The human papillomaviruses (HPV) are a diverse group of DNA virus belonging to the family of the Papillomaviridae and represents one of the most common infections of sexual transmission. Papillomaviruses cannot be cultivated on tissue culture, so the DNA diagnostics is the main and accurate method for the detection of clinical specimens. Commonly used methods in laboratories include the use of the polymerase chain reaction. Conventional methods often do not specify the types of HPV and only distinguish the presence of high-risk or low-risk type of HPV.

In recent decades, the number of techniques is based on diagnostic of human papillomavirus using nanotechnologies, such as nanoparticles, their modification, magnetic separation or antisense therapy.

In the focus of this research is the preparation of a composite material, consisting of graphene oxide and nanoparticles of zinc oxide (ZnO), silver phosphate (Ag₃PO₄) and silver. Graphene oxide was prepared by Hummers method, involving the oxidation of graphite flake in sulfuric acid with permanganate with subsequent addition of the necessary components for the formation of metal nanoparticles. Physicochemical methods are considered for their characterisation. The size of nanoparticles ranges from 50 – 200 nm. Subsequently, the antibacterial effect of composites was tested by disk method on bacterial cultures of S.aureus, E.coli, methicilin-resistant S. aureus (MRSA). Selenium nanoparticles exhibit the highest antibacterial activity from selected nanoparticles containing graphene oxide composite with an inhibitory zone with the size 5 ± 1 mm. Silver nanoparticles display also a distinguishable antibacterial effect with inhibition zones of 2 ± 1 mm. Graphene oxide which is modified by zinc oxide nanoparticles shows no inhibitory effect. The obtained results show suitability of the prepared composite materials as candidates for alternative antimicrobial materials.

**Keywords:** Diagnostic; human papillomavirus; nanotechnologies
1. Human papillomavirus

Sexually transmitted human papillomavirus (HPV) infection has been identified as a cause of cervical cancer, and it is now widely recognized as being responsible for more than 95% of cervical cancer causes [1]. Since the discovery of HPV 16 and 18 DNA in cervical cancer tissue, more than 120 different types of the human papillomavirus have been isolated; N40 (E1-derived peptide) infect the epithelial lining of the anogenital tract and other mucosal areas. Epidemiological data from the U.S. National Health and Nutrition Examination Survey determined that the prevalence of HPV infection in a representative sample of women was highest in those aged 20–24 years (44.8%). Thus, cervical HPV infection is one of the most common sexually transmitted infections (STIs) in women.

Human papillomaviruses are small circular, double-stranded DNA viruses infecting epithelial tissues. HPV types can be classified both as high-risk and low-risk. Of the more than 120 different identified types of HPV, the majority are involved in infections of the genital tract, cancer or cervix, vulva, vagina and penis, and of non-anogenital localizations, such as the head and neck areas. From the point of view of the infection, human papillomaviruses have developed several molecular mechanisms to suppress apoptosis in infected cells [2].

The transmission of HPV infection occurs most often through the sexual contact. The newborn child infectin might be experienced through the contact with infected cells lining birth canal [3].

Viral particles penetrate by tiny cracks in mucosa or skin and infect the basal epithelial cells. Infected cells have characteristic cytological features. These include pycnosis of nuclei hyperchromasia and especially perinuclear vacuolation called coilocytosis. Such findings are typical indicators of viral infection [4].

The discovery of human papillomavirus DNA in cervical cancer by Harald zur Hausen sparked 30 years of research that established that persistent cervical infection by certain HPV genotypes causes cervical cancer. This research has led to revolutionary technical advances for the prevention of cervical cancer: prophylactic HPV vaccination and sensitive molecular HPV testing for screening. These promising technologies can be used to complement or enhance established cervical cancer prevention programs, and to provide robust solutions in low-resource settings without screening programs [5].

The available studies show that the prevalence of asymptomatic genital human papillomavirus infections in the population is very high. Bauer and his coworkers investigated university students by the method PCR (polymerase chain reaction) and at 46% have demonstrated HPV infection. A study of 1992 estimated incidence of HPV infections in the US for more than 30 million cases [6].

