3D CHIP AS A TOOL FOR ISOLATION AND DETECTION OF INFLUENZA VACCINE HEMAGGLUTININ

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ABSTRACT

The aim of this study was an isolation and detection of influenza antigen using 3D fabricated chip. Surface of influenza virion is equipped with two antigens: hemagglutinin (HA) and neuraminidase (NA), which are responsible for virus life cycle and interaction with host cell. In this study, vaccine HA, labeled by quantum dots (QDs) was used for better specification. The 3D chip assay was divided in two parts: paramagnetic particles (MPs) based isolation and electrochemical detection of isolated product. Our results show, that 3D fabricated chip is useable tool for MPs based isolation and electrochemical detection influenza hemagglutinin.

Key words: 3D chip, voltammetry, influenza, hemagglutinin, quantum dots, paramagnetic particles

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INTRODUCTION

Influenza is probably the most powerful member of the group of potential pandemic agents, because of the high speed of constant mutational changes in surface antigens, hemagglutinin (HA) and neuraminidase (NA). HA is a trimeric glycoprotein expressed on the membrane of influenza virus (Suenaga, Mizuno et al. 2012). It binds to SA receptors on the surface of the host cell and subsequently mediates fusion of the viral and host membranes (McCullough, Wang et al. 2012). For this reason, HA is considered to be the main target for antibodies upon vaccination as well as infection (Lingwood, McTamney et al. 2012). For this reason HA is utilized for vaccine preparation (Wang, Ni et al. 2012). Quantitative and qualitative analysis of vaccine antigens is a turning point before the vaccine is placed on the pharmaceutical market and used for immunization (Williams, Pirkle et al. 2012).

Nowadays, new sensors based on magnetic beads separation are coming to the foreground (Gijs 2004). Paramagnetic particles (MPs) are the excellent tool with many advantageous features, such as easy handling and possibility for separation by the magnetic field. The easily modified surface is the next great advantage. Because of these reasons, these particles have found many applications to enhance the selectivity, sensitivity, and speed of isolation methods (Krejcova, Dospivova et al. 2012). In current study, the microfluidic assay based on MPs isolation and HA indirect detection by QDs is described. Microfluidic device for isolation and detection of HA-QDs complex were fabricated using three-dimensional (3D) printing, which is an example of additive manufacturing or of solid freeform fabrication technology (Polzin, Spath et al. 2013).

EXPERIMENTAL SECTION

Chemicals

Tris(2-carboxyethyl)phosphine (TCEP) was purchased from Molecular Probes (Oregon, USA). $Co(NH_3)_6Cl_3$ and other chemicals were purchased from Sigma Aldrich (Sigma-Aldrich, USA) unless indicated otherwise. Deionised water was used for rinsing, washing, and preparation of buffers. Stock solutions were prepared from ACS water.

Hemagglutinin

As the sample of Influenza hemagglutinin was used vaccine Vaxigrip (Sanofi Pasteur, France), which contained three strands (A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2) and B/Wisconsin/1/2010) of inactivated and splitted influenza virions. Vaxigrip contains 15 µg of all three HA per 0.5 mL.

CdS QDs

CdS QDs were prepared by a slightly modified method published by Li and co-workers (Li, Shih et al. 2007). The obtained yellow solution was stirred for 1 h. Prepared CdS QDs were stored in the dark at 4 ºC and were used for labeling of vaccine HA.

Labeling of vaccine HA by QDs

Vaxigrip (500 µL) was reduced and washed with water (5 x 400 µL) on an centrifugal filter device - Amicon 3k (Millipore, Massachusetts, USA) and mixed with a QDs solution (500 µL), mixture was shaken (24 h, room temperature) on a Biosan Orbital Shaker OS-10 (Biosan Ltd. Riga, Latvia). Volume of solution was reduced to 100 µL (Amicon Ultra 3k), washed, diluted to 1 mL and used for isolation.

Fabrication of 3D microfluidic chip

The first step in fabrication of microfluidic chip was its 3D processing in the modeling program Blender 2.65. Product of this software was exported in STL format and further edited in netFabb programme (Germany). The STL format was opened in the program G3DMAKER (DO-IT, Czech Republic) 3D printing by EASY 3D MAKER (DO-IT s.r.o., Czech Republic). Chip was printed with an accuracy of [x,y,z] 0,1/0,1/0,08 mm. As a material was used polylactide (PLA) (DO-IT, Czech Republic), which was applied by extrusion (melting head) at temperature 210 °C on a heated surface (40° C), printing time of the chip was 94 min. Every printed chip was machined from minor impurities, fitted with tubes with a diameter of 2.1 mm and three electrodes (working glassy carbon microelectrode (GCm), reference graphite lead with a diameter of 0.5 mm, auxiliary platinum wire). Attachment the plastic film (thickness 0.7 mm) at top of chip was the last step.

Microfluidic analysis

A microfluidic analysis system (3D chip) equipped with devices for electrochemical detection was proposed and constructed. Procedure included two basic steps: isolation and electrochemical detection. The isolation procedure was done as follows: 10 µL of streptavidin modified MPs (Dynabeads M-270, Invitrogene) was dosed by a peristaltic pump in the reaction chamber in 3D chip. Using an external magnet MPs was anchored in the reaction chamber, stored solution from the MPs was aspirated and MPs were washed by 1500 µL of phosphate buffer (PB) (0.3M, pH 7.4). Thereafter, MPs were modified by 20 µL biotinyled Glycan (50 µg/ml); this step was followed by washing (1500 µl of PB). Last part of isolation was binding of HA-CdS (20 µL, concentration 45 µg/mL) onto glycan-modified MPs, again followed by a washing with PB (1500 µL). After that, chip with MPs-glycan-HA-CdS was immersed in an ultrasonic bath and complex was fractionated. Isolated HA was detected indirectly due to electroactivity of Cd(II), respectively CdS from isolated HA-CdS complex.

