CHAPTER I

INTRODUCTION

Human papillomaviruses (HPV) belongs to the *Papillomaviridae* that, is a non-enveloped and has icosahedral symmetry. HPV particles are 52-55 nm in diameter. The 943genome consists circular double-stranded DNA approximately 8 kb in length. The viral genome is divided into early (E1, E2, E4, E5, E6 and E7), late (L1 and L2) and noncoding (URR) regions (1-5). Early genes encode proteins that are involved with regulation of replication, transcription and transformation. The late genes, L1 and L2 encode the major and minor capsid proteins, respectively. The E1-E4 are viral regulatory proteins that involved in viral DNA replication , controlling viral transcription and viral egress. The oncogenic proteins E5, E6 and E7 proteins play important role in malignant transforming of cervical cancer and immortalized human foreskin keratinocytes. The E6 and E7 proteins can bind and inhibit function of tumor suppressor proteins p53 and pRb, respectively. The noncoding region has many binding sites for repressors and activators that regulate DNA replication.

Previous studies, HPV associated to cervical cancer because HPV DNA is found in more than 90% of cervical cancer patients (6).

The classification of human papillomavirus is based on degree of genetic relatedness. HPV type are defined as having less than 90% nucleotide sequence homology within the E6, E7 and L1 open reading frame of all other known types. More than 100 HPV types have been detected but to date, only 85 of HPV genotypes have been fully sequenced and characterized (1, 2, 5, 7). HPV also are grouped according to potential for malignant transformation. Two groups of HPV can be separated to high-risk group such as HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, and low-risk group such as HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP108. HPV type 16 and 18 are the most closely associated with cervical carcinoma that more than 50% of

squamous cell carcinoma found HPV16 DNA and more than 50% of adenocarcinoma found HPV18 DNA.

Cervical cancer is the second leading cause of cancer deaths in women worldwide. More than 470,000 cases are diagnosed each year (8). Development of cervical cancer is associated with persistent of high-risk HPV infection especially type 16 and 18 (6, 9). Now, methods for screening cervical cancer can be divided into detection of cervical cell dysplasia and detection of HPV DNA. The clinical appearance of cervical cancer is based on cytology (Pap smear) and histology screening for change in cell of transformation zone of cervix. However, this method has some limitations. False negative rates as high as 20-30% have been reported due to clumping of cells (10). HPV cannot routinely be cultured from clinical specimens and immunology assays are not established for detection of HPV infection. Therefore, molecular methods to detect HPV DNA are used to confirm the presence of HPV in clinical specimens. Molecular methods for detection of HPV DNA can be divided into amplified techniques and non-amplified techniques. Target amplified technique such as type-specific primer polymerase chain reaction (PCR) and general primer PCR. Type-specific PCR assays are based on the E6 or E7 gene of HPV subtypes. The sensitivity of these assays is 10-200 HPV copies per sample depending on HPV type (10). General primer PCR is a majority of studies using consensus primers to amplify a broad spectrum of HPV type in a PCR amplification.

Since previous studies strongly indicated that HPV is a major causative agent for cervical cancer development, early detection of HPV has been important for prevention and treatment. Moreover, the molecular methods for detecting HPV DNA required special instruments, experience technician, and very expensive. Beside HPV DNA detection, an attempt to develop a new nanotechnology assay for HPV antigen detection was done. With nanotechnology, increase sensitivity of HPV antigen detection was expected.

Nanotechnology is the study of controlling of matter on the nanometer-length scale. Nanotechnology is the creation and utilization, devices with a vast range of applications, such as in life sciences, medicine, electronics and energy production. Application in life sciences are described in detail under term of nanobiotechnology. Nanobiotechnology used in molecular diagnostics fall under the broad category of biochips/microarrays, nanoparticles labelled, nanoscale visualization, nanopore, and nanobiosensor (11, 12).

Nanoparticles have high surface areas and unique physicochemical properties that making them ideal candidates for developing biomarker platforms. There are several nanoparticles used in diagnostics. Gold nanoparticles, quantum dot, magnetic nanoparticles are the most frequently used. Goldnanoparticles based diagnostics can be broadly advantages over conventional detection methods used in clinical diagnostics. The first approach is based on color changes of goldnanoparticles upon aggregation. The second approach is that the goldnanoparticle can be tailored with a wide variety of surface functionalization to selectively bind biomarkers. And the last approach is to use electrochemical based methods that can be coupled with metal deposition for signal enhancement (11-14).

Previous studies, used gold nanoparticles (AuNPs) in microbiology detection, for example, gold nanoparticle based immunochromatographic test for detection of *Staphylococcus aureus* from clinical specimens showed sensitivity and specificity of 100% and 94.7-100%, respectively (15). A piezoelectric immunoagglutination assay for *Toxoplasma gondii* antibodies (16) which principle of the technique is that the specific agglutination of antigen coated gold change could be monitored by piezoelectric device. The results from this assay demonstrated that sensitive to dilution ratio of *Toxoplasma gondii* antibody as low as 1:5500 could be detected. In addition, HBV DNA can be detected in serum by dot blot hybridization with gold nanoparticle gene probe (17). Based on a two-probe sandwich hybridization/nanoparticle amplification/silver staining enhancement method, gold nanoparticle gene probe could detect as low as 10^{-11} mol/L composite HBV DNA molecules on a nylon membrane and the PCR product

of HBV DNA was visualized. HPV type 16 and 18 detection were performed by sequence sandwich hybridization with oligonucleotide- functionalized gold nanoparticle from cervical mucous of patients (18). Specificity of oligonucleotide probes as designed for the sequence detection within the L1 gene of HPV-16 and 18, and the probes were capped onto AuNPs. This method can observe with naked-eye by a change of color. The result of this method was comparable to TaqMan real-time PCR assay for HPV-16. The sensitivity and specificity of AuNPs probes sandwich hybridization reached 95% and 90%, respectively.

In this study, an optical detection assay of HPV type 16 antigens using gold nanoparticle based on immunoagglutination was developed. The assay used AuNPs conjugated with either HPV-16L1 or HPV-16E6 polyclonal antibodies for detecting HPV-16 L1 or E6 proteins directly from clinical specimens. And the results could be visibly detected by an agglutinate of the reaction.