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Antimicrobial Efficacy of Silver Nanoparticle Gel in Preventing *Bacillus*-Mediated Biodeterioration of Historical Wall Paintings, in Prince Youssef Kamal Palace, Upper Egypt

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1. Abstract

This study investigates the application of silver nanoparticle (AgNPs) gel as an antimicrobial treatment to preserve wall paintings in the Palace of Prince Youssef Kamal, Upper Egypt. The wall paintings surface, susceptible to microbial deterioration were sampled, and the isolated bacteria underwent treatment with synthesized AgNP gel. The gel was produced through a two-stage process involving sonication and precipitation, yielding nanoparticles with a size range of 10–50 nm, which were confirmed by Transmission Electron Microscopy (TEM). Antimicrobial efficacy was tested through the well diffusion method, to indicate a dose-dependent inhibition of *Bacillus* growth, with the largest inhibition zone observed at the highest concentration (500 ppm). In addition, the study evaluated the impact of AgNPs gel on pigment stability using colorimetric measurements in the CIELAB color space. Results indicated that while most pigments retained their color, some, particularly yellow ochre and black, exhibited moderate discoloration post-treatment. These findings highlight both the potential and the limitations of AgNPs gel for conservation, suggesting its suitability for microbial control while noting the need for caution regarding pigment sensitivity.

Key words: Silver nano particles (AgNPs), Antimicrobial, Biodeterioration, Conservation, wall paintings.

2. Introduction

The conservation of cultural heritage, particularly ancient wall paintings, is a critical challenge for archaeologists, conservators, and microbiologists(Cappitelli, Cattò, & Villa, 2020; Piñar & Sterflinger, 2021). Wall paintings are an invaluable aspect of humanity's cultural legacy, showcasing historical, artistic, and socio-cultural narratives of bygone eras(Chalmers, 1996). In Upper Egypt, the Palace of Prince Yusuf Kamal in Nag Hammadi stands as an emblem of such heritage, featuring intricate wall paintings that depict historical motifs and embody the unique artistry of the time(El-Taher, 2022; Faisal, Kandil, & Maher; Rahman). However, these artworks are susceptible to degradation by various factors, particularly microbial colonization, which leads to bio-deterioration. Bacterial communities that thrive on wall paintings in humid environments can cause severe structural and aesthetic damage over time, threatening the preservation of this cultural heritage(Ciferri, 1999, 2002; Ferrari et al., 2015; Gambino, Ahmed, Villa, & Cappitelli, 2017; Stanaszek-Tomal, 2020; Wu, Gu, Li, Feng, & Wang, 2022).

The presence of bacteria on historical surfaces is attributed to environmental factors like humidity, temperature, and light exposure, as well as to the inherent properties of the wall materials themselves(Dakal & Cameotra, 2012; Horve et al., 2020). Egypt's climate, with seasonal high temperatures and significant humidity variations near the Nile River, creates an environment that promotes microbial growth(Abd El-Azeem, 2020; El-Ramady, El-Marsafawy, & Lewis, 2013). The paintings in Youssef Kamal Palace are no exception, as these environmental conditions have made them particularly vulnerable to bacterial deterioration. In recent decades, *Bacillus* species have been frequently identified on ancient artworks and architectural surfaces due to their spore-forming ability, which enables them to endure extreme conditions(da Silva, 2017; Mesquita, 2014). While these bacteria naturally occur in the environment, their presence on wall paintings can lead to disintegration of pigment layers, discoloration, and eventually, loss

of the artwork's original appearance(Cappitelli et al., 2020; Petersen & Klocke, 2020).

The wall paintings at Youssef Kamal Palace represent a unique heritage that has unfortunately been subject to microbial-induced degradation. The bacterial colonies on these wall paintings introduce enzymes and organic acids that can alter and dissolve mineral pigments, penetrate paint layers, and cause visible discoloration. Additionally, *Bacillus sp* are known to produce biofilms, which further trap moisture, particulate matter, and other microbial species, exacerbating the deterioration process (Ciferri, 1999; Pei et al., 2023; Wu et al., 2022).

