



FACULTY OF VETERINARY MEDICINE DEPARTMENT OF MICROBIOLOGY

Biomimetic immunoassay based on polymeric virus imprints for diagnosis of Foot and Mouth Disease virus (FMDV) serotypes

A THESIS

Presented to the graduate school

Faculty of Veterinary Medicine, Alexandria University

In Partial Fulfillment of the Requirements for the Degree

OF

Ph.D of Veterinary Sciences

In

Microbiology

SPECIALIZATION

(VIROLOGY)

By

Heba Ahmed Hussein Ahmed

(B.V.Sc.Fac.Vet.Alex.Univ. 2010) (M.V.SC. Fac.Vet.Alex.Univ. 2015)

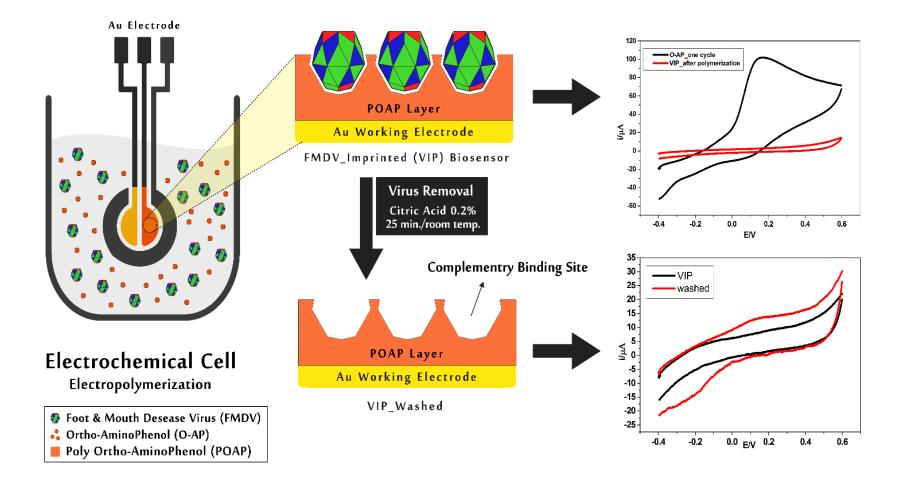
(2019)

Abstract

Foot and mouth disease virus (FMDV) is a highly contagious virus due to its ease of transmission. FMDV has seven genetically distinguished serotypes with many subtypes within each serotype. The traditional diagnostic methods of FMDV have demonstrated many drawbacks related to sensitivity, specificity, and cross-reactivity. In the current study, a new viral imprinted polymer (VIP)-based biosensor was designed and fabricated for the rapid and selective detection of the FMDV. The bio-recognition components were formed via electrochemical polymerization of the oxidized O-aminophenol (*O*-AP) film imprinted with FMDV serotype O on a gold screen-printed electrode (SPE). The overall changes in the design template have been investigated using cyclic voltammetry (CV) and atomic force microscopy. Optimal conditions were achieved through investigating the capturing efficiency, binding stability, selectivity and lifetime of the developed biosensor. The results depicted a high selectivity of the biosensor to the serotype O over all other genus serotypes A, SAT2 and Lumpy skin disease virus (LSDV) as well as the inactivated serotype O. The limits of detection (LOD) and quantification (LOQ) were around 2 ng/mL and 6 ng/mL, respectively, in addition to the tested repeatability and reproducibility values with a variance coefficient of 1.0% and 3.6%, respectively. In comparison with the reference methods (ELISA and PCR), the analysis of saliva real samples using the developed affordable biosensor offered 50 fold better LOD with the possibility of on-line monitoring in the field with no prior sample treatment.

Keywords: Foot and mouth disease virus (FMDV), Virus imprinted polymers (VIPs), Screen printed electrode (SPE), Biomimetic virus biosensors

Graphical abstract:



List of content

Subject	Page
1. Introduction	1
2. Review of Literature	5
2.1. FMDV	5
2.1.1. The disease and the affected animals	5
2.1.2. The disease distribution	6
2.1.3. The virus	8
a. Classification, serotypes, capsid, genome, and culture criteria	8
b. Effect of pH and chemicals on the virus capsid and infectivity	9
2.1.4. FMDV diagnosis	11
a. Virus isolation on cell culture (BHK21)	11
b. Enzyme-linked immunosorbent assay	12
c. Polymerase chain reaction (PCR)	13
2.1.5. Molecularly imprinted polymers (MIPs)	14
2.1.6. Biosensors	16
2.1.7. Ortho-aminophenol (o-AP)	19
2.1.8. Viral imprinted polymers (VIPs) and biosensors	20
3. Material & Methods	22
3.1. Materials	22
a. Biological materials	22
b. Chemicals & reagents	23
c. Apparatus	25
3.2. Methods	27
3.2.1. Virus molecular identification by modified sanger method	27
3.2.2. Virus propagation	27
3.2.3. Fabrication of viral imprinted polymer (VIP) biosensor	27
a. Electrode preparation	27
b. Optimization of FMDV molecular imprinted polymerization	27 27
c. Testing of the conductive solution for investigation of the current	27 28
signals	20

3.2.4. The washing step to remove the virus particles from the imprinted matrix	28
3.2.5. The efficacy of the created attachment sites of the VIP and the Calibration curve	28
3.2.6. The selectivity testing of the proposed virus biosensor	29
3.2.7. Bench application of the proposed FMDV biosensor with the real field samples	29
3.2.8. Confirmation using routine diagnostic methods, Enzyme-linked immunosorbent assay (ELISA) and Polymerase chain reaction (PCR)	30
4. Results	31
5. Discussion & Conclusion	45
6. English Summary	52
7. References	54
8. Abstract	66
9. Arabic summary	68

Lists

List of Figures

Figure	Caption							
1	Applied number of cycles for the electrochemical polymerization of <i>O</i> -AP with FMDV particles (VIP).	32						
2	Cyclic voltammetry records the declined signal of the proposed electrode from first cycle polymerization and after the complete polymerization technique of NIP and VIP.							
3	The VIP responses in KCl and FCN towards the FMDV capturing.	34						
4	The investigated washing intervals using citric acid.	35						
5	 AFM images (non-contact mode). 2D AFM images illustrate the surface roughness of the VIP 	36						
	surface. 3: 2D AFM images of washed VIP show the biomimetic attachment cavities for the virus.	37						
6	SEM images of gold electrode surface.	38						
	FT-IR spectroscopy of the NIP, drop casted solution of <i>O</i> -AP, and bare gold.	39						
8	Calibration curve.	40						
9	Selectivity test of the developed VIP biosensor	41						
10 10	Application of the VIP biosensor on real field samples.	43						

<u>List of tables</u>

Table	Title						na i na i na t	Page	
• • • • • • • • • • • • • • • • • • •	Application comparison				• 1	0	biosensor	in	44

1994 - 1994