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Electron Microscope Scanning, and Antibacterial Activity of Tio_2 Nanoparticles on pathogenic strains of *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background: Nanoparticles are increasingly being used as an alternative antibiotic to target microorganisms, particularly for treating bacterial infections. In current study it was recognized processes such as metal ion release, induction of oxidative stress, and non-oxidative mechanisms. Developing resistance to nanoparticles is challenging for bacterial cells due to the multiple simultaneous actions against microorganisms, and require several simultaneous gene alterations within the same bacterial cell.

Objective: To determine the effect of the titanium dioxide nanoparticles (TiO2NPs) on the activity of the same bacteria that participate in dental caries (*Staphylococcus aureus* and *Escherichia coli*).

Patients and Methods: The study included 50 patients who attended the dental clinic and took the samples by the wooden swap from the molar area (cervical area of inflamed gingiva and same samples taken from occlusal surface of molar). The collected samples were cultured on "Mannitol salt agar, Blood agar, and MacConkey's agar plates" for separation of the needed bacteria (*Staphylococcus aureus* and *Escherichia coli*) that showed wide spreading in the oral cavities and dental carries. TiO2NPs are then created using pulse laser ablation, followed by coating preparation the nanoparticles materials (using a hybrid sol-gel and organ silicate nanoparticles), and finally the prepared nanoparticles to the coating solution and local paint were used for antimicrobial activity of the separated bacteria using different concentrations of TiO2 in the cultures.

Results: Characterization showed the phase of the TiO2NPs was spherical, with very few irregularly shaped particles, and had an average small size in millimeters. Antimicrobial activity results showed a strong bactericidal effect against Gram-positive bacteria and demonstrated greater sensitivity to TiO2 nanoparticles at lower concentrations when compared to Gram-negative bacteria.

Conclusion: It was shown that nanotechnology promise in treating various infections caused by bacteria, including dental caries. Notably, nanoparticles have demonstrated broad-spectrum antibacterial effects against Gram-positive bacteria.

Keywords: TiO_2, nanoparticles, Staphylococcus aureus, and *E. coli*

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Introduction

Dental caries is a highly prevalent chronic infectious disorder worldwide [1,2]. The etiology of dental caries is explained by the ecological plaque hypothesis, which offers three main explanations. The specific plaque theory suggests that the disease is primarily caused by a small number of distinct species, such as *Staphylococcus aureus* and E. coli. In contrast, the nonspecific plaque hypothesis proposes that caries result from the overall activity of the entire plaque microflora, consisting of numerous bacterial species. According to the ecological plaque hypothesis, changes in the local environment lead to a shift in the equilibrium of local bacteria, ultimately causing caries [3].

Historically, approaches based on culture have been used to identify caries-associated bacteria, excluding species that have not previously been grown. Molecular methods for bacterial identification and enumeration are now frequently used in order to more thoroughly study bacterial species connected to dental caries, even those that are not yet cultivable [4]. In a previous study, the bacterial species found in young children with caries were compared to those found in young children without caries. The species Streptococcus sanguinis has been associated with well-being. On the other hand, caries has been associated with S. mutans, other Streptococcus species, Veillonella species, Actinomyces species, Bifidobacterium species, and Lactobacillus fermentum [5].

A frontier of medical science has been the search for new antimicrobial drugs against microorganisms that promote den tal caries. Streptococcus mutans has long been thought to be the cause of dental caries, but several other bacteria, including *E. coli*, P. aeruginosa, and S. aureus, are also to blame for spreading this mouth condition and other dangerous illnesses in people. Recently, Lactobacilli strains have developed into infections, especially in children, where they can cause tooth decay and subsequent dental dysfunction [6].

Staphylococcus aureus is a common bacterium in our body's microbiota. It's a round-shaped, Gram-positive bacterium and it can be found in the upper respiratory tract, oral cavity, and skin. This bacterium can do catalase and nitrate reduction, and it can grow with or without oxygen since it's a facultative anaerobe [7]. *Staphylococcus aureus* is usually a harmless member of the bacteria that live on our bodies, but it can turn into a harmful germ. It commonly causes food poisoning, sinus infections, and various skin and respiratory infections, such as abscesses. Pathogenic strains of Staph.aureus produces strong toxins and a protein that can neutralize antibodies, helping them spread diseases. About 20%–30% of people are long-term carriers of *Staph. Aureus* [8].

