Virology 513 (2018) 85–90

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

An easy way to detect dengue virus using nanoparticle-antibody conjugates

Caroline R. Basso[a], Claudia C. Tozato[b], Bruno P. Crulhas[a], Gustavo R. Castro[a], João Pessoa A. Junior[b], Valber A. Pedrosa[a,*]

[a] Department of Chemistry and Biochemistry, Institute of Bioscience, UNESP-Botucatu, SP 18618-000, Brazil
[b] Department of Microbiology and Immunology, Institute of Bioscience, UNESP-Botucatu, SP 18618-000, Brazil

ARTICLE INFO

Keywords:
Infectious diagnostics
Biosensor
Fast detection

ABSTRACT

The aim of the present research is to propose a new method based on localized surface plasmon resonance (LSPR) for fast dengue virus detection. A pool with four dengue serotypes (DENV-1, -2, -3, -4) was detected through antigen-antibody binding using gold nanoparticles (AuNPs) as signaling antibody carriers. Such result was confirmed through surface plasmon resonance (SPR), transmission electron microcopy (TEM), and dynamic light scattering (DLS) techniques. The limit of detection was calculated for $TCID_{50}$ 10^7 demonstrating a linear correlation between viral concentration and number of cells with an r^2 value of > 0.993. The assay presented good sensibility and reproducibility of results and the negative controls were not mistakenly detected. This design requires no pretreatment or high trained person. In the future, it can be used in commercial antibody detection kits.

1. Introduction

Dengue is an infectious viral disease caused by one of its four distinct serotypes (DENV-1, -2, -3 or -4). This is a flavivirus (belonging to family *Flaviviridae*) transmitted to humans through the bite of female mosquitoes belonging to the species *Aedes aegypti* and, to a lesser extent, through the bite of *Aedes albopictus* female mosquitoes (Jahanshahi et al., 2014). The genetic material of the dengue virus, RNA positive-sense single-strand containing approximately 10,700 bases, encodes three structural proteins (capsid [C], membrane protein [M] and glycoprotein for viral envelope [E]) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) (Ramos-Castañeda et al., 2017). Data from the World Health Organization (WHO) indicate that the dengue virus remains a major health problem in tropical and subtropical regions worldwide. It leads to 390 million dengue infections per year, and 96 million clinical manifestations of the disease. Estimates foresee that 3.9 billion people are at risk of dengue virus infection in 128 countries (Ramos-Castañeda et al., 2017; World Health Organization, 2017). The dengue virus causes dengue fever (DF), which main symptoms are: high fever (40 °C/104 °F), severe headache; pain behind the eyeballs, in the muscles and joints; nausea; vomiting; swollen glands or rash. It can develop into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Kumbhat et al., 2010). These symptoms are reported approximately 10 days after virus inoculation in humans. The virus incubation period in the host lasts from 4 to 10 days. Infection with one of four dengue serotypes DENV 1–4 leads to life-long protective immunity against this serotype, but only partial cross-protection against the other serotypes. For example, if a person contracts DENV 1, they may still be infected with DENV 2, 3 and 4 throughout their lifetime (Zhang et al., 2015).

The dengue virus diagnosis is primarily done through the clinical signs presented by the patient and It is confirmed through laboratory tests such as Immunoglobulin M (IgM) and Immunoglobulin G (IgG) (Nunes et al., 2011; Wang and Sekaran, 2010), enzyme-linked immune sorbent assay (ELISA) (Wiwanitkit and Wiwanitkit, 2015), reverse transcription polymerase chain reaction (RT-PCR) (Saxena et al., 2008) and non-structural protein 1 (NS1) detection and point-of-care (POC) test (Jahanshahi et al., 2014). However, none of these exams are fast, specific and sensitive enough to be used as stand-alone diagnostic tool, and some of them require few days to get the results, besides their high cost (Wiwanitkit and Wiwanitkit, 2015; Saxena et al., 2008; Guzman and Kouri, 2004). Moreover, the requirement for specialized training and equipment and time consuming nature of these assays limits their widespread utility for virus detection, compromising the rapid diagnosis of viral infections.