Human papillomavirus (HPV) is linked to anal cancer through high HPV DNA-detection rates. Here, in one of the largest international studies to date, HPV DNA was detected in more than 88% of anal cancers and more than 95% of anal intraepithelial neoplasias grades 2/3 [7].

2. General diagnostic of human papillomavirus

Papillomaviruses cannot be cultivated on tissue culture, so the DNA diagnostics is the main and accurate method for the detection of clinical specimens.

Commonly used methods in laboratories include the use of the polymerase chain reaction. Conventional methods often do not specify the types of HPV and only distinguish the presence of high-risk or low-risk type of HPV.

Diagnosis of HPV infection can be carried out more often visually or by different types of tests: i) vinegar (acetic acid) solution test; ii) Pap test or iii) DNA test.

Using of vinegar solution test turns HPV-infected genitals to white color. This may help to identifying of difficult-to-see flat lesions.

Pap tests can reveal abnormalities that may lead to cancer. These tests are performed from collection of cells from cervix or vagina. The principle of the Pap test is to wipe the cells from the outer opening of the cervix of the uterus and the endocervix. Swab is then microscopically examined to look for abnormalities in the sample [8]. The Pap test complements tissue biopsy;
the tests do not compete. Each has some advantages and limitations. A Pap test is inexpensive, rapid, and very simple to obtain and process. It produces no injury to tissues. This allows frequent repetition of cellular sampling, which is especially important in the evaluation of the progressive or posttreatment regression of a disease. It contains samples of cells originating from a wider surface area than that obtained by a biopsy [9].

DNA test recognize the DNA of the high-varieties of HPV that have been linked to genital cancers. The test is conducted on a sample from cells taken from cervix. It is recommended for women older than 30 in addition to the Pap test.

Clinical forms of HPV infection are therefore defined as lesion visible to the naked eye. Subclinical form of infection can be detected by colposcopy, cytology and histology. Latent form of HPV infection causes no morphological changes in the squamous epithelium and for its detection are necessary to use methods of molecular biology. Cytology uses so-called Panicalaou’s classification (PAP IV) and smears from the cervix.

Histology classifies the biopsy as a cervical intraepithelial neoplasia (CIN) grade I to III and invasive carcinoma (INCA). Newer classification system from 1988 value the finding of lesions as a low-grade (low squamous intraepithelial traditional treatment lesions (LSIL)), high-grade (high squamous intraepithelial lesions (HSIL)), invasive carcinoma and atypical cells (atypical squamous cells of unknown significance (ASCUS)/atypical glandular cells of unknown significance (AGUS)) [10]. Morphological methods, especially cytology are important screening methods. However, many studies points out some shortcomings of cytology. Above all, it has a poor reproducibility, false negativity (15 – 50%) and false positivity (10%) [11, 12]. The success of these screening programs depends on their good organization and capture in the widest possible population [13, 14].

The molecular-biological methods can help detect also the latent form of infection. Among them are two methods, whose sensitivity and reproducibility is sufficient that their use can be envisaged in routine diagnostic. One is polymerase chain reaction (PCR), when HPV DNA is amplified and thus high sensitivity is achieved. The second method is test based on a direct hybridization test with complementary DNA probes with chemiluminescent signal amplification (HC = hybrid capture), which is now commercially available (distributed by Abbott). Using PCR method was HPV DNA detected in up to 100% of biopsies INCA and CIN III. This method is fast and relatively simple. It must be strictly comply to some principle in order to
avoid possible contamination. Due to robustness of PCR method various materials can be investigated. The method of direct detection by hybridization is somewhat less sensitive than the PCR method. New improved HC system on microplates (HCM) brought compared to the original system carried out in test tubes (HCT) the improvement of sensitivity (HCT = 74 – 94%, HCM = 95%). Preliminary studies indicate that a combination of methods HC with PAP smears can achieve sensitivity up to 91 – 100% [15].

Use of HR HPV detection in gynecology is currently very serious issue around the world. Current data suggest that the introduction of HR HPV detection as a secondary diagnostic test for women with the cytology finding ASCUS/AGUS in women younger than 35 years would allow up to 100% capture of women with HSIL lesions. The introduction of testing on HR HPV together with cytology is also considered for a primary screening for women older than 35 years. In the long term so conceived screening program should bring in particular the reduction of incidence and mortality for cervical cancer, while this program should be economically recoverable.