The lower intestines of warm-blooded creatures often harbor Escherichia coli, a type of Gram-negative, facultative anaerobic, rod-shaped coliform bacteria [9]. About 0.1% of the gut microbiota consists of facultative anaerobes, such as E. coli. The primary mode of transmission for pathogenic strains of this bacterium is through fecal-oral contact, which spreads diseases. Interestingly, these cells can survive outside the body, making them useful indicator organisms for assessing fecal contamination in environmental samples [10]. There have been several studies

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that have examined environmentally persistent *E. coli*, which can survive outside of a host for several days [11].

One of the most potent methods for combating numerous clinical difficulties, such as infectious diseases, is nanotechnology [12]. Titanium dioxide nanoparticles, referred to as ultrafine, nanocrystalline, or microcrystalline titanium dioxide, are tiny particles of titania (TiO_2) with a diameter smaller than 100 nm. These particles are used in sunscreens because they can block UV radiation while remaining transparent on the skin. To prevent any potentially harmful effects, ultrafine TiO has a rutile crystal structure and is coated with silica and alumina. Although there is limited knowledge about the health impacts of ultrafine TiO_2, it is believed to be safer than other UV blockers when applied topically to intact skin [13].

Due to their supramolecular structure and capacity to interact with biological components like cell membranes and related proteins, the metal oxide nanoparticles have shown significant antibacterial potential [14]. In general, the nano size and functionality of nanoparticles at the atomic and molecular level make them extremely important for larger applications in electronics, pharmaceuticals, and healthcare systems, as well as the creation of novel materials and sensor designs [15]. Because of their enhanced biological activity, which is linked to the production of more reactive oxygen species, the TiO2Nps are regarded as potent antibacterial agents for bacteria, fungi, and parasites [16].

TiO_2Nps have low thermal conductivity and exceptional resistance to abrasion, attrition, fatigue, corrosion, tarnish, and wear in the oral cavity, making them suitable for various dental applications. These NPs release various active radicals and peroxides, such as OH-, H2O2, and O-, that destroy DNA, protein, cell membrane, cell wall, and organelles of pathogenic bacteria [17,18].

Patients and Methods

Subject population

Subjects attended to dental clinic with severe dental caries and the patient age-matched between (25-60) years old, samples were recruited from subjects with caries in secondary teeth (posterior part of the upper and lower arch) who attended the private dental clinic in Diyala province- Baqubah-AL Khalis and according to the ethical approval of College of Medicine, University of Diyala Committee (2023MAH791) from 1st October 2022 to 1st March 2023. A Patients' samples totaling (50) were gathered. Some samples were obtained by rubbing a local lesion or gum edge attached to teeth with a wooden stick, while the other samples were made up of teeth extracted by dentists at a dental clinic. Samples were obtained using aseptic methods with the assistance of a dentist. The samples were put in sterile containers that were used for this objective and then transmitted to the central private Microbiology laboratory.

Isolation and Identification of Microorganisms

On Mannitol salt agar, Blood agar, and MacConkey's agar plates, the obtained samples were cultivated. Then, the plates were all incubated at 37°C for 24 hours. Visual growth was seen on the cultured plates when the incubation period was over, and the colony morphology was recorded. Gram

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staining, morphological analysis of the colonies, staining, and biochemical features, such as tests for coagulase and catalase were all employed to identify the isolates (19,20). Levofloxacin LEF (5 mcg) and Amikacin AK (10 mcg) were used as positive controls for each bacterium.

Preparation of TiO_2

By adding dropwise from titanium tetrachloride TiCl4 in ethanol with a 1:10 ratio, TiO2 nanoparticles can be produced. Absolute ethanol CH3CH2OH (99.99%) and titanium tetrachloride TiCl4 (99.99%) are more interesting raw materials. The reaction was conducted in a fume hood at room temperature while stirring due to the high concentrations of Cl2 and HCl. This reaction produced a yellow solution, which was then allowed to sit and return to room temperature. The pH of the solution was then determined to be between (1-2). At 80°C, the final solution was dried till gel formed. When the temperature was raised to 900 ℃, the TiO2 powder underwent a phase transition from anatase to rutile. The resulting TiO_2 powder was calcined for two hours at 500 ℃ in an ambient environment in the box furnace [21].