Recently the use of nanotechnologies for the development of biosensors has been gaining more space by significantly optimizing the sample volume used, the time of analysis, the limit of detection and the possible detection of analytes in unusable samples in classical methods. The use nanoparticles makes it possible to detect infectious diseases

* Corresponding author.
E-mail address: vpedrosa@ibb.unesp.br (V.A. Pedrosa).

http://dx.doi.org/10.1016/j.virol.2017.10.001
Received 24 August 2017; Received in revised form 28 September 2017; Accepted 2 October 2017

outside laboratories in a more sensitive, economical and fast way, because they present high specificity to different pathogens (Vaculovicova et al., 2017). The development of assays based on metallic nanoparticles has been widely used to diagnose human-related diseases (Urdea et al., 2006). The gold nanoparticles (AuNPs) have attracted the attention of researchers, and used as diagnostic apparatus due to their easy synthesis, low cost, simplicity, practicality, size, shape, optical properties and to the functionality of their surface (antibodies, enzymes, aptamers, nucleic acids) (Basso et al., 2015; Cordeiro et al., 2016). The use of gold in immunoassay production differs from the use of other materials due to its high sensitivity and to the collective oscillation of gold atoms' conductive electrons, known as localized surface plasmon resonance (LSPR) (Cordeiro et al., 2016; Pelton et al., 2008). The LSPR is formed by two plasmon absorption bands; the first one refers to the longitudinal plasmon, which is responsible for light scattering and absorption along the long axis of the particle. This axis is related to the visible region of IR, which stays close to the electromagnetic spectrum. The other band corresponds to the transverse plasmon, which is responsible for light scattering and absorption along the short axis of the particle. Often, the gold nanoparticles in the visible region of the electromagnetic spectrum show maximum absorption at ca 520 nm in this region of the particle (Basso et al., 2015; Cordeiro et al., 2016; Pelton et al., 2008; Wang and Irudayaraj, 2008). The AuNPs solution gets an intense red color when it is dispersed. The extinction band redshifts generated a bluish/purple solution after bioconjugation, due to the formation of aggregates (Liu et al., 2013). This visual change in the color has been used in the development of several visual colorimetric biosensors applied to detect: Alzheimer disease (Cao et al., 2011), *Mycoplasma pneumonia* (Xianyu et al., 2014), and bacteriophages (Endo et al., 2005). It is also used to detect a prostate specific antigen (PSA) and HIV-associated protein (Rica and Stevens, 2012), *E. coli* and *Salmonella* (Ma and Sui, 2002), influenza virus (Li et al., 2007), and to detect analytes from nucleic acids of small molecules (Wang et al., 2009), nuclease activity (Haes et al., 2005), immunoglobulin (Tang et al., 2013) and pathogens (Wang and Irudayaraj, 2008). The noble metal solution can also be quantitatively measured in an ultraviolet-visible (UV–Vis) spectrophotometer in order to generate higher sensitivity and lower detection limits (Liu et al., 2011).

The aim of the present study is to propose a new, simpler and easier methodology for early detection of dengue virus through LSPR immunosensors. The methodology allows detecting all four dengue serotypes presenting high specificity and selectivity. The biosensor principle and format is shown in Fig. 1. Our innovative system should be a helpful tool for the detection of DENV since it solves many of the limitations of current virus detection. This immunosensor and subsequent analysis is cost effective, simple to perform, and the assay components are highly stable at temperatures above 30 °C enabling easy storage at room temperature

2. Experiments

2.1. Materials

The following materials were purchased at Sigma-Aldrich (USA): 11-mercaptoundecanoid acid, 95% (MUA); N-hydroxysuccinimide (NHS) 98% and N-(3-dimethylaminopropyl)-N′ethylcarbodiimide hydrochloride (EDC); phosphate-buffered saline (PBS); sodium citrate dehydrate 99%; gold (III) chloride trihydrate 99.99% ($HAuCl_4$); absolute ethanol 99%; bovine serum albumin solution (BSA) 98% (1 mg/mL^{-1} in 10 mM PBS, pH 7.4); and glycine–HCl (pH 3.0). The water used in all solution preparations came from a Millipore unit (USA). All working solutions were prepared with analytical grade chemicals.

