ISSN: 0067-2904

Titanium Dioxide Nanoparticles: A Novel Approach for Inhibiting Human Papillomavirus

Sharafaldin Al-Musawi[1]*, Abbas. K. Almansorri[2], Hider. M. H. Al-Shirifi[3], Bara B Ahmed[2], Adawiya J. Haider[4]*

[1]College of Food Sciences, Al-Qasim Green University, Babylon, Iraq
[2]Faculty of Biotechnology, Al-Qasim Green University, Babylon, Iraq
[3]Dept. of Environmental Health, College of Environmental Sciences, Al-Qasim Green University, Babylon, Iraq
[4]Laser Science and Technology Branch, Department of Applied Sciences, University of Technology, Baghdad, Iraq

Received: 22/11/2022 Accepted: 13/11/2023 Published: 30/3/2024

Abstract

Nanotechnology products such as titanium dioxide nanoparticles (TiO_2-NPs) can be used for viral infections because of their unique characteristics. The current study aimed to determine the impact of TiO_2-NPs on HPV type 1 and 2 infections. The characterization of these NPs was performed using dynamic light scattering (DLS), field emission scanning electron microscopy (FESEM), high-resolution transmission electron microscopy (HRTEM), and X-ray diffraction (XRD). The MTT assay was used to determine the toxic impacts of TiO_2-NPs on BHK-21 cells. The efficiency of TiO_2-NPs was performed using several parameters, including TCID50 and RT-PCR assays. An indirect immunofluorescence assay (IFA) was performed to estimate the inhibitory impact of TiO_2-NPs on viral antigen expression, and Acyclovir was used as a reference medicine. When the human papilloma type 1 and 2 viruses exposed to TiO_2-NPs at high doses (100 µg/mL) produced 0.3, 1.1, 2.3, and 3.3 log10 TCID50 decreases in infective virus load when compared with control viruses ($P<0.0001$), these TiO_2-NPs doses were related to 24.9%, 35.1%, 47.2%, 59.5%, and 66.6% inhibition percentages that were determined depending on the viral titer as compared to virus control. It is concluded that TiO_2-NPs have strong potential for the treatment of face and labial lesions caused by papillomaviruses 1 and 2 and could be used in topical formulations.

Keywords: Papillomavirus, Titanium oxide, nanoparticles, Antiviral Activity, drug-resistant

جزيئات ثاني أكسيد التيتانيوم النانوية: نهج جديد لتثبيط فيروس الورم الحليمي البشري

شرف الدين الموسوي[1]*, عباس المنصوري[2], حيدر الشريفي[3], براء أحمد[2], عدوية ج حيدر[4]

[1]كلية علوم الأغذية, جامعة القاسم الخضراء, بابل, العراق

[2]كلية التقانات الاحيائية, جامعة القاسم الخضراء, بابل, العراق

[3] كلية علوم البيئة, جامعة القاسم الخضراء, بابل, العراق

[4] قسم العلوم التطبيقية, فرع علوم و تكنولوجيا الليزر, الجامعة التكنولوجية, بغداد, العراق

*Email: dr.sharaf@biotech.uoqasim.edu.iq

الخلاصة

يمكن استعمال منتجات تقنية النانو مثل الجسيمات النانوية لثاني أكسيد التيتانيوم للعدوى الفيروسية بخصائصها الفريدة. تهدف الدراسة الحالية إلى تحديد تأثير أكسيد التيتانيوم على عدوى فيروس الورم الحليمي البشري من النوع 1 و 2. تم إجراء توصيف الجسيمات النانوية باستعمال تشتت الضوء الديناميك، المجهر الإلكتروني لمسح الانبعاث الميداني، المجهر الإلكتروني الانتقالي عالي الدقة وانحراف الأشعة السينية. تم استعمال اختبار MTT لتحديد التأثيرات السامة للجسيمات النانوية على خلايا BHK−21. تم إجراء كفاءة أكسيد التيتانيوم باستعمال العديد من المعلمات، بما في ذلك فحوصات TCID50 و RT−PCR. تم إجراء مقايسة الكفاءة المناعية غير المباشرة لتحفيز التأثير المثبط لأكسيد التيتانيوم على تعبير المستضد الفيروسي، واستعمال الأسيكلوفير كدواء مرجعي. ينتج عن فيروسات الورم الحليمي البشري من النوع 1 و 2 التعرض لأكسيد التيتانيوم جرعة عالية (100 ميكروغرام / مل) 0.3 ، 1.1 ، 2.3 ، 3.3 لوغاريتم 10 TCID50 ينخفض في الفيروسات المعدية عند المقارنة مع فيروسات التحكم ((P <0.0001، ارتبطت جرعات TiO2−NPs بنسبة 24.9٪ ، 35.1٪ ، 47.2٪ ، 59.5٪ ، و 66.6٪ نسبة تثبيط، والتي تم تحديدها اعتمادًا على العيار الفيروسي مقارنةً بالسيطرة على الفيروسات. يمكن استعمال علاج آفات الوجه والشفرين الناتجة عن فيروس الورم الحليمي 1 و 2 في التركيبات الموضعية.