Bio-deterioration is not only limited to the visible, but structural damage also caused by bacteria(Sulymon, Bello, Nwaigwe, & Bello). The biochemical mechanisms that bacteria use to metabolize pigments and other materials of the wall paintings can result in a loss of historical detail, as many of these pigments were created with rare or locally sourced minerals and dyes that are irreplaceable(Dhami, Reddy, & Mukherjee, 2014; Koestler, 2002)(Strzelczyk, 2004).

In recent years, nanotechnology has emerged as a promising approach in the conservation of cultural heritage, with silver nanoparticles (AgNPs) gaining particular attention due to their potent antimicrobial properties and relatively low toxicity to non-target surfaces(Husain et al., 2023). Silver has been used for centuries for its antimicrobial efficacy, and as a nanomaterial, it offers a unique advantage due to its high surface-area-to-volume ratio, enhancing its interaction with microbial cells(Dakal, Kumar, Majumdar, & Yadav, 2016; Rai, Yadav, & Gade, 2009). Studies have demonstrated that AgNPs are effective against a wide range of bacterial strains, including *Bacillus* species, by disrupting cellular membranes, generating reactive oxygen species (ROS), and interfering with DNA replication, ultimately leading to cell death(Liao, Li, & Tjong, 2019; Tripathi & Goshisht, 2022). Unlike traditional biocides, silver nanoparticles do not readily degrade or lose efficacy over time, which makes them suitable for long-term

protection of artwork surfaces)(Reidy, Haase, Luch, Dawson, & Lynch, 2013).

The application of AgNPs in the form of a gel provides several benefits for cultural heritage conservation(Chobba et al., 2023). First, the gel medium allows for a more controlled and uniform application, which can be spread over delicate painted surfaces without causing mechanical damage. Second, the gel can be formulated to release silver ions gradually, providing sustained antimicrobial effects over time without the need for frequent reapplication(Lak, Mohammadi, & Ghadam, 2024). This controlled release is particularly important in heritage conservation, as repeated applications can increase the risk of damaging fragile surfaces(Lak et al., 2024). Finally, the gel matrix itself can be formulated to be transparent and minimally invasive, ensuring that it does not visually alter the artwork after application(Ben Chobba, Weththimuni, Messaoud, Urzi, & Licchelli, 2024).

Studies in similar conservation scenarios have shown that AgNPs-based gels can effectively inhibit microbial growth without impacting the underlying material(Ogunsona, Muthuraj, Ojogbo, Valerio, & Mekonnen, 2020). For example, (Chobba et al., 2023)demonstrated that silver nanoparticles applied to painted surfaces provided effective antimicrobial action without compromising the integrity of the artwork. Another study by (Carrillo-González, Martínez-Gómez, González-Chávez, & Hernández, 2016) showed that silver nanoparticles successfully reduced biofilm formation on stone surfaces exposed to microbial colonization, suggesting that they could also be effective in biofilm inhibition on wall paintings. Given these findings, AgNPs gel presents a promising, minimally invasive approach for inhibiting microbial growth on the wall paintings of Yusuf Kamal Palace.

While AgNPs gels offer many advantages, it is essential to consider potential drawbacks, particularly concerning pigment interactions and color stability. The unique composition of historical pigments could make them susceptible to slight alterations when exposed to nanoparticles. Therefore, it is necessary to conduct thorough testing of AgNPs gel formulations to ensure that they maintain the aesthetic

and structural integrity of the pigments used in these wall paintings. By selecting appropriate nanoparticle sizes and optimizing concentrations, the gel formulation can be tailored to minimize potential side effects while maximizing its antimicrobial efficacy.

This research aims to develop and apply a silver nanoparticle-based gel as an antimicrobial treatment to control the bacterial degradation of the mural paintings of Youssef Kamal Palace. The primary objectives are as follows: isolate and identify bacterial strains on the deteriorating surfaces of wall paintings in Yusuf Kamal Palace, focusing on determining the presence and prevalence of *Bacillus* species known to contribute to bio-deterioration, synthesize and characterize a silver nanoparticle gel tailored for conservation, focusing on particle size, concentration, and controlled release properties that will provide effective microbial inhibition without damaging the artwork, evaluate the antimicrobial efficacy of the AgNPs gel against the identified bacterial strains using controlled laboratory experiments, particularly focusing on the inhibition of *Bacillus* sp., a common bacterium in deteriorative microbial communities on historic artwork and analyze the color stability of pigments after treatment with the AgNPs gel, ensuring that its application does not lead to undesirable alterations in the aesthetic properties of the paintings.