The monoclonal antibody [D1-11(3)] of the dengue virus types 1, 2, 3 and 4 were purchased at GeneTex (USA). Mice were immunized with a mixture of dengue 1, 2, 3 and 4. Spleen cells were used to prepare hybridomas and clones selected based on the ability of the antibody they produced to react with each dengue serotype (datasheet provided by the manufacturer). The antibody was chosen from the ability to react with the different serotypes.

2.2. Instruments

The UV–Vis absorption spectra were collected in a Biochrom Lira S11 spectrophotometer (Biochrom Ltd, England) at 1 cm glass cell, wavelength 400–800 nm, with 1 nm step; speed 500 nm min^{-1}, and wavelength accuracy of 0.5 nm. The molecular interactions between the monoclonal antibody and the dengue virus were investigated through Surface Plasmon Resonance (SPR) (AutoLab Springle®, Eco-Chemie, Netherlands). The planar gold sensor SPR discs (17 mm diameter) were purchased from Autolab. All experiments were carried out at 22 °C. The AuNPs diameter and their dispersity in solution were analyzed through dynamic light scattering (DLS) in a DynaPro Titan apparatus (Wyatt Technology Corporation). This technique made it possible estimating the hydrodynamic radius of the particles. Samples were filtered in 0.22 μm Millex-GV filters (Millipore®).

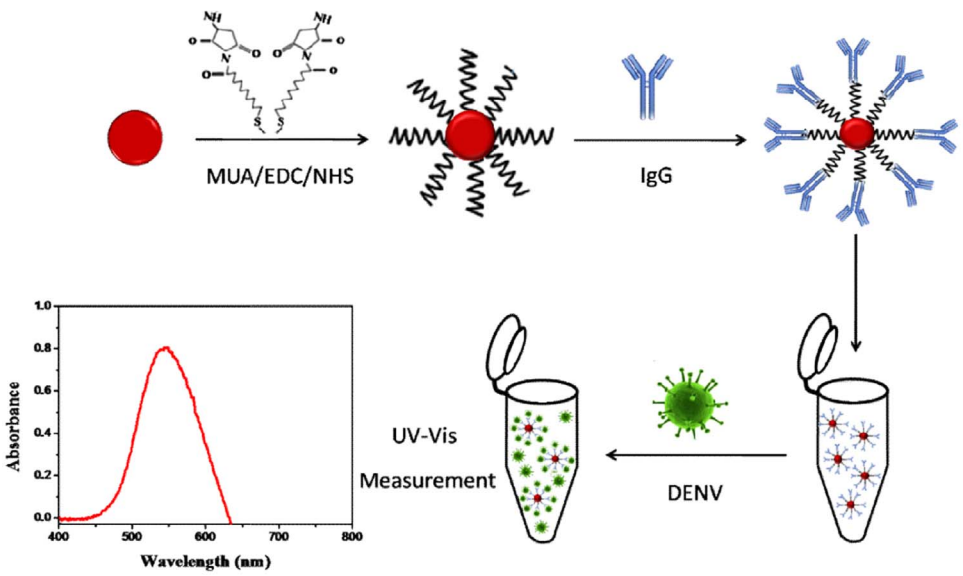

Fig. 1. Schematic representation of the detection of the dengue virus. The design shows the surface modification of the AuNP with the formation of the self-assembled monolayers conjugated to the antibodies for virus detection demonstrated by the UV–Vis spectrum.