1. Introduction

Human papillomavirus (HPV) is considered one of the most sexually transmitted diseases in the world. It is a small, epitheliotropic, non-enveloped dsDNA virus that infects epithelial cells in a wide variety of higher vertebrates and induces cellular proliferation [1, 2]. HPVs have been found in more than 199 different types, with approximately half of them infecting the genital tract [3]. Some types of HPV have been linked to cervical cancer, while others have only been linked to a small number of malignancies in a large number of cases. This has led to the designation of high-risk and low-risk HPVs. In addition, these types of skin cancers are present in both normal skin and non-melanoma skin cancers [4]. HPV does not spread via toilet seats, although certain warts do so through the floor [5]. HPV (1 and 2) are responsible for developing common warts in certain infected persons, as shown in our work [6]. Cultivated warts on the skin are frequent in youngsters and are caused by HPV (1 and 2), although they disappear on their own after a few weeks to many months [6]. A regular occurrence is the reoccurrence of a wart infection. During the last decade, nanoscience has been widely employed worldwide in various applications [7]. NPs were created to treat infectious disorders and have special physical characteristics [8–10]. The main causes of these are the NP scale, which affects bioavailability and blood circulation time, and the wide-ranging surface-to-volume proportion. Such characteristics make the NPs potentially ideal for research and improving therapeutic effects [11–15]. It has been observed that the efficiency of traditional medicines is rapidly eroding, particularly in the case of viral infections, owing to the development of resistance, which might be related to a quicker adaptation in peripheral protein sequence that results in a new-fangled viral strain [16–20]. Because they have shown better and unique characteristics than their bulk material counterparts, titanium oxide nanoparticles (TiO_2-NPs) have attracted considerable attention. These nanoparticles exhibit quantum size effects, which are characterized by the fact that materials' chemical and physical characteristics are highly reliant on the size of the particles [21–24]. When exposed to nonlethal ultraviolet light less than 385 nm, TiO_2 NPs decompose organic, photocatalytic substances by generating and constant liberation of hydroxyl radicals and superoxide ions. TiO_2 NPs' antimicrobial effect is related to their crystal structure, size, and shape. The mechanism postulated for TiO_2 NPs is oxidative stress generated by ROS. As a consequence, ROS induce site-specific DNA damage [25]. Madhubala et al. exhibited that the cell viability of some human cells treated with TiO2 NPs was significantly reduced at higher doses, as observed by the MTT assay. So the low

concentration of these NPs didn't have any toxic effects on the cells [26]. The use of nanometer-sized TiO_2 particles has been shown to increase the antimicrobial activity of TiO_2 [27]. The study's goal was to create methodologies for estimating the antiviral effects of TiO_2-NPs against HPV. In this study, for the investigation of the cytotoxic effects of TiO_2-NPs on BHK-21 cells, the MTT test was utilized. The efficiency of TiO_2-NPs was measured using a variety of criteria, including the TCID50 and RT-PCR, among others. To assess the preventing effect of TiO_2-NPs on viral antigen expression, IFA was carried out with Acyclovir serving as a reference medication.

2. EXPERIMENTAL WORK

2.1 Materials

TiO2-NPs was given by Merck, Germany. In order to create varying amounts of concentration, suspensions of the NPs in Dulbecco's modified Eagle (Shangdong, China) were prepared and oltra-sonicated to reduce the aggregation. Acyclovir was given by Sigma (USA), added to DMEM, and used as a reference drug against HPV at various levels in this study.

2.2. Characterization of TiO_2 NPs

DLS (Malvern Instruments Ltd., Malvern, UK), FESEM (Hitachi S-4160, Japan), HRTEM (Carl Zeiss AG-Zeiss EM900, Germany), and XRD (SIEMENS-D5000) analysis were utilized to evaluate the NP scale morphology and structure.

2.3. Culture of virus and cell:

BHK-21 cells were provided by the ATCC. BHK-21 cells were grown in DMEM supplemented with 10% fetal bovine serum from Gibco, USA. 1 mM sodium pyruvate, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 g/mL streptomycin (Sigma, USA). The cells were cultivated at 37 °C in an incubator with 5% CO2 humidity. It was decided to use an existing stock of the papillomavirus from Tehran University's Virology Department in Tehran, Iran. The viruses were grown in BHK-21 cells and leveled using the tissue culture infectious dose 50% (TCID50) technique [28], then stored in vials at 70 °C.

2.4. Cytotoxicity assay:

Based on our previous study [16], the MTT assay was used to find out how TiO2-NPs affected the health of BHK-21 cells. A total of 100.000 cells/mL of BHK-21 cells were seeded on a flat-bottomed microtiter plate 96-well (Nalge Nunc (Naperville, IL)) and incubated at 37°C for one day. Each of the three plates was treated with different levels of TiO2-NPs (20–140 g/mL). Afterwards, incubation of the plate for three hours at 37°C in an atmosphere without light after two days of incubation at 37°C with 10 L of MTT reagent (5 mg/mL) (Roche, Germany) added to each well It was next necessary to remove the MTT solution. Then, 50 L of plain dimethyl sulfoxide (Sigma, USA) was added to each well of the plate and agitated for 10 minutes at (25%). Finally, reading of the plate was done by the microplate reader at 550 nm (Synergy 4, USA), and the rate of cell survival for the used levels was computed concerning the normal cells.

2.5. Antiviral activity:

BHK-21 cells in confluent monolayers on a 96-well microtiter plate were treated with 100 TCID50/mL papillomavirus for 60 minutes at 37 °C in a humidified atmosphere containing 5% CO2. Afterwards, the viral inocula were removed, and the monolayers were washed several times with PBS to eliminate any remaining viruses. Afterwards, the infected cells were treated with 100 L of various noncytotoxic doses of TiO2-NPs, then incubated on the plate for two days at 37 °C with 5% CO2. Additionally, cell control and viral control were studied. Our

experiment was carried out for Acyclovir. After that, the incubation time is specified, treating the cells with a single freezing-thawing cycle to release the virus particles connected with the cells. Finally, the lysates were collected from the wells and used for the TCID50 and quantitative RT-PCR tests, as previously described [11].