3. Materials and Methods

3.1. Site and Sampling

The bacterial samples were collected from the painting surface at the Palace of Prince Youssef Kamal, located in Nag Hamadi, Upper Egypt (Fig.1) (Faisal et al.). Sterile cotton swabs were gently rolled across various areas of the paintings, focusing on regions showing visible signs of deterioration or discoloration. Care was taken to avoid damaging the delicate painted surfaces during the sampling process. The swabs were then immediately transferred to sterile tubes containing a nutrient broth to preserve the viability of the collected microorganisms. Each sample was labeled with the specific location within the palace and the date of collection. The samples were promptly transported to the laboratory under

controlled temperature conditions to ensure the integrity of the microbial populations for subsequent analysis and identification.

Fig 1. Current Location of the palace.
Google mapshttps://maps.app.goo.gl/3CjAz1k1Q19MDUoK7

3.2. Identification of the isolated bacteria

The identification of the isolated bacteria was primarily conducted using morphological techniques. Following the initial isolation and purification of the bacterial cultures, a series of morphological examinations were performed. These included microscopic observations of cell shape, size, and arrangement, as well as macroscopic evaluation of colony characteristics on various culture media. Gram staining was carried out to determine the cell wall structure and to classify the isolates as either Gram-positive or Gram-negative. Additionally, the presence or absence of endospores was investigated, as this is a key characteristic of certain bacterial genera. characteristics results of these morphological analyses, including the rod-shaped appearance of the cells, the presence of endospores, and the characteristic colony morphology.

3.3. Preparation of Silver Nanoparticle Gel

According to (El-Banna et al., 2022)The creation of silver nano-composite gel involves two primary stages. The initial phase focuses on generating silver nanoparticles through a precipitation method enhanced by ultrasonication. This process begins with heating 125 mL of 0.002 M AgNO3 to its boiling point. Subsequently, 10 mL of 1% trisodium citrate is introduced gradually. The resulting mixture

undergoes ultrasonication using a Hielscher UP400S (400 W) device, with parameters set at 73% amplitude and a 0.81 cycle for a duration of 15 minutes at 90°C. This continues until the solution exhibits a pale-yellow hue. Following this, the mixture is allowed to cool to ambient temperature while shielded from light exposure. The chemical reaction for silver nanoparticle formation can be represented as:

$4Ag+ C6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag0 + C_6H5O7H3 + 3Na+ + H+ + O_2\uparrow$

The second phase involves the formulation of the silver nano gel. This stage commences with the dissolution of 0.75 g of Carbopol 940 in 350 mL of double deionized water. This solution is then combined with 100 mL of silver nanoparticles (50 ppm). The mixture undergoes sonication using a device manufactured by Hielscher Company for 400 seconds, with settings at 71 amplitude and 91% cycle.

3.3.1. Characterization of silver nanoparticles gel

3.3.1.1. Investigation of silver nanoparticles gel by Transmission Electron Microscopy (TEM)

The examination of the Silver Nanoparticle Gel was conducted utilizing a Transmission Electron Microscope (TEM) Jeol 1010 for TEM observations. A small droplet of this thinly dispersed solution was carefully placed onto a staining mat. Subsequently, a copper grid coated with carbon was inserted into the droplet, ensuring that the coated side of the grid was facing upwards. After a period of approximately ten minutes, the grid was delicately removed from the droplet and allowed to dry in air.

4. Antimicrobial efficacy of Silver Nanoparticle Gel.

The isolated bacteria strain Bacillus sp. underwent evaluation against Silver Nanoparticle Gel utilizing the well diffusion technique. The pure cultures were subjected to subculturing in Muller Hinton broth for a duration of 24 hours at a temperature of 37°C. On Muller Hinton agar plates, wells measuring 5 mm in diameter were created using a gel puncture tool. The bacterial strain was uniformly distributed across the plates through the application of sterile cotton swabs. Employing a sterile micropipette, a quantity

of 20 µL (equivalent to 0.002 mg) of the Silver Nanoparticle Gel sample three concentrations (500 pp, 250ppm and 125 ppm) were introduced into each well on the plate. Following an incubation period of 24 hours at 35°C, the varying extents of the zone of inhibition of each concentration were subsequently measured and recorded.