Synthesis of TiO_2 NP powder via the Sol-Gel method

Tetrachloride of Titanium (TiCl4) (99.99%) and Ethanol-CH3CH2OH (99.99%) were used as starting materials. The synthesis process is accomplished by adding a series of droplets from TiCl4 into the absolute ethanol with a ratio of 1:10. The reaction was carried out using a magnetic stirrer in the chemical fume hood to expel unwanted toxic gases, such as cloud [22,23]. By applying a temperature of 80°C for 24 hours, the resulting solution was changed to a gel condition. The anatase and rutile phases of TiO_2 were created during the calcination process. The rutile phase is produced under (900) ℃ over 1.5–2 hr, whereas the anatase phase is produced under (400–450) Co over (1.5–2) hr (21).

Antimicrobial Assay

Agar well diffusion assay was used to test the TiO_2 nanoparticles' antibacterial abilities. The effectiveness of the nanoparticles' antibacterial properties was assessed against eight common bacterial species. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) measurements were performed. Bacterial cultures were prepared in sterile distilled water to match the McFarland 0.5 standard's turbidity (1.5 ×108 CFU/mL), then inoculated on Mueller-Hinton agar before the assay.TiO2 nanoparticle concentrations of “0 µg, 10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60 µg, 70 µg, 80 µg, 90 µg and 100 µg/ml” were produced by ten serial dilutions. 6 mm-diameter wells were cut from each into the culture medium at 20 mm distance, and filled with various concentrations of TiO_2 nanoparticles (0 %, 0.5%, 1%, 1.5%, 2%, and 2.5%) were added to each well. After that, the cultures were incubated at 37ºC for 24 hrs under aerobic circumstances. After the time of incubation, the inhibition zone of bacterial growth around the wells (mm) was measured (24,25). The lowest nanoparticle concentration preventing the growth of bacteria is known as the “minimum inhibitory concentration (MIC)” and the “minimum bactericidal concentration (MBC)” was determined by using Mueller-Hinton agar.

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Results

The shape and size of TiO_2 Nano-sol can be described by the morphologic examination of nanoparticles that were examined using scanning electron microscopy. Demonstrates TiO_2 nanoparticles from the initial process Small nanoparticles with a size of less than 10 nm Figure (1, 2) that have spherical forms are possible.

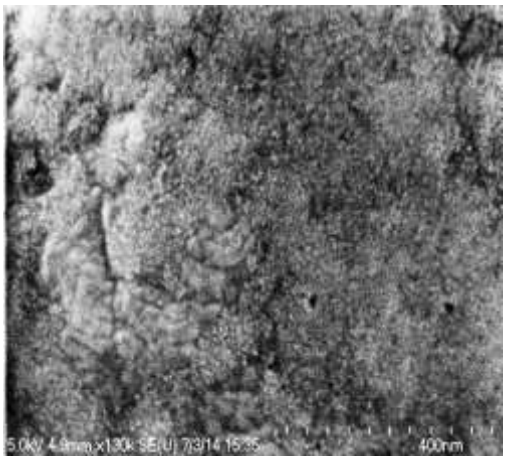

Figure (1): Electron Microscope Scanning images (SEM) for the small-size nanoparticles TiO_2

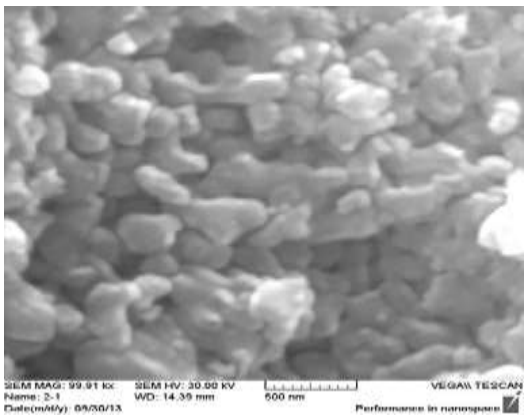

Figure (2): Electron Microscope Scanning images (SEM) to powder samples of TiO_2 nanoparticles

Due to the agglomeration of initial particles, TiO_2 nanoparticles made using the Sol-Gel approach had irregular particle shapes and had an average diameter of roughly 35 nm for the anatase phase and 65 nm for the rutile phase. These findings indicated that the anatase phase's particle size was smaller than that of the rutile phase. There have been several methods used to create TiO_2 nanoparticles that are more easily synthesized, less expensive, and devoid of harmful chemicals [26]. Sol-gel production of TiO_2 nanoparticles was used in our investigation. After 24 hours, changes in the color of the solution suggest the creation of TiO_2 nanoparticles, and UV-visible spectroscopy data showed that the synthesis of nanoparticles was indicated by an apparent broadening beak at 450 nm as shown in Figure (3).