2.6. RT-PCR:

DNA extraction of papillomavirus is carried out by the gDNA Extraction Mini Kit (Qiagen, UK) by using a pair of primers (5′-GAGAACTGCAATGTTTCAGGACC3-3′ and 5′-TGTATAGTTGTTTGCAGCTCTGTGC3-3′) on a 65-bp fragment of the L1 region by quantitative RT-PCR. The temperature conditions are adapted as [29–30]. RT-PCR was done by Rotor-Gene Q (Qiagen, UK). The plasmid includes 65-bp DNA, while the L1 gene is represented as a template. Amplification of the segment was then cloned into the pGH vector by Generay Biotech (a Shanghai company, China). The stock solution was used as a standard solution, which is made from a template (4) µg with dilution buffer (40) µL. The template DNA concentration was evaluated by NanoDrop (Fisher, USA), and the total number of DNA copies was calculated by the website (http://cels.uri.edu/gsc/cndna.html). The serial dilution of the stock solution was tenfold so as to form the curves. Papillomavirus copies were determined as standard references.

2.7. Indirect immunofluorescence assay:

Sterile glass (Sigma-Aldrich, USA) was used to put into the wells of a 24-well growth plate to cultivate BHK-21 cells (Qiagen, UK). For 60 minutes at 37 ºC, the cells were treated with 200 L of a papillomavirus solution containing 100 TCID50/mL papillomavirus solution. It was then replaced with ZnO-NPs at the greatest noncytotoxic concentration feasible, and the plate was incubated at 37 °C for a day. Controls were employed in this experiment as cell controls and virus controls. The cells were treated with papillomavirus antibody after 14 hours of incubation, followed by 15 minutes of acetone fixation and 45 min at ambient temperature, before the cells were rinsed triplicate in PBS and incubated for forty min at 25 °C with goat anti-human IgG conjugated with (FITC) from Sigma-Aldrich (USA). The cells were then washed in triplicate in PBS and treated for another 40 minutes at 25 °C (Tokyo, Japan) [21, 31–33].

2.8. Statistical analysis:

The mean of three different experiments is represented by the data, while the error bars represent the SD. GraphPad Prism was used to conduct a one-way analysis of variance and Tukey's multiple test for forming significant differences at p-values less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Characterization

The structural and morphological evaluation of TiO2-NPs performed by FE-SEM and TEM revealed different particle shapes and sizes, with a spherical shape with a mean size of 50 nm (Figure 1).

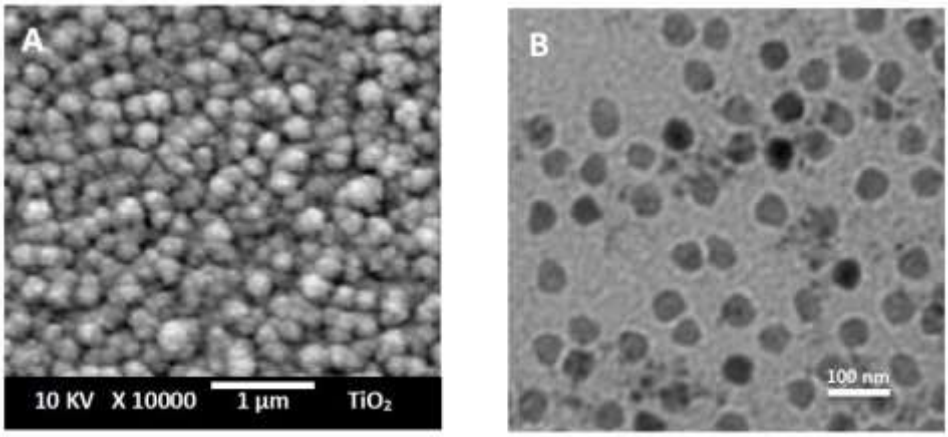

Figure 1: FESEM image of TiO_2-NPs (10 kx) (A); TEM image of TiO_2-NPs (B). The morphological and structural examination of TiO_2-NPs showed particle shape and size, with a semi-spherical shape with a mean size of 50 nm

3.2. MTT results:

The findings demonstrate that when the concentration of TiO_2-NPs was increased to 120 g/mL, the cell viability decreased to 60.03% compared to control cells (Figure 2). The MTT test was used to determine the cytotoxic impact of TiO_2 nanoparticles on BHK-21 cells that had been infected with the papillomavirus. (See Figure 2 for an example.) That demonstrates the vitality of BHK-21 cells when exposed to concentrations of 20, 40, 60, 80, 100, and 120 g/mL of TiO_2-NPs (P = 0.0001), respectively. As a consequence of increasing the TiO_2-NPs concentration to 120 g/mL, the viability of the cells was reduced to 48.32% compared to the control cells. Vertical lines in a graph show the median values of three separate trials. As previously reported, antiviral experiments were performed at TiO_2 nanoparticle concentrations with less than 10% cytotoxic impact [37–40].