5. Colorimetric measurements

To assess colorimetric alterations, the National Institute of Standards (NIS) in Cairo, Egypt, employed the Optimatch 3100® from SDL Company to measure color changes in experimental painting samples treated with a silver nanoparticle gel, both before and after treatment. The color variations were recorded using the CIE L* a* b* system, where the L value indicates brightness, the "a" value represents the red-green axis, and the "b" value corresponds to the yellow-blue axis. The total color changes (ΔE) before and after treatment were calculated using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where L (lightness), a (red/green axis), and b (yellow/blue axis) values were recorded.

6. Results and discussions

6.1. Identification of the isolated bacteria

The isolated bacteria were identified as belonging to the genus Bacillus. Given the limitations of morphological identification alone, the isolation was conservatively classified as Bacillus sp.,

6.2. characterization of silver nanoparticles gel

As shown in figure 2, this image shows transmission electron microscopy (TEM) micrographs of silver nanoparticles at two different magnifications. Image (a) on the left is taken at 80,000x magnification, while image (b) on the right is at a higher magnification of 200,000x. Both images were captured using an accelerating voltage of 80 kV.

The nanoparticles appear as dark spots against a lighter background. They exhibit varying sizes and shapes, with some particles appearing spherical while others have more irregular morphologies. Some particles seem to form small clusters or aggregates.

The images use colored annotations to highlight specific features: Red dotted circles: These highlight larger clusters or aggregates of nanoparticles. The higher magnification in (b) allows for a more detailed view of the particle arrangement within these clusters. Purple squares: These focus on smaller groupings or individual particles. The higher magnification in (b) reveals more detail about the morphology of these particles. The scale bars at the bottom of each image indicate 100 nm, providing a reference for particle size estimation. Most particles appear to be in the range of 10-50 nm in diameter, though there is considerable size variation.

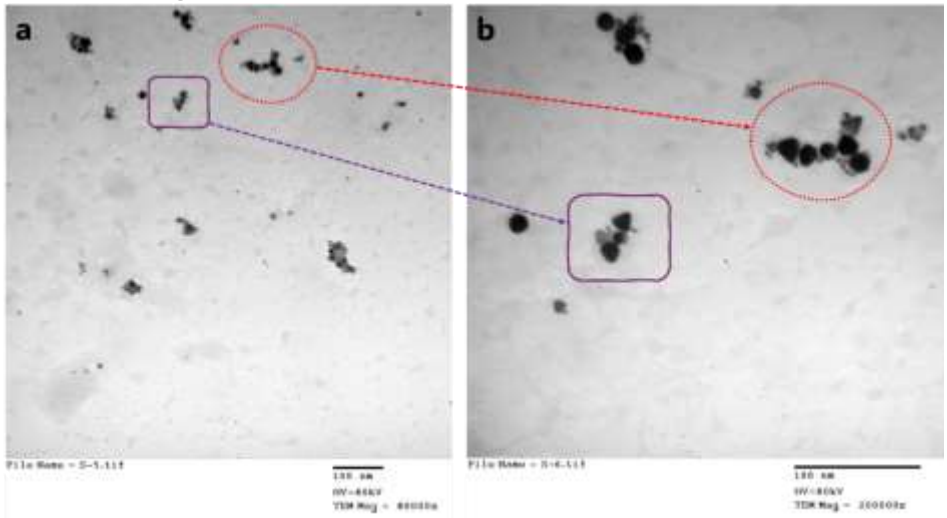

Fig 2. Transmission Electron Microscopy Analysis of Silver Nanoparticles show Morphology and Size Distribution

6.3. Antimicrobial efficacy of Silver Nanoparticle Gel.

The data shows the antimicrobial efficacy of Silver Nanoparticle Gel against Bacillus sp., a bacterial strain isolated from deteriorated archaeological wall paintings. The gel was tested at three concentrations: 500 ppm, 250 ppm, and 125 ppm. The results demonstrate a clear dose-dependent response in the inhibition of bacterial growth. At the highest concentration of 500 ppm, the gel produced a 2 cm inhibition zone, indicating strong antimicrobial activity. The medium concentration of 250 ppm resulted in a 1.5 cm inhibition zone, showing moderate efficacy. Even at the lowest concentration of 125 ppm, the gel maintained some antimicrobial effect, producing a 0.7 cm inhibition zone as shown in table 1 and

figure 3. This gradual decrease in inhibition zone size correlates directly with the decreasing concentration of silver nanoparticles in the gel. The effectiveness of the gel even at lower concentrations suggests its potential usefulness in preserving archaeological artifacts like wall paintings, where minimal intervention is often preferred.