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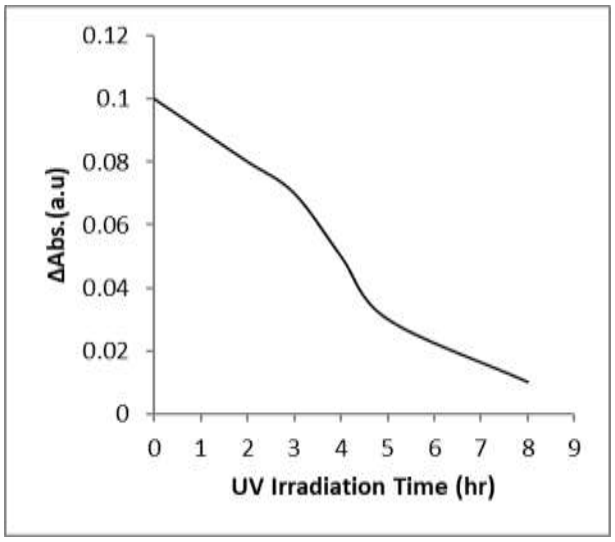

Figure (3): Absorbance changes (Δ Abs.) as a function of UV exposure Time

Antibacterial properties of TiO_2 nanoparticles

The generated TiO_2 NPs were utilized to test the antibiotic sensitivity at various doses. Using a well diffusion experiment in culture plates, the diameter of the inhibitory zone was evaluated after the addition of various TiO_2 nanoparticle concentrations and before measuring the growth of the bacteria. The most contagious nosocomial disease agents are *E. coli* and *S. aureus*, both of which are resistant to wide-spectrum antibiotics as a result of excessive antibiotic use [27,28]. Due to their potent germicidal properties, many studies have questioned the viability of utilizing metal oxide nanoparticles as an alternative to antibiotics. The effects of TiO_2 nanoparticles' antibacterial activity on bacterial development in liquid media as shown in Figure (6) that shows the Gram-positive bacteria illustrated higher TiO_2 nanoparticles sensitivity to lesser concentrations in contrast to Gram-negative bacteria.

A **B** **C**

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D E F

Figure (4): Illustrate different images of different concentrations of TiO2 nanoparticles and PBS with *Escherichia coli* and *Staphylococcus aureus*, respectively, (a) *E-coli* with PBS, (b) 10_{-3} TiO_2 nanoparticles, PBS and *E- coli* bacteria, (c) 10^{-5} TiO_2 nanoparticles, PBS and E- coli bacteria, (d) *Staph. aurous* with PBS, (e) 10_{-1} TiO_2 nanoparticles, PBS and *Staph. aureus* bacteria, (f) 10-3 TiO_2 nanoparticles, PBS and *Staph. aurous* bacteria

The test of sensitivity showed that Staph. aureus (2.0 cm), and (2.2 cm) sensitivity to, Staph. aureus at TiO*2* -600, and (2.0 cm) for *E. coli* while rutile shows sensitivity to Staph. aureus (2.1cm). The TiO_2 shows significant antimicrobial activity compared with TiO_2 -600 and rutile TiO*2*. TiO_2 NPs' antibacterial activity is decreased as a result of the increased particle size brought on by calcination. In this experiment, varying quantities of TiO_2 nanoparticles were added to Muller Hinton medium to inoculate the strains of *E. coli* and Staph. aureus. The MIC values of TiO_2 nanoparticles were 30 µg/ml, and 40µg/ml for *E.coli*, *Staph. aureus* respectively. When compared to the control, *E. coli* and *S. aureus* growth was generally reduced, and the results were inversely related to increasing the TiO_2 nanoparticle concentration. The agar diffusion techniques used to corroborate these findings emphasize the bacterial susceptibility to TiO_2 nanoparticles.