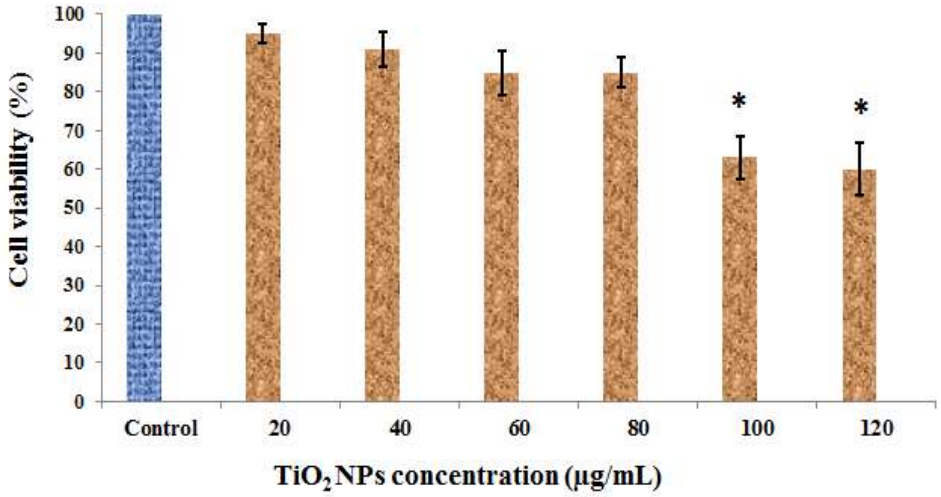

Figure 2: toxic effect of TiO_2-NPs on BHK-21 cell by MTT techniques showed an increase in TiO_2-NPs concentration to 120 µg/mL, while the cell viability decreased to 60.03%. The vertical lines are the mean values of the three experiments

3.3. Evaluation of antiviral activity

When compared to acyclovir, both the viability of BHK-21 cells and the TCID50 test used to measure how well TiO_2-NPs killed the infectious papilloma titer were higher. Papilloma

virus-infected BHK-21 cells were exposed to TiO_2 nanoparticles at levels of 20, 40, 80, and 100 g/mL. Compared to the viral control, the infectious titer of Papillomavirus dropped by 0.3, 1.1, 2.3, and 3.3 log10 TCID50, respectively (Figure 3)

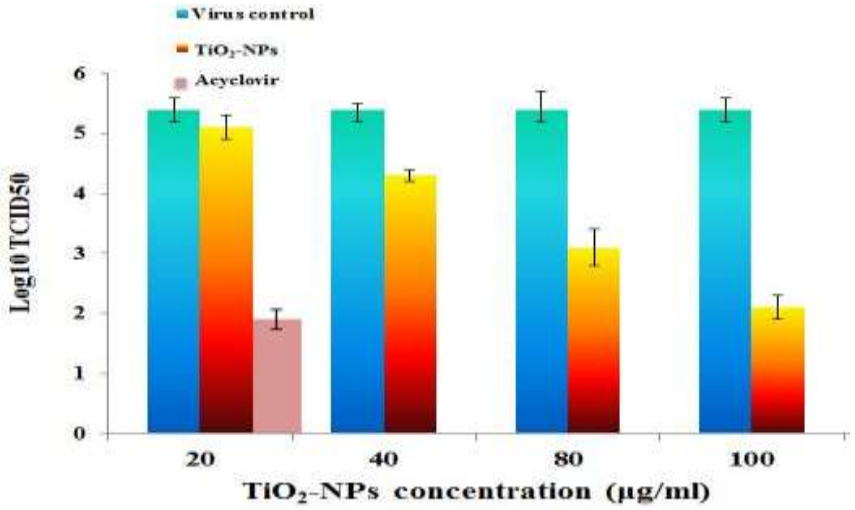

Figure 3: Antiviral activity of TiO_2-NPs on Papilloma virus by TCID50 technique as compared with Acyclovir. The exposed Papillomavirus of the infected cells with 20, 40, 80, and 100 µg/mL TiO_2-NPs produced 0.3, 1.1, 2.3, and 3.3 log10 TCID50 decreases in the Papillomavirus as compared to virus control

3.4. Cytopathic effects:

Pathological effects on BHK-21 cells caused by Papillomavirus infection were observed in this work, indicating that infected Papillomavirus BHK-21 cells with 100 g/ml of TiO_2-NPs exhibited the presence of TiO_2-NPs in the pathologically affected cells. The creation of syncytia and cell rounding are three separate cytopathic effects that occur in the organism's body. Infected Vero cells with Molluscum contagiosum were treated with TiO_2-NPs, and the cytotoxic effects of the infection were minimized. Notably, the TiO_2-NP at the highest level tested (100 g/ml) was linked with a cytotoxic impact on the BHK-21 cells in the range of 10% to 15% cytotoxicity [41–43]. As a result, the morphology of the cells has been changed in specific locations compared to the control cells, which is not connected to the papilloma virus-induced cytopathic effects (Figure 4).

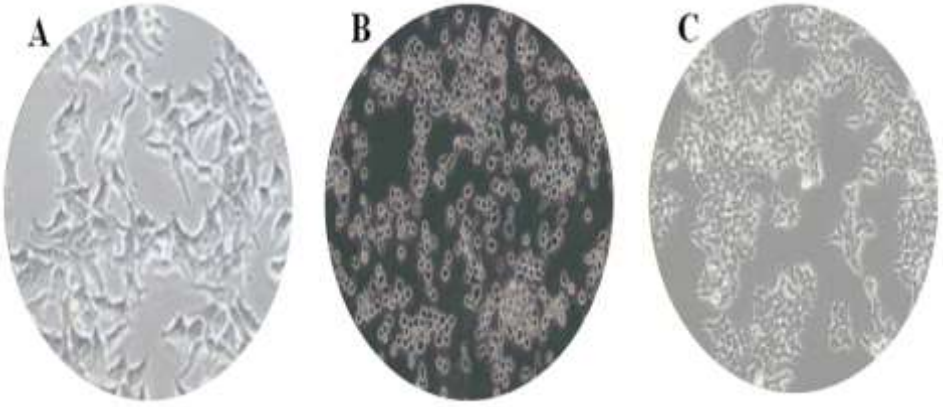

Figure 4: TiO_2-NPs were used to inhibit the papilloma virus-induced cytopathic effect on BHK-21 cells in vitro. TiO_2-NPs were used to treat papilloma virus-infected cells. (A) cell control; (B) viral control; and (C) papilloma virus-infected cells with 100 g/ml of TiO_2-NPs

3.5. Real Time PCR:

RT-PCR was used to analyze the influence of TiO_2-NPs on the papilloma viral load. A 127-bp fragment of the US3 gene of the papillomavirus was amplified using SYBR Green Quantitative PCR Master Mix to determine the effect of TiO_2-NPs on the papilloma viral load. This is seen in Figure 5, which depicts the effect of TiO_2-NPs on papilloma viral load as assessed by real-time PCR. When applied to Papillomavirus viral load, TiO_2-NPs at the levels of 20, 40, 60, 80, and 100 g/mL resulted in 24.9%, 35.1%, 47.2%, 59.5%, and 66.6% inhibition rates, respectively, which were assessed based on the viral load.