Table 1. Antimicrobial Efficacy of Silver Nanoparticle Gel Against Bacillus sp.: Inhibition Zone Measurements.

Microorganisms	Concentrations PPM / inhibition zone cm		
	500	250	125
Bacillus sp	2 cm	1.5 cm	0.7 cm

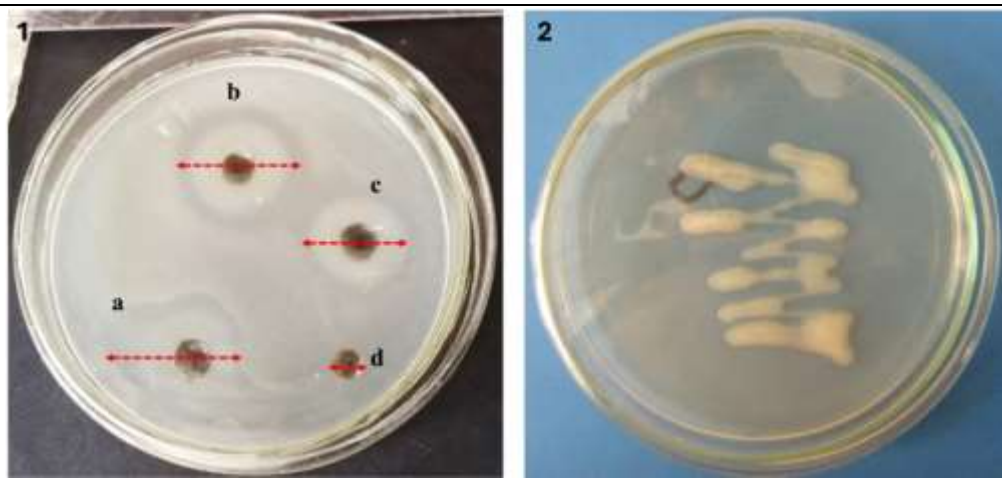

Fig.3. the antimicrobial efficiency of silver nanoparticles gel against *Bacillus sp* in different concentrations (a:500PPM, b: 250 PPM, c: 125 PPM and d: control), **Fig 3**. *bacillus sp* in petri dishes

6.4. Colorimetric measurements.

Table 2. Colorimetric Parameters (L*, a*, b*) and Color Differences (ΔL, Δa, Δb, ΔE) of Pigments Before and After Treatment with Silver Nanoparticles Gel.

Table 2. Colorimetric Parameters (L*, a*, b*) and Color Differences (ΔL, Δa, Δb, ΔE) of Pigments Before and After Treatment with Silver Nanoparticles Gel.

	L	a	b	Δ l	Δ a	Δ b	ΔE
Pigments	Before treatment						
Yellow	44	11	43	-14	1	5	14.9
Dark red	19	19	13	-6	3	6	9
Blue	40	-14	-16	-17	2	3	17.3
White	92	-5	10	4	-5	0	6.4
Black	16	-5	2	-13	-5	-8	16.1
	After treatment						
Yellow	42	12	45	-16	2	7	17.6
Dark red	19	21	14	-6	5	7	10.5
Blue	44	-16	-13	-13	0	6	14.3
White	93	-5	8	5	-5	-2	7.3
Black	15	-7	2	-14	-7	-8	17.5

