Development of a Columbimetric Immunosensor for the Detection of Human Cardiac Troponin I

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Biosensors play an important role in the biomedical research, drug and therapy discovery and health care. Two of the main application fields are in the personalized therapeutics for adjust the therapeutic agents dose (i.e. the glucose sensor for insulin dependent diabetes) and in point of care testing (POCT) devices. The main objective in this work is the development of an electrochemical immunosensor used to detect cardiac troponin I (cTnI) which is considered the golden biomarker for the diagnosis of acute myocardial infarction (AMI).

cTnI play an important role in the accurate diagnosis of AMI and more importantly, is a powerful tool for assessing risk and give good therapies improving clinical outcomes (Aldous 2013). cTnI is released to the bloodstream after AMI episode and elevated levels of this biomarker mean severe and probably irreversible damage of the myocardium. Therefore an early detection of cTnI levels in blood could allow to us to make an accurate diagnostic of the disease and to predict patient outcome (Jillian R. Tate 2008). Aditionally, cTnI is a good marker for risk stratification (MEMBERS, Morrow et al. 2007). Values of cTnI exceeding the 99th percentile of a reference control group are closely related with AMI episodes. These values are around 0.02 – 0.08 ng/mL (Eggers, Jaffe et al. 2009).

We present in this work a columbimetric immunoassay for the detection of cTnI. Different polyclonal antibodies were produced against cTnI detection, ones by immunization of the whole proteins and others by immunization of selected sequences of different epitopes of cTnI. It was done taken into account the different areas of the protein avoiding susceptible areas to be eclipsed by preventing its immunodetection. All of these antibodies were tested by ELISA assay, and after their evaluation, they were used to develop a columbimetric immunodevice. The selected antibodies were As220 used as a capture antibody and produced against the whole cTnI, and As260 used as detection antibody produced against a selected cTnI epitope. The immunosensor presented uses a screen-printed electrodes (SPE), biofunctionalized magnetic µ-particles with a capturing antibody Ab220 and electrochemical nanoprobes prepared by labeling the detection antibody Ab260 with CdS nanoparticles (CdSNP) (Valera, Muriano et al. 2013). The different stages of the assay are showed in Figure 1. The immunoreactions took place in one step by the incubation of the sample (containing cTnI) Ab260 labeled with CdSNP and Ab220 immobilized to the magnetic beads. After this incubation step, the complex was washed 3 times and resuspended in the appropriate volume of measuring buffer and deposited on the working electrode. The signal were provide taking advantatge of the characteristic redox potential of the Cd, applying a stripping voltammetry, The intensity of this peak is directly related with cTnI concentration, showing a good dose-dependence. Due to the amplification effect on the amperometric/coulombimetric signal produced by the CdSNP, a high detectability can be reached such as a LOD of 0,004 ng/mL in buffer. In order to study the reproducibility of the assay, it has been repeated three different days, showing in all the cases good results (Figure 2).

References

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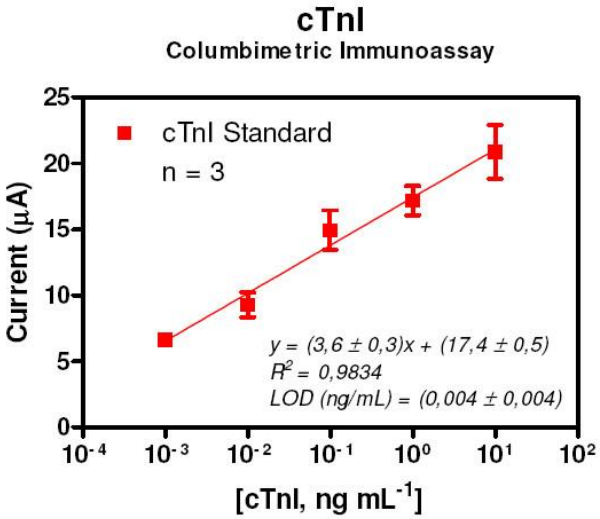

Figure 2. Cardiac Troponin calibration curve developed by columbimetric immunosensor.

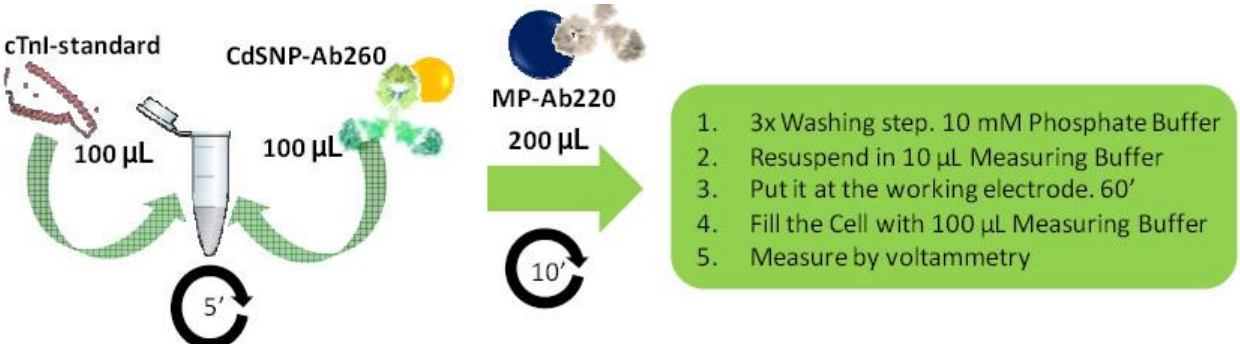

Figure 1. Different Steps of the columbimetric immunosensor developed.