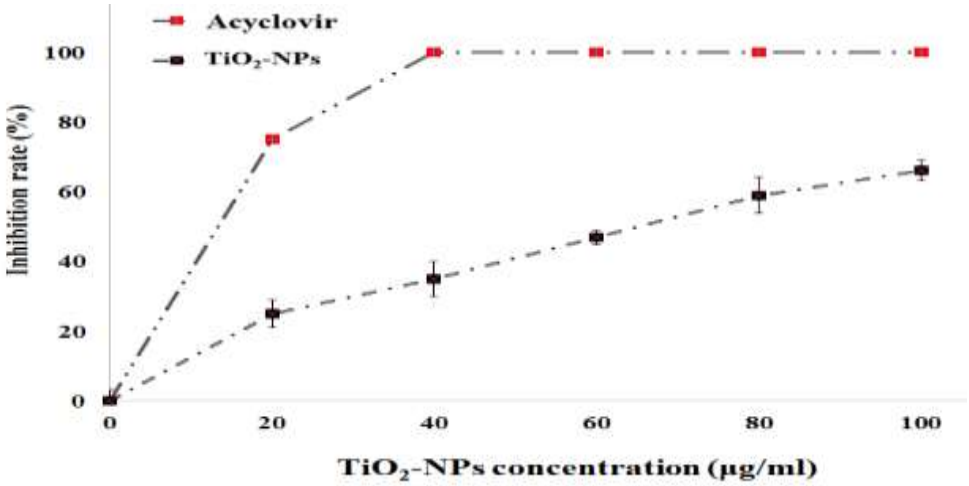

Figure 5: shows the effects of TiO_2-NPs against the papilloma virus using RT-PCR. TiO_2-NPs at the 20, 40, 60, 80, and 100 µg/mL concentrations led to 24.9%, 35.1%, 47.2%, 59.5%, and 66.6% inhibition percentages, which were estimated to depend on the papilloma viral load

3.6. IFA assay:

It was decided to use an IFA test to evaluate the inhibitory effects of TiO_2-NPs on the antigens expressed by the papillomavirus on the surface of the BHK-21 cells. By employing the immunofluorescence test, we were able to determine the influence of TiO_2-NPs on the expression of Molluscum contagiosum antigens on BHK-21 cells in this experiment (IFA). According to the results of virus control, cell control, and papilloma-infected cells with 100 g/mL TiO_2-NPs experiments, the level of fluorescence signals in Papilloma virus-infected cells with TiO_2-NPs was lower than the level of fluorescence signals in the virus-control cells, showing that TiO_2-NPs had a substantial antiviral effect on the expression of Papillomavirus antigens. Green dots in Figure 6C represent viral antigens expressed in distinct cell compartments stained with anti-IgG coupled with fluorescein isothiocyanate to visualize viral antigen expression (FITC) [44–51].

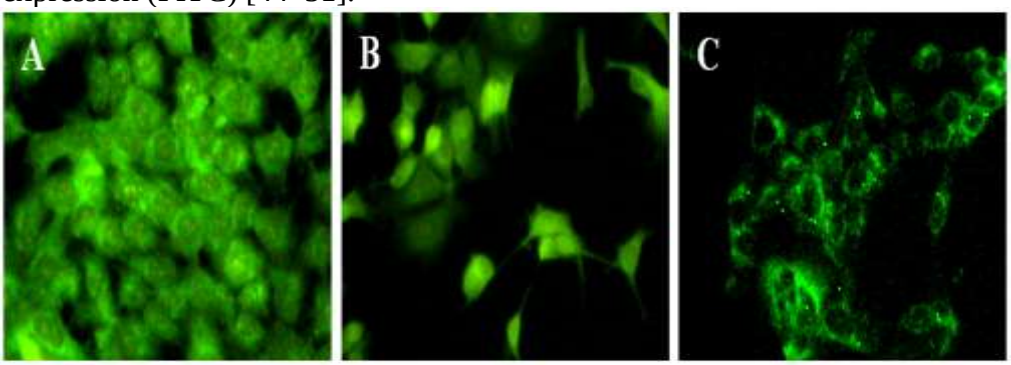

Figure 6: Immunofluorescence assay (A): treated infected cells with HPV; (B) and (C): intensity of fluorescence signals in papilloma virus-infected cells with TiO_2-NPs