Discussion

The hydrodynamic size combination of predominantly round and slightly the particles showed irregular form with a size of around “65 nm” was discovered in the modification of conventionally manufactured new TiO2Nps. According to CLSI recommendations, these TiO_2 NPs demonstrated appropriate antibacterial efficacy against a variety of bacterial strains known to promote dental caries, including “P. aeruginosa, *E. coli*, L. acidophilus, and *S. aureus*”. First, the alteration of the titanium tetrachloride color from black to white solution confirmed the creation of TiO_2Nps agreeing with a study done at Pakistan Institute of Engineering and Applied Sciences showed antimicrobial action as bactericidal action against Pseudomonas aeruginosa (20mm), *Escherichia coli* (19mm) and Lactobacillus acidophilus (19nm) while comparatively less activity against Staph. aureus (16mm) [12].

Both ions of oxygen and titanium were present in the structure of TiO2 NPs without the inclusion of any other element, which would have increased their effectiveness. The current investigation showed that TiO_2Nps effectively inhibited the growth of *S. aureus* and *E. coli*. When compared to Gram-positive bacteria, TiO_2Nps had more

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antibacterial action against gram-negative bacteria for two plausible reasons, the gram-negative bacteria's cell wall is made up of a thin peptidoglycan layer, which these NPs may be able to dissolve easily, and charge difference between these Nps (+ve) and the bacteria (-ve) may increase the force of attraction between them, leading to the oxidation and death of the bacteria and this results agree with [29]. Numerous investigations have found a connection between the germicidal properties of the proteins on the cytoplasmic membrane's negatively charged thiol group (-SH), and processes of TiO_2 nanoparticles by releasing positively charged ions into the reaction medium [30, 31]. They create holes or pits in the bacterial cell wall that may be connected to absorbed particles, increasing permeability and cell death [32,33]. In other words, the difference in electrical charges between contaminated metals and living things creates an attraction between them, which causes the living thing to accumulate heavy metals in its body and eventually perish". Due to their small size and high surface-to-volume ratio, TiO_2 nanoparticles interact with bacterial cell surfaces more intensely than bigger particles, which leads to a high antibacterial activity [34].

The TiO_2 shows significant results of antimicrobial activity compared with TiO_2 -600 and rutile TiO_2. TiO_2 NPs' antibacterial activity is decreased as a result of the increased particle size brought on by calcination. The current study reported that criteria for effective antibacterial activity against infections include the presence of light, TiO_2 content, and NP size. Moreover, TiO_2 NP samples indicated very good oppositional activity against Staphylococcus aureus. TiO_2 NPs' antibacterial effect is caused by the breakdown of bacterial outer membranes by reactive oxygen species (ROS), particularly hydroxyl radicals (OH), which leads to phospholipid peroxidation and ultimately cell death [35]. ROS produced by the TiO_2 photocatalytic action causes severe cell membrane damage, which is followed by the loss of vital activities [36].

The results of this work differ from other references due to the study was conducted using the disk diffusion technique, as opposed to numerous studies carried out in a liquid medium that may have favored the close interaction between the suspended nanoparticles and the Gram-positive microbial cells, which could better attach and anchor to the microbial cells' surfaces, causing structural changes and altering the response of bacterial species to TiO_2 NPs at various concentrations [37].

On the other hand, by interfering with transcription factors, TiO_2 NPs can oxidize elements of cell signaling pathways directly and even alter the expression of the gene. These results implied that TiO2 NPs have an impact on microorganisms through oxidative damage, bacterial aggregation, and biofilm development, all of which directly affect pathogenicity [38].

In hospital settings where resistant bacteria could swiftly spread and infect patients with surgical incisions and burns, TiO_2 NPs could be used as the appropriate disinfectant. In order to decrease the rate of infection in patients, cotton materials containing the antibacterial TiO_2 might also be utilized to make sutures or wound bands [39].

Conclusions

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TiO_2Nps can be made in the necessary size with the use of modifications in the nanoparticle production process, which may improve their antibacterial efficacy when used against strains of *E. coli* and *Staph. aureus* that promote tooth decay. According to our findings, titanium-based NPs may be employed as a potential replacement for traditional antibacterial treatments to ban dental caries in the oral cavity. Titanium dioxide nanoparticles showed strong broad-spectrum antibacterial efficacy against all the indicator pathogens—the potential for deploying TiO_2 nanoparticles as a significant antibiotic replacement is made possible by their outstanding features. Medical device-related biofilms and other microbial infections cannot be cured by antibiotic therapy alone. Antibiotics and the promising TiO2 nanoparticles agent may be combined in the future to eradicate the infection of these microorganisms.