Electrochemical detection of isolated HA-CdS complex

Measurements were carried out using three-electrode set up. As the working electrode was used a glassy carbon microelectrode (GCm), as the reference electrode was used a graphite lead and as the auxiliary electrode a platinum wire was used.

The differential pulse voltammetry (DPV) was used for the detection with the following parameters: initial potential -1.3 V, end potential -0.1 V, deposition potential -1.3 V, deposition time 85 s, modulation amplitude 0.1 V, step potential 0.005 V, scan rate 0.05 V/s. Acetate buffer (0.2M, pH 5) was used as the electrolyte. The experiments were carried out at 20 °C. The signal was detected by a PGSTAT101 Autolab potentiostat (Metrohm, The Netherlands) and the results were evaluated by the Software NOVA 1.8 (Metrohm, The Netherlands).

RESULTS AND DISCUSSION

We focus on implementation of the method for isolation and detection of HA-CdS by magnetic field controllable microfluidic 3D chip. The isolation is stable due to the continual washing process. The flow was discontinued in two steps: conjugation of glycan on MPs and conjugation of HA-CdS on glycan-modified MPs. Using above mentioned conditions, the isolation process was very specific because non-specifically bounded substances were eliminated by the washing process better than in stationary design.

The beads-based isolation of HA-CdS complex was the cornerstone of the procedure. Glycan-conjugated (modified) beads bound vaccine HAs, which could be recognized specifically and linked onto the surface of the glycan-modified MPs (Fig. 1).

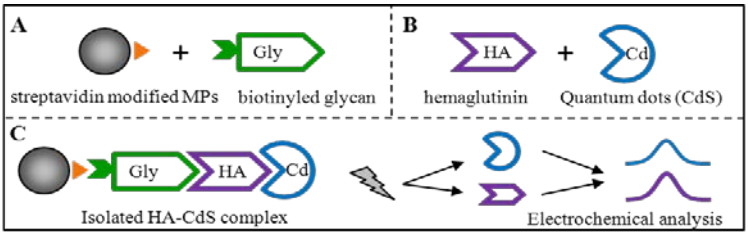

Fig. 1: Scheme of 3D chip isolation and electrochemical detection of HA-CdS complex. A Biotinyled glycan bounds on streptavidin-modified MPs. B Labeling of hemagglutinin by CdS. C Isolation of HA-CdS and ultrasound breaking of complex and electrochemical analysis of Cd from complex.

Microfluidic analysis

Optimization of electrochemical detection of Cd(II) in microfluidic conditions was the first step. Flow rate and time of accumulation were the optimized parameters. Sample was dosed by peristaltic pump in the operating range from 0 to 1200 µL/min. The highest response of signal was achieved by the flow rate 480 µL/min (Fig. 2 / B), as the best time of accumulation was established 65 s (Fig. 2/C). Reproducibility of method was tested with five samples with identical concentration of HA-CdS complex (concentration of cadmium 1 mM) (Fig. 2 / D). Reproducibility of method was higher than 80 %. Influence of the dependence of HA-CdS concentration (Cd concentration respectively) in the range from 0.06 to 0.5 mM was investigated too. The increasing dependence in this concentration interval has parameters as follows: y = 158.9x, R^2 = 0.9842, n =4 (Fig. 2 / E).

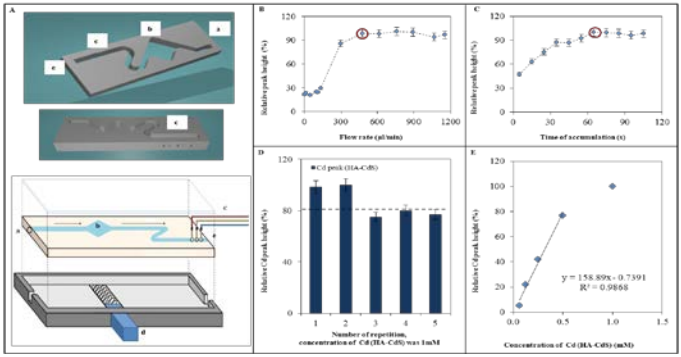

*Fig.: 2 **A** Scheme and model of 3D chip: injection (**a**), reaction cell (**b**), three electrode setup (**c**), magnet (**d**), eflux (**e**). **B+C** Optimization of isolation parameters. **B** Influence of flow rate (µl/min) on Cd relative peak height (%). **C** Dependence of relative peak height (%) on time of accumulation (s). **D** Dependence of relative Cd peak height (%) on concentration of Cd (HA-CdS) (1mM). **(E)** Dependence of relative Cd peak height (%) on different concentration of Cd (HA-CdS).*

CONCLUSIONS

We described 3D microfluidic chip for vaccine hemagglutinines labeled by CdS quantum dots. Our results show that electrochemical determination of isolated hemagglutinin using CdS is very effective and have potential to become an alternative way as a rapid, sensitive, and specific detection of influenza hemagglutinin, influenza virus respectively.

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