4. Discussion:

Nanoparticles have been extensively investigated for their potential uses in various sectors, including medication delivery systems and antibacterial agents [50]. Furthermore, nanoparticles have been shown to have broad-spectrum antiviral activity, suggesting that they may have a multi-targeting mode of action [51–53]. It has been reported that the antibacterial activity of TiO_2 nanoparticles against multi-antibiotic-resistant strains of *E. coli*, *P. aeruginosa*, *S. aureus*, and *P. putida*, as well as exposed spores of Bacillus to UV and fluorescent rays, increased with an increase in the TiO_2-NPs level. The effects of UV were more successful due to the appropriate bandgap energy of UV light and the greater hydroxyl radical level on the coated film surface. TiO_2 showed photocatalytic effects. Therefore, it is used in several applications, such as waste water and air purification, as a self-cleaning or antimicrobial agent, or for self-disinfecting substances [54]. Because of their considerable side effects and the rising occurrence of medicine-resistant strains throughout therapy [55], the present antiviral medications against HPV are weakening, and the production of new anti-HPV agents is needed. Comparing nanoparticles for therapeutics to traditional treatments, there are various benefits, including efficiency at lower doses, powerful antiviral action against medicine-resistant viruses, cheap cost of manufacturing, and appropriateness for various coating types [56, 57]. According to the findings of this research, TiO_2 nanoparticles effectively inhibit the activity of papillomavirus types 1 and 2. The antiviral activity of TiO_2 nanoparticles was investigated using HPV types 1 and 2. During the inhibition tests, BHK-21 cells and/or virus solutions were treated with TiO_2 nanoparticles at various periods to determine the various phases of viral infection that may be prevented. The results demonstrated that TiO_2 NPs decrease HPV infections by blocking the attachment, preventing the virus entrance inside the cells, and preventing the virus from spreading among the cells.

5. Conclusions:

In summary, according to our findings, we show for the first time that TiO2-NPs are associated with significant antiviral potency against HPV. TiO2-NPs appear to possess wide anti-HPV efficacy, presenting new pharmaceutical possibilities. The antiviral behavior of TiO2-NPs is considered to be described by a number of experimental processes, including the TCID50 test, antiviral efficacy, inhibiting the Papilloma virus-induced cytopathic effect, gene expression inhibition percentage, and antigen expression. It is concluded that TiO2-NPs have strong potential for the treatment of face and labial lesions caused by papillomaviruses 1 and 2 and could be used in topical formulations.

Acknowledgments: The authors appreciate the technical assistance from the University of Technology.

References

[1] Forman, David et al., "Global burden of human papillomavirus and related diseases," *Vaccine*, vol. 30, no. 5, pp. 12-23, 2012. doi:10.1016/j.vaccine.2012.07.055

[2] A. M. Mohammed, I. K. Al-Khateeb, A. J. Haider, R. A. Rahim, and U. Hashim, "Preparation of DNA biosensor application from fuel oil waste by functionalization and characterization of MWCNT," *Sens. bio-sensing Res.*, vol. 16, pp. 1–5, 2017.

[3] A. Schneider, M. Hotz, and L. Gissmann, "Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women," *Int. J. Cancer*, vol. 40, no. 2, pp. 198–201, 1987.

[4] N. Li, S. Franceschi, R. Howell-Jones, P. J. F. Snijders, and G. M. Clifford, "Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication," *Int. J. cancer*, vol. 128, no. 4, pp. 927–935, 2011.

[5] M. L. Gillison *et al.*, "Distinct risk factor profiles for human papillomavirus type 16–positive and human papillomavirus type 16–negative head and neck cancers," *J. Natl. Cancer Inst.*, vol. 100,

no. 6, pp. 407–420, 2008.

[6] H. M. Ockenfels, "Therapeutic management of cutaneous and genital warts," *JDDG J. der Dtsch. Dermatologischen Gesellschaft*, vol. 14, no. 9, pp. 892–899, 2016.

[7] S. A. Mahdi *et al.*, "Gene expression and apoptosis response in hepatocellular carcinoma cells induced by biocompatible polymer/magnetic nanoparticles containing 5-fluorouracil," *Electron. J. Biotechnol.*, vol. 52, pp. 21–29, 2021.

[8] L. A. Yaaqoob, R. W. Younis, Z. K. Kamona, M. F. Altaee, and R. . M. Abed, "Biosynthesis of Nio Nanoparticles Using Prodigiosin Pigment and its Evaluate of Antibacterial Activity Against Biofilm Producing MDR- Pseudomonas Aeruginosa," *Iraqi Journal of Science*, vol. 64, no. 3, pp. 1171–1179, Feb. 2023.

[9] J. A. S. Salman, A. A. Kadhim, and A. J. Haider, "Biosynthesis, characterization and antibacterial effect of ZnO nanoparticles synthesized by Lactobacillus Spp," *J Glob. Pharma Technol*, vol. 10, no. 03, pp. 348–355, 2018.

[10] S. Al-Musawi, A. J. Hadi, S. J. Hadi, and N. K. K. Hindi, "Preparation and characterization of folated chitosan-magnetic nanocarrier for 5-fluorouracil drug delivery and studying its effect in bladder cancer therapy," *J. Glob. Pharm. Technol*, vol. 11, pp. 628–637, 2019.

[11] H. J. Abdul-Ameer, M. F. AL-Hilli, and F. T. Ibrahim, "The Performance of V2O5:Ag Nanoparticles as Thin Film and Bulk Pellet Sensor for NO2 and NH3 Detection," *Iraqi Journal of Science*, vol. 64, no. 2, pp. 630–642, Feb. 2023.

[12] M. A. Al-Kinani, A. J. Haider, and S. Al-Musawi, "High uniformity distribution of Fe@ Au preparation by a micro-emulsion method," in *IOP Conference Series: Materials Science and Engineering*, IOP Publishing, 2020, p. 12013.

[13] M. A. Al-Kinani, A. J. Haider, and S. Al-Musawi, "Design and synthesis of nanoencapsulation with a new formulation of Fe@ Au-CS-CU-FA NPs by pulsed laser ablation in liquid (PLAL) method in breast cancer therapy: in vitro and in vivo," *Plasmonics*, vol. 16, no. 4, pp. 1107–1117, 2021.