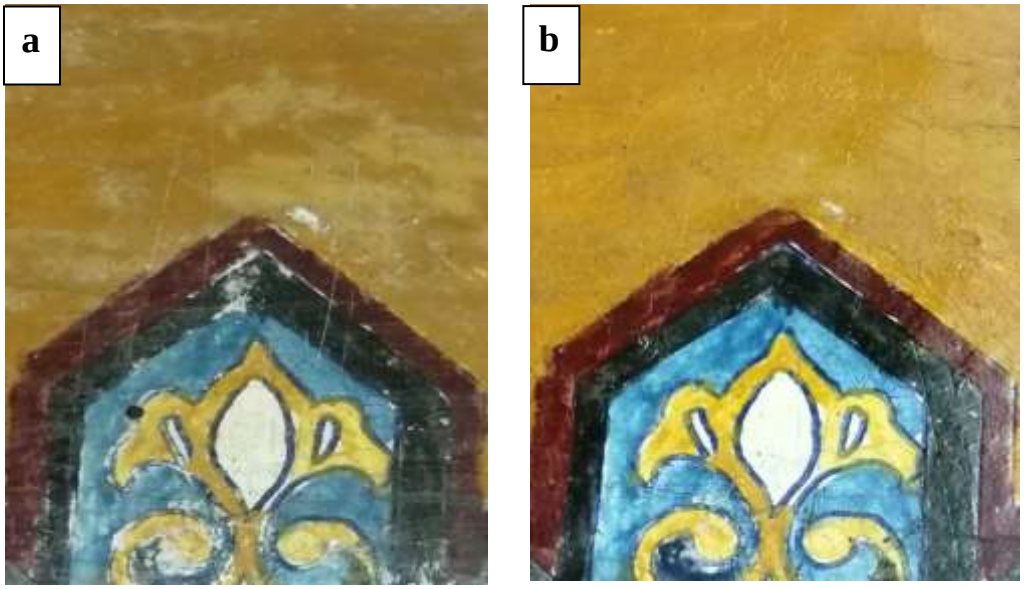

Fig 4. Show the Experimental sample before treatment (a) and after treatment (b)

As shown in table 2, the analysis of the colorimetric changes before and after silver nanoparticles gel treatment reveals varying effects across different pigments. The yellow pigment showed a moderate increase in total color difference, with ΔE increasing from 14.9 to 17.6, primarily due to changes in lightness (L value) and a slight increase in the b value (yellow-blue axis). The dark red pigment experienced a small increase in color difference, with ΔE rising

from 9.0 to 10.5, mainly due to changes in a and b values (red-green and yellow-blue axes). The blue pigment showed an improvement in color stability, with ΔE decreasing from 17.3 to 14.3, indicating better preservation of its original color after treatment. The white pigment maintained relatively stable characteristics with a slight increase in color difference, ΔE changing from 6.4 to 7.3, primarily due to minor changes in lightness (L value) from 92 to 93. The black pigment showed the most notable change among all pigments, with ΔE increasing slightly from 16.1 to 17.5, mainly due to changes in lightness (L value) and a-b coordinates. Overall, while all pigments showed some degree of color change after treatment, the changes were relatively moderate, with black and yellow showing the most significant alterations, while white demonstrated the best color stability. The experimental analyzed samples (5*10 cm) consist of natural oxide pigments: yellow ochre (iron oxide yellow), red ochre (iron oxide red), azurite (copper carbonate), white (gypsum), and black (Charcoal black). Color measurements were performed using the CIELAB color space system, where L* represents lightness (0-100), a* indicates red-green coordinates (+a* = red, -a* = green), and b* indicates yellow-blue coordinates (+b* = yellow, -b* = blue). ΔE* represents the total color difference between before and after treatment.

7. **Discussion**:

7.1. Identification of Isolated Bacteria.

The isolated bacteria, identified as belonging to the genus Bacillus, were found on deteriorated sections of the artwork. In alignment with the present findings, several studies have reported Bacillus species as common microorganisms on degraded cultural heritage sites, often due to their resilience and ability to survive in extreme conditions(Caselli et al., 2018; Pavić et al., 2015). The genus Bacillus is known for its adaptability, particularly in the formation of spores, which help these bacteria thrive on diverse surfaces and under varying environmental conditions(Checinska, Paszczynski, & Burbank, 2015). Ensuring more precise classification within the Bacillus genus, a step taken by other researchers to validate

morphological findings (Berkeley, Logan, Shute, & Capey, 1984; Claus & Fritze, 1989).