Recommendations

According to the results of a recent study, it was recommended using the TiO_2Nps and studying their effect on the other microorganisms. In addition, it was recommended using of NPS to treat the infection with strains of *E. coli* and *Staph. aureus* and to reduce the causes of antibiotic resistance. TiO_2 nanoparticles could be employed as the proper disinfectant. Cotton textiles containing the antibacterial TiO_2 might also be used to construct wound bands or sutures to lower the rate of infection in patients.

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Ethical clearance: This study was conducted according to the approval of College of Medicine/ University of Diyala and in accordance with the ethical guidelines of the Declaration of ethical committee of the College (document no.2023MAH791).

Conflict of interest: Nil

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التشخيص المجهري الالكتروني والفعالية المضادة للبكتيريا لجسيمات ثاني اوكسيد التيتانيوم النانوية على السلالات المسببة للأمراض للمكورات العنقودية الذهبية والإشريكية القولونية

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الملخص

خلفية الدراسة: حديثا تم استخدام لجسيمات ثاني اوكسيد التيتانيوم النانوية بشكل كبير كبديل للمضادات الحيوية ضد الكائنات الحية الدقيقة، وخاصة في علاج الالتهابات البكتيرية. في حين أن الية العمل المضادة للبكتيريا للجسيمات النانوية ليست مفهومة تمامًا بعد، فإن الأبحاث الحالية تتعرف على عمليات مثل إطلاق أيون المعدن، وتحريض الإجهاد التأكسدي، والآليات غير المؤكسدة. يمثل تطوير المقاومة للجسيمات النانوية تحديًا بالنسبة للخلايا البكتيرية بسبب الإجراءات المتزامنة المتعددة ضد الكائنات الحية الدقيقة، الأمر الذي يتطلب العديد من التعديلات الجينية المتزامنة داخل نفس الخلية البكتيرية.

اهداف الدراسة: لتحديد تأثير جزيئات ثاني أكسيد التيتانيوم النانوية على نشاط البكتيريا المسببة لتسوس الأسنان (المكورات العنقودية الذهبية والإشريكية القولونية).

المرضى والطرائق: اشتملت الدراسة ٥٠ مريضاً راجعوا عيادة الأسنان الخاصة وتم أخذ العينات بواسطة العيدان الخشبية من الضرس المولي (منطقة عنق اللثة الملتهبة ونفس العينات مأخوذة من اجزاء السن الأخرى). تم زراعة العينات المأخوذة على طبق ملح مانيتول وطبق الدم وطبق ماكونكي لفصل البكتيريا المطلوبة (المكورات العنقودية الذهبية والإشريكية القولونية) التي أظهرت انتشارًا واسعًا في الآفة الفموية. تم بعد ذلك تحضير جسيمات نانوية من ثاني أكسيد التيتانيوم باستخدام الليزر النبضي، يليها تحضير طلاء مواد الجسيمات النانوية (باستخدام هجين محلول وجل جسيمات نانوية لسيليكات عضوية وأخيرًا الجسيمات النانوية المحضرة ثم تحديد النشاط المضاد للميكروبات للبكتيريا بتراكيز مختلفة من ثاني أكسيد التيتانيوم النانوية في المزارع البكتيرية المحضرة.

النتائج: أظهرت النتائج أن مرحلة الجسيمات النانوية لثاني أكسيد التيتانيوم كانت كروية، مع عدد قليل جدًا من الجزيئات غير المنتظمة الشكل، وكان متوسط حجمها صغيرًا بالمليمتر. أظهرت نتائج النشاط المضاد للميكروبات تأثيرًا قويًا مضادا للجراثيم ضد البكتيريا الموجبة لصبغة غرام وأظهرت حساسية أكبر لجسيمات النانوية بتركيزات أقل مقارنةً بالبكتيريا سالبة لصبغة الغرام.

الاستنتاجات: تم قبول اللقاح لدى أقل من نصف المشاركين، وكان معدل القبول مرتبطًا بشكل كبير بجنس الذكر والتعليم والمهنة ودخل الأسرة والوضع الاجتماعي والاقتصادي.

الكلمات المفتاحية: جسيمات ثاني اوكسيد التيتانيوم النانوية , المكورات العنقودية الذهبية , الإشريكية القولونية

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