3. Diagnostic of human papillomavirus using micro- and nanotechnologies

In recent decades, the number of techniques for detection of DNA, RNA or proteins, has been developed. For these purposes are used so-called biochips or microarrays. This technique became widely used for diagnosis of diseases, in the discovery of novel genes and drugs [16]. DNA microarray is a powerful tool for the parallel determination of nucleic acids and other biologically significant molecules [17]. DNA microarray technology can be efficiently used for the simultaneous detection and identification of many biological contaminants and health risky agents [18]. This technique was commonly used for diagnosis of HPV infection in patients using the Greiner Bio-OnePapilloCheck IDNA chip assay [19].

Among the most common used nanomaterials for the diagnostic of human papillomavirus belong nanoparticles. In order to overcome the shortcomings of the fluorescence-based detection technique, many researcher adopt nanoparticles to label biomolecules instead of fluorescence dyes [20-23]. Nanoparticles can be modified in various ways. For example, in the study of Piao et. al. [24] were used fluorescent nanomaterials, such as quantum dots, fluorophoredoped silica nanoparticles and metal nanoparticles for the diagnosis of human papillomavirus DNA.

To speed up the diagnostic technique can be used the method ultra-fast PCR based primers nanoparticles conjugates. It runs one thermal cycle in 8.5 s or one complete 40-cycle PCR protocol in 5 min and 40 s [25]. The polymerase chain reaction (PCR) technique is very important technique in the modern life science. Accurate and especially rapid diagnosis of the causative agents is built into the foreground of detection methods in terms of early treatment of patients [26]. PCR is often used for its speed, sensitivity and specificity in the detection of pathogens [27]. Currently, the technique of polymerase chain reaction with respect to the speed of determination dramatically reduced from three hours to few minutes. This area is requires improvement and further development for certain applications [28]. This technique was also used in many studies for diagnosis of viral particles [29, 30].

Other nanotechnology ways for papillomavirus diagnosis are magnetic microparticles. Magnetic microparticles have attracted vast attention because they have good biocompatibility and can be readily separated from reaction mixtures with the aid of an external magnetic field [31]. Magnetic microparticles have been extensively used for DNA hybridization, cell separation, immunoassay, protein and enzyme immobilization, and drug delivery [32], which can be further employed for fast and accurate HPV diagnostic in complex biological matrices. Using of magnetic microparticles for detection of presence of nucleic acids/protein from viruses was also patented [33].

For the diagnosis based on nanotechnologies can be also used the basic enzyme-linked immune sorbent assay (ELISA), which can be modified
by conjugated antibodies with quantum dots. Utilization of magnetic particles and antibodies can be also used together for diagnosis of HPV [34]. Recently, attention has been focused on the development of biolabels in the field of nanomaterials. For this purpose have been studied previously mentioned quantum dots [35]. Between the greatest advantages of quantum dots in nanotechnologies undoubtedly belong their unique optical properties, long-term imaging and “multiplexing” (simultaneous detection of multiple signals) [36]. Quantum dots with regard to nanotechnologies and microarrays have three disadvantages: fluctuations of photoluminescence and thereby reducing of quantum efficiency; toxicity of quantum dots containing heavy metals; their excitation maximum in the UV region [17].

Other way is antisense therapy. Antisense oligodeoxynucleotides (AS-ODNs) are designed to specifically downregulate target gene expression utilizing Watson-Crick pairing rules [37]. Antisense moieties have been widely used for gene silencing and therapeutic purposes. Márquez-Gutiérrez for example developed two AS-ODNs (419 and 434) directed to region that resulted in the destruction of HPV-16 E6/E7 mRNA in vitro and in vivo, inhibition of tumor cell proliferation, anchorage-independent growth, and tumor growth in an animal model [38].

4. Conclusions

There exist a number of analytical or molecular biological methods that can be used for the diagnosis of human papillomavirus. Given the need to accelerate the process of identifying the causative agent several available methods are described. They range from classical such as PCR and DNA/RNA hybridization to new identification methods based on nanotechnologies. Methods based on nanotechnologies discussed in this review represent a choice selection of available techniques for diagnostic applications related to human papillomaviruses.

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