7.2. Characterization of Silver Nanoparticles Gel

The silver nanoparticles gel (AgNPs gel) was synthesized through a two-step procedure involving precipitation and ultrasonication. In this study, the size of the silver nanoparticles was confirmed using Transmission Electron Microscopy (TEM), which revealed particles predominantly within the range of 10-50 nm(Jadhav, Dhamecha, Bhattacharya, & Patil, 2016). Studies suggest that smaller nanoparticles (1-50 nm) are particularly effective in antimicrobial applications due to their larger surface area-to-volume ratio, allowing greater interaction with microbial cells(Tessema, Gonfa, & Hailegiorgis, 2024). Similarly, demonstrated that AgNPs with sizes between 10 and 40 nm showed higher biocidal efficiency against pathogenic microbes(Agnihotri, Mukherji, & Mukherji, 2014), aligning well with the particle characteristics in this study(Carrapiço, Martins, Caldeira, Mirão, & Dias, 2023; Gutarowska, Skora, Zduniak, & Rembisz, 2012).

7.3. Antimicrobial Efficacy of Silver Nanoparticle Gel

The antimicrobial activity of the AgNPs gel was tested against the isolated Bacillus strain using the well diffusion method. Results indicated a dose-dependent inhibition, with higher concentrations (500 ppm) creating larger inhibition zones (2 cm). This result is consistent with other studies showing silver nanoparticles' broad-spectrum antimicrobial effects(Kabeerdass et al., 2021). For instance, found a significant correlation between AgNPs concentration and microbial inhibition(Kabeerdass et al., 2021), particularly with Gram-positive bacteria like Bacillus, which often require higher nanoparticle concentrations due to their thicker peptidoglycan layer.

Moreover, observed that AgNPs exert their antimicrobial effect by attaching to bacterial cell membranes, disrupting permeability, and ultimately leading to cell death(Dakal et al., 2016). Such a mechanism may explain the effectiveness observed in this study, especially as the AgNPs gel demonstrated a consistent inhibition zone across concentrations(Bellissima, 2014).

The study's findings align with the emerging consensus that AgNPs are effective antimicrobial agents for cultural heritage preservation (Carrillo-González et al., 2016). However, some researchers, such as(Chobba et al., 2023), advise caution, as prolonged or repeated exposure to high AgNPs concentrations could lead to nanoparticle accumulation on art surfaces, potentially altering optical properties over time(Cappitelli et al., 2020; Du, Pan, Zheng, Zhang, & Hu, 2024).

7.4. Colorimetric Measurements of Pigment Alterations Post-Application of AgNPs Gel

The effect of AgNPs gel on the color stability of various pigments was analyzed using the CIELAB color space system, measuring parameters like lightness (L*), red-green axis (a*), and yellow-blue axis (b*). Table 2 in the study indicated that pigments exhibited varying degrees of color change, with the yellow and black pigments showing the most notable changes post-treatment. This observation suggests that certain pigments may be more sensitive to AgNPs application, a consideration for conservators aiming to minimize visual alterations.

(Fouda et al., 2023; Mostafa, Hamed, Afifi, & Mohamady, 2019), documented similar concerns with nanoparticles used in conservation, particularly regarding color changes in pigments with high chromatic sensitivity. The changes in ΔE (total color difference) noted in the study, particularly for yellow ochre (ΔE increased from 14.9 to 17.6), indicate that AgNPs may interact more with certain oxides, potentially due to surface adsorption effects or slight shifts in reflectance. As further validation, research by(Becerra, Mateo, Ortiz, Nicolas, & Zaderenko, 2019; Fistos, Fierascu, & Fierascu, 2022) explored the compatibility of nanoparticles with mineral pigments, noting that while AgNPs generally preserve chromatic stability, specific formulations may be required to protect pigments sensitive to metal exposure(Becerra et al., 2019).

In contrast, blue and white pigments maintained greater stability, with ΔE changes remaining relatively low. White pigments, based

on gypsum, displayed high resilience to AgNPs exposure(Franco-Castillo, Hierro, de la Fuente, Seral-Ascaso, & Mitchell, 2021).

