection limit ($ng\ mL^{-1}$)
cTn T [9]	Dual-QCM; immobilization of anti-cTn T on self-assembled monolayer by cysteamine via glutaraldehyde	100 μL Human serum	~18 min	0.025–4.0 0.008
cTn T [10]	QCM; spray coating technique of polyvinyl chloride-COOH with attachments of cross-linker reagents	1 mL Standard solution	60 min	5–50000 5
cTn T [11]	Cyclic voltammogram; molecularly-imprinted human cardiac troponin sensors based on electropolymerisation of *o*-phenylenediamine on a gold electrode in the presence of cTn T as a template; signal is response in a suppression of the ferro/ferricyanide redox process	Standard solution	~10–15 min	0.009–0.8 0.009
cTn T [12]	Potentiometric sensor by molecular imprint on the surface of multiwalled carbon nanotubes	200 μL Standard solution	–	1410–20860 160
cTnI [13]	Optical biosensing platform by CO_2 laser ablation technique for rapidly prototyping on-chip planar lenses, in conjunction with capillary action based autonomous microfluidics. The device has been used in conjunction with a miniaturised and bespoke fluorescence detection platform to create a complete, palm sized system (≈60 × 80 × 60 mm) capable of performing fluoro-immunoassays	No information	No information	0.08
cTnI [14]	Conductometry; Chemiresistive biosensor based on mercaptopropionic acid capped gold nanoparticles (GNPs) functionalized single walled carbon nanotube hybrid; the cTnI antibody was covalently immobilized on GNPs through capping agent using carbodiimide coupling reaction	No information	No information	0.01–10 0.01
H-FABP [15]	On-step immunochromatography using the same mouse antihuman H-FABP monoclonal antibodies as used for the sandwich-ELISA	150 μL aliquot of whole blood with anticoagulant	15 min	If concentration of H-FABP is at or over 6.2 ng/mL, a red line appears at the detection line; if concentration is below 6.2 ng/mL, only the red control line develops This is qualitative test
cTnI H-FABP [This Letter]	Proposed QCM immunosensor; on-step analysis in real time, without multiple labeling procedures and without additional chemical modifications of electrode surface	1 μL Human serum or plasma with EDTA; standard solution	2 min	cTnI: 0.4–5.08 0.16 H-FABP: 0.5–5.0 1.26

recombinant human heart fatty acid binding protein (H-FABP, 50 $\mu g\ mL^{-1}$, HC2105) were purchased from Hycult biotech (The Netherlands). The antibodies solutions of (100 and 118) ng μL^{-1} to H-FABP and cTnI were used for immunosensors preparation. All the reagents were analytical grade. Standard solutions of cardiac markers were prepared daily by dissolving lyophilized cTnI or H-FABP in 0.1 M phosphate buffer (PBS, pH = 7.4).

2.2. Plasma samples

Plasma samples were obtained from patients with acute myocardial infarction (AMI-X; Central Clinical Hospital of the Russian Railways). Informed signed consent was obtained from each of the patients. Plasma was collected after centrifugation of blood with EDTA as anticoagulant for 10 min at 3000 rpm. Cardiac troponin I concentrations were determined in each sample with the bench-top lateral flow immunoassay RAMP® (Response Biomedical Corp) according to the instruction. The qualitative determination of H-FABP in plasma samples was analyzed using an immunochromatographic hand-held device 'CardioH-FABP' (Research and Manufacturing Association 'Biotest', Russia, www.biotst.ru).

2.3. QCM measurements

The quartz crystal microbalance measurements were performed using the Nova software and the Autolab module QCM with AT-Cut, 6 MHz quartz piezoelectric crystals and gold-plated electrode (diameter 0.67 cm, 1000 E thickness) on both sides mounted in a conventional QCM cell (Eco Chemie, The Netherlands). Prior to modification with antibodies, the quartz crystals with gold electrodes were rinsed with ethanol and then immersed in 1:3 (v/v) 30% H_2O_2/H_2SO_4 'piranha' solution for 5 min at room temperature. Then quartz piezoelectric crystals were rinsed with water and ethanol and dried in the air.

Solutions of monoclonal antibodies (1 μL, 100 ng μL^{-1} to H-FABP and 118 ng μL^{-1} to cTnI) were immobilized onto the gold electrode surface by drop casting. Then the resonance frequency was checked up. A shift of <1 Hz min^{-1} showed the stability of the system. For the registration of target protein binding, 1 μL plasma sample was added to the antibody layer.

All measurements were carried out in 2 μL of total volume in a conventional QCM cell at room temperature. The time-dependent frequency changes in all experiments were referred to the average responses of the immunoreactions with the corresponding confidential interval of triplicate measurements which was calculated by Student's *t*-test ($t_{0.05} = 2$).

3. Results and discussion

3.1. Cardiac markers' monitoring by use of QCM immunosensor

Registration of the immunochemical reaction of the cardiac marker with the monoclonal antibody was based on the differences in the kinetics of specific interaction and nonspecific sorption of plasma components thereby permitting the exclusion of separation and washing steps.

The use of small volume of human plasma is often desirable in clinical practice. For biosensor construction, it is crucial to use