[14] A. Haider, R. Al-Anbari, G. Kadhim, and Z. Jameel, "Synthesis and photocatalytic activity for TiO2 nanoparticles as air purification," in *MATEC web of conferences*, EDP Sciences, 2018, p. 5006.

[15] D. Bzhalava, P. Guan, S. Franceschi, J. Dillner, and G. Clifford, "A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types," *Virology*, vol. 445, no. 1–2, pp. 224–231, 2013.

[16] S. Albukhaty, L. Al-Bayati, H. Al-Karagoly, and S. Al-Musawi, "Preparation and characterization of titanium dioxide nanoparticles and in vitro investigation of their cytotoxicity and antibacterial activity against Staphylococcus aureus and Escherichia coli," *Anim. Biotechnol.*, vol. 33, no. 5, pp. 864–870, 2022.

[17] F. Jalili *et al.*, "Assessing the impact of mailing self-sampling kits for human papillomavirus testing to unscreened non-responder women in Manitoba," *Curr. Oncol.*, vol. 26, no. 3, pp. 167–172, 2019.

[18] A. A. Yousif, N. F. Habubi, and A. A. Haidar, "Nanostructure zinc oxide with cobalt dopant by PLD for gas sensor applications," *Journal of Nano-And Electronic Physics*, vol. 4. no. 2, p. 02007, 2012.

[19] A. J. Haider, F. I. Sultan, M. J. Haider, and N. M. Hadi, "Spectroscopic and structural properties of zinc oxide nanosphere as random laser medium," *Appl. Phys. A*, vol. 125, no. 4, p. 260, 2019.

[20] I. Benammar, R. Salhi, J.-L. Deschanvres, and R. Maalej, "Study of the Physico-Chemical Properties of Sol-Gel (Er, Yb) Doped TiO2 Nanoparticles Prepared with a Novel Protocol," *J. Appl. Sci. Nanotechnol.*, vol. 3, no. 2, pp. 1-17, 2023. doi: 10.53293/jasn.2023.6799.1208

[21] F. Haghighi, M. ShR, P. Mohammadi, M. Eskandari, and S. Hosseinkhani, "The evaluation of Candida albicans biofilms formation on silicone catheter, PVC and glass coated with titanium dioxide nanoparticles by XTT method and ATPase assay," *Bratisl. Lek. Listy*, vol. 113, no. 12, pp. 707–711, 2012.

[22] C. Maneerat and Y. Hayata, "Antifungal activity of TiO2 photocatalysis against Penicillium expansum in vitro and in fruit tests," *Int. J. Food Microbiol.*, vol. 107, no. 2, pp. 99–103, 2006.

[23] A. J. Haider, A. A. Jabbar, and G. A. Ali, "A review of pure and doped ZnO nanostructure production and its optical properties using pulsed laser deposition technique," in *Journal of Physics: Conference Series*, IOP Publishing, 2021, p. 12015.

[24] R. Al-Obaidy, A. J. Hadier, S. Al-Musawi, and N. Arsad, "Study of the Effects of Solution Types

on Concentration of Iron Oxide by Pulsed Laser Ablation in Liquid," *J. Appl. Sci. Nanotechnol.*, vol. 3, no. 1, pp. 137–150, 2023.

[25] H. Iqbal *et al.*, "Breast cancer inhibition by biosynthesized titanium dioxide nanoparticles is comparable to free doxorubicin but appeared safer in BALB/c mice," *Materials (Basel).*, vol. 14, no. 12, p. 3155, 2021.

[26] V. R. Lopes, V. Loitto, J.-N. Audinot, N. Bayat, A. C. Gutleb, and S. Cristobal, "Dose-dependent autophagic effect of titanium dioxide nanoparticles in human HaCaT cells at non-cytotoxic levels," *J. Nanobiotechnology*, vol. 14, p. 22, 2016.

[27] C.-L. Cheng *et al.*, "The effects of the bacterial interaction with visible-light responsive titania photocatalyst on the bactericidal performance," *J. Biomed. Sci.*, vol. 16, no. 1, p. 7, 2009.

[28] C.-Ș Adochiție *et al.*, "Synthesis and Investigation of Antibacterial Activity of Thin Films Based on TiO2-Ag and SiO2-Ag with Potential Applications in Medical Environment," *Nanomaterials*, vol. 12, no. 6, p. 902, 2022.

[29] R. L. Finnen, K. D. Erickson, X. S. Chen, and R. L. Garcea, "Interactions between papillomavirus L1 and L2 capsid proteins," *J. Virol.*, vol. 77, no. 8, pp. 4818–4826, 2003.

[30] G. Palomino-Vizcaino *et al.*, "Effect of HPV16 L1 virus-like particles on the aggregation of non-functionalized gold nanoparticles," *Biosens. Bioelectron.*, vol. 100, pp. 176–183, 2018.

[31] P. Chaudhary, F. Fatima, and A. Kumar, "Relevance of nanomaterials in food packaging and its advanced future prospects," *J. Inorg. Organomet. Polym. Mater.*, vol. 30, pp. 5180–5192, 2020.

[32] A. K. Chaturvedi *et al.*, "Human papillomavirus and rising oropharyngeal cancer incidence in the United States," *J. Clin. Oncol.*, vol. 29, no. 32, p. 4294, 2011.

[33] M. Shen *et al.*, "Antiviral properties of R. tanguticum nanoparticles on herpes simplex virus type I in vitro and in vivo," *Front. Pharmacol.*, vol. 10, p. 959, 2019.