8. Conclusion.

This study underscores the promising role of silver nanoparticle (AgNPs) gel as a microbial inhibitor in the conservation of historic artwork, particularly in protecting wall paintings from bacterial deterioration. Through a series of controlled experiments, the AgNPs gel demonstrated effective antimicrobial action against *Bacillus* strains commonly found on deteriorated wall painting surfaces, with inhibition correlating positively with gel concentration. Furthermore, colorimetric analysis revealed that while AgNPs gel minimally altered most pigments. Overall, AgNPs gel offers a viable method for microbial control in cultural heritage preservation, but further studies are recommended to refine formulations and mitigate potential pigment sensitivity for long-term applications.

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9. References:

1. Abd El-Azeem, S. A. E.-M. M. (2020). Impacts of climate change on microbial activity in agricultural Egyptian soils. *Climate Change Impacts on Agriculture and Food Security in Egypt: Land and Water Resources—Smart Farming—Livestock, Fishery, and Aquaculture*, 97-114.
2. Agnihotri, S., Mukherji, S., & Mukherji, S. (2014). Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *Rsc Advances, 4*(8), 3974-3983.
3. Becerra, J., Mateo, M., Ortiz, P., Nicolas, G., & Zaderenko, A. P. (2019). Evaluation of the applicability of nano-biocide treatments on limestones used in cultural heritage. *Journal of cultural heritage, 38*, 126-135.
4. Bellissima, F. (2014). Investigations on the use of titanium dioxide and silver nanoparticles to inhibit biodeterioration of stone materials.
5. Ben Chobba, M., Weththimuni, M. L., Messaoud, M., Urzi, C., & Licchelli, M. (2024). Recent Advances in the Application of Metal Oxide Nanomaterials for the Conservation of Stone Artefacts, Ecotoxicological Impact and Preventive Measures. *Coatings, 14*(2), 203.

6. Berkeley, R., Logan, N., Shute, L., & Capey, A. (1984). 12 Identification of Bacillus Species *Methods in microbiology* (Vol. 16, pp. 291-328): Elsevier.
7. Cappitelli, F., Cattò, C., & Villa, F. (2020). The control of cultural heritage microbial deterioration. *Microorganisms, 8*(10), 1542.
8. Carrapiço, A., Martins, M. R., Caldeira, A. T., Mirão, J., & Dias, L. (2023). Biosynthesis of metal and metal oxide nanoparticles using microbial cultures: Mechanisms, antimicrobial activity and applications to cultural heritage. *Microorganisms, 11*(2), 378.
9. Carrillo-González, R., Martínez-Gómez, M. A., González-Chávez, M. d. C. A., & Hernández, J. C. M. (2016). Inhibition of microorganisms involved in deterioration of an archaeological site by silver nanoparticles produced by a green synthesis method. *Science of The Total Environment, 565*, 872-881.
10. Caselli, E., Pancaldi, S., Baldisserotto, C., Petrucci, F., Impallaria, A., Volpe, L., . . . Sassu, G. (2018). Characterization of biodegradation in a 17th century easel painting and potential for a biological approach. *PLoS One, 13*(12), e0207630.
11. Chalmers, F. G. (1996). *Celebrating pluralism: Art, education, and cultural diversity* (Vol. 5): Getty Publications.
12. Checinska, A., Paszczynski, A., & Burbank, M. (2015). Bacillus and other spore-forming genera: variations in responses and mechanisms for survival. *Annual review of food science and technology, 6*(1), 351-369.
13. Chobba, M. B., Weththimuni, M. L., Messaoud, M., Urzi, C., Maalej, R., & Licchelli, M. (2023). Silver Nanoparticles in the Cultural Heritage Conservation *Self-Assembly of Materials and Their Applications*: IntechOpen.
14. Ciferri, O. (1999). Microbial degradation of paintings. *Applied and environmental microbiology, 65*(3), 879-885.
15. Ciferri, O. (2002). The role of microorganisms in the degradation of cultural heritage. *Studies in conservation, 47*(sup1), 35-45.
16. Claus, D., & Fritze, D. (1989). Taxonomy of bacillus *Bacillus* (pp. 5-26): Springer.
17. da Silva, M. T. C. (2017). *Novel Biocides for Cultural Heritage.* Universidade de Evora (Portugal).
18. Dakal, T. C., & Cameotra, S. S. (2012). Microbially induced deterioration of architectural heritages: routes and mechanisms involved. *Environmental Sciