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主　論　文　の　要　旨

論文題目　ZnO Nanoarchitecture-Based Devices for Urinary Dengue Virus NS1 Protein Detection
(尿中デングウイルス NS1 タンパク質検出のための ZnO ナノ構造デバイス)

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論　文　内　容　の　要　旨

Dengue virus is a viral infection transmitted by the bite of an infected female Aedes mosquito. It is one of the most dangerous illnesses for humans and becoming a world public health concern due to its increasing prevalence in tropical and subtropical regions. [1-5] Dengue virus is enveloped, single-stranded RNA, and formed by three structural proteins. Protein C (capsid) encloses genome which forms a nucleocapsid that is surrounded by a lipid bilayer in which are anchored protein M (membrane) and protein E (envelope). These three structural proteins derive from the N-terminal part of the polyprotein inside the genome and are followed by seven nonstructural proteins: NS1, NS1A, NS2B, NS3, NS4A, NS4B, and NS5.

Dengue infection is caused by four distinct serotype (DENV 1-4). They are antigenically distinct, and all four types may cause different forms of the disease, ranging from an asymptomatic infection and febrile illness to the more severe dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), and their related complications. Dengue NS1 glycoprotein is a 50 kDa glycoprotein which has been investigated as the important role in causing the vascular leakage symptoms in DHF/DSS. [6] Dengue NS1 is a protein that is released along with virus particles during

infection. It exhibits various functions and exists in different oligomeric forms both inside and outside the cells. Intracellularly, NS1 exists as a dimeric form associated with the cell membrane and is involved in the viral replication complex. In contrast, extracellularly, NS1 is secreted and exists in higher oligomeric forms. These distinct forms of NS1 serve different roles in the context of the infection. The soluble form of Dengue NS1 protein is initially detected in the serum during the acute phase of Dengue infection and serves as a diagnostic indicator for acute Dengue infection. Elevated levels of secreted Dengue NS1 have been correlated with greater disease severity. Moreover, the secreted Dengue NS1 has the ability to directly bind host complement components and inhibit complement activation both in solution and on the surface of cells. Hence, the investigation and advancement of Dengue NS1 detection remain a critical and challenging area of research for scientists worldwide.

Biodeteciton in non- invasive samples interested researchers due to its safety, comfort to the patients, especially in the insufficient laboratory instruments area. Besides the Dengue NS1 protein investigation in serum, there are some reports study the Dengue NS1 in other body fluid, for examples, saliva or urine. [7-11] The Dengue NS1 in urine showed the correlation to the DHF patient. The higher positive rate of Dengue NS1 in urine could be due to the plasma leakage or the high possibility of kidney infection by the viral antigen. [7] The Dengue NS1 in urine might benefit in terms of the long durability of the Dengue NS1 in urine has been found longer than the Dengue NS1 in serum. Saito, et. al. reported that they found the Dengue NS1 in urine after two weeks of symptom on set while the Dengue NS1 in serum had not been found. [10] A majority of studies focusing on the detection of Dengue NS1 protein in urine have utilized commercially available test kits that are primarily designed for serum or blood samples. However, when these assays are applied to non-invasive samples, they frequently exhibit low sensitivity due to the low concentration of the target antigen and the presence of complex contaminating substances in the matrix. As a result, it is imperative to undertake further evaluation and refinement of detection assays that are specifically designed to address the challenges associated with non-invasive sample analysis. By developing assays that are tailored to overcome these limitations, we can enhance the sensitivity and reliability of non-invasive detection methods.

Zinc oxide nanowires (ZnO) have garnered significant attention as fluorescence enhancement materials, particularly in biosensing applications, owing to their distinctive optical and electrical properties stemming from their wide direct bandgap. [12-15] Notably, ZnO nanowires have been found to boost fluorescence intensity by amplifying electromagnetic energy and generating a robust evanescent field, thereby

enhancing molecule excitation. [16-19] Additionally, gold (Au) nanoparticles possess intriguing biocompatibility features and are renowned for their fluorescence enhancement capabilities due to plasmon polarization, which generates a localized electromagnetic field that improves fluorophore excitation rates. Furthermore, Au nanoparticles serve as electron acceptors, enhancing the binding affinity and chemisorptive properties of thiolate molecules. Consequently, the combination of ZnO and Au has emerged as an ideal material for biosensing and analytical purposes, particularly in the field of medical diagnosis. [19-22]

To overcome all those circumstances, developing of the high sensitivity for the Dengue NS1 protein detection in urine in one of necessary topic for Dengue diagnosis. Here, the utilization of ZnO nanowires for detection Dengue NS1 protein in urine is focusing. Combining the plasmonic properties of ZnO nanowires and Au nanoparticles is used to play main role for enhance the fluorescence intensity in Dengue NS1 tracking. This thesis is successfully in device fabrication, characterization, and investigation in cases of the Dengue NS1 protein activities beyond the picogram and femtogram level in urine. The thesis is divided into five chapters.

Chapter 1 offers a comprehensive introduction to the significance of Dengue NS1 diagnosis and detection. It outlines the current state of detection techniques in non-invasive matrices, emphasizing the existing challenges associated with accurate detection. Additionally, the chapter highlights the main strategies aimed at achieving state-of-the-art Dengue NS1 detection in urine.

Chapter 2 demonstrates the ZnO nanowires which decorated the Au nanoparticles for fluorescence enhancement in fluorophore-linked immunosorbent essay (FLISA). The ZnO/Au nanowire FLISA platform was developed as well plate based platform for the Dengue NS1 detection in urine. This method provided a limit of detection (LOD) in the picogram level and showed high performance for Dengue NS1 detection in urine. This platform revealed the high efficiency in cases of high specificity and sensitivity combined with user-friendly method, well-plate reader.

Chapter 3 presents the success of branched ZnO nanowires with no additional seeding steps. The branched ZnO nanowires (BZnO) was fabricated via two-steps hydrothermal assisted with ammonia at specific concentration. Moreover, Zn^{2+} precursor played an important role to the branch character uniform dispersion. The BZnO was used for nanowires fluidic platform to detect the Dengue NS1 protein by using Au nanoparticles decoration on the surface. This BZnO/Au nanowire fluidic platform reveals further step for the Dengue NS1 detection with the LOD at femtogram level. This impressive LOD level was achieved through a combination of double chaotic

mixing, high surface area branched nanowires, and fluorescence enhancement from Au nanoparticles. Utilization of the BZnO/Au nanowire fluidic platform compared to the gold standard, Dengue NS1 ELISA, the fluidic device showed impressive results to detect the target in day-one onset urine. This BZnO/Au nanowire fluidic platform is one of the interesting platform for the early state detection which benefit to the medical treatment and management.

Chapter 4 demonstrates the summary of the thesis which shows finding of the development in fabrication and utilization of the materials for Dengue NS1 detection in urine.

Chapter 5 ends with future perspective on the future development of the Dengue NS1 protein detection technology.

This thesis aims to develop and utilize materials for the detection of Dengue NS1. It specifically focuses on new methods for non-invasive detection and overcoming challenges associated with early-stage detection. This research contributes to innovative approaches that benefit early diagnosis and effective management of Dengue infection.