[34] M. Ahmadi Kamalabadi, A. Neshastehriz, H. Ghaznavi, and S. M. Amini, "Folate functionalized gold-coated magnetic nanoparticles effect in combined electroporation and radiation treatment of HPV-positive oropharyngeal cancer," *Med. Oncol.*, vol. 39, no. 12, p. 196, 2022.

[35] T. Wiest, E. Schwarz, C. Enders, C. Flechtenmacher, and F. X. Bosch, "Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control," *Oncogene*, vol. 21, no. 10, pp. 1510–1517, 2002.

[36] R. J. Kimple *et al.*, "Enhanced radiation sensitivity in HPV-positive head and neck cancer," *Cancer Res.*, vol. 73, no. 15, pp. 4791–4800, 2013.

[37] J. M. M. Walboomers *et al.*, "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide," *J. Pathol.*, vol. 189, no. 1, pp. 12–19, 1999.

[38] M. Jit, K. Prem, E. Benard, and M. Brisson, "From cervical cancer elimination to eradication of vaccine-type human papillomavirus: Feasibility, public health strategies and cost-effectiveness," *Prev. Med. (Baltim).*, vol. 144, p. 106354, 2021.

[39] T. Giroglou, L. Florin, F. Schäfer, R. E. Streeck, and M. Sapp, "Human papillomavirus infection requires cell surface heparan sulfate," *J. Virol.*, vol. 75, no. 3, pp. 1565–1570, 2001.

[40] D. G. Valencia-Reséndiz, G. Palomino-Vizcaino, J. V. Tapia-Vieyra, M. L. Benítez-Hess, A. G. Leija-Montoya, and L. M. Alvarez-Salas, "Inhibition of human papillomavirus type 16 infection using an RNA aptamer," *Nucleic Acid Ther.*, vol. 28, no. 2, pp. 97–105, 2018.

[41] X. Wang *et al.*, "Therapeutic potential of delivering arsenic trioxide into HPV-infected cervical cancer cells using liposomal nanotechnology," *Nanoscale Res. Lett.*, vol. 11, no. 1, p. 94, 2016.

[42] C. Ripolles-Avila, M. Martinez-Garcia, A.-S. Hascoët, and J. J. Rodríguez-Jerez, "Bactericidal efficacy of UV activated TiO2 nanoparticles against Gram-positive and Gram-negative bacteria on suspension," *CyTA-Journal Food*, vol. 17, no. 1, pp. 408–418, 2019.

[43] B. Serrano, M. Brotons, F. X. Bosch, and L. Bruni, "Epidemiology and burden of HPV-related disease," *Best Pract. Res. Clin. Obstet. Gynaecol.*, vol. 47, pp. 14–26, 2018.

[44] L. C. P. Vilas Boas, M. L. Campos, R. L. A. Berlanda, N. de Carvalho Neves, and O. L. Franco, "Antiviral peptides as promising therapeutic drugs," *Cell. Mol. Life Sci.*, vol. 76, pp. 3525–3542, 2019.

[45] N. A. Parmin *et al.*, "Voltammetric determination of human papillomavirus 16 DNA by using interdigitated electrodes modified with titanium dioxide nanoparticles," *Microchim. Acta*, vol. 186, p. 336, 2019.

[46] D. Lembo, M. Donalisio, A. Civra, M. Argenziano, and R. Cavalli, "Nanomedicine formulations

for the delivery of antiviral drugs: a promising solution for the treatment of viral infections," *Expert Opin. Drug Deliv.*, vol. 15, no. 1, pp. 93–114, 2018.

[47] A. J. Haider, T. Alawsi, M. J. Haider, B. A. Taha, and H. A. Marhoon, "A comprehensive review on pulsed laser deposition technique to effective nanostructure production: Trends and challenges," *Opt. Quantum Electron.*, vol. 54, no. 8, p. 488, 2022.

[48] A. J. Haider, A. D. Thamir, D. S. Ahmed, and M. R. Mohammad, "Deposition of silver nanoparticles on multiwalled carbon nanotubes by chemical reduction process and their antimicrobial effects," in *AIP Conference Proceedings*, AIP Publishing, vol. 1758, no. 1, p. 030003, 2016.

[49] L. Chen and J. Liang, "An overview of functional nanoparticles as novel emerging antiviral therapeutic agents," *Mater. Sci. Eng. C*, vol. 112, p. 110924, 2020.

[50] M. Chakravarty and A. Vora, "Nanotechnology-based antiviral therapeutics," *Drug Deliv. Transl. Res.*, vol. 11, pp. 748–787, 2021.

[51] F. Y. Ahmed, U. Farghaly Aly, R. M. Abd El-Baky, and N. G. F. M. Waly, "Comparative study of antibacterial effects of titanium dioxide nanoparticles alone and in combination with antibiotics on MDR Pseudomonas aeruginosa strains," *Int. J. Nanomedicine*, vol. 15, pp. 3393–3404, 2020.

[52] K. P. Priyanka, T. H. Sukirtha, K. M. Balakrishna, and T. Varghese, "Microbicidal activity of TiO2 nanoparticles synthesised by sol–gel method," *IET nanobiotechnology*, vol. 10, no. 2, pp. 81–86, 2016.

[53] A. Fradet-Turcotte and J. Archambault, "Recent advances in the search for antiviral agents against human papillomaviruses," *Antivir. Ther.*, vol. 12, no. 4, pp. 431–451, 2007.

[54] P. R. Jubu *et al.*, "An Alternative Method for Estimating the Phase Fraction of Multiphase Nanomaterials: Analysis from X-ray Diffraction," *J. Appl. Sci. Nanotechnol.*, vol. 3, no. 3, pp. 1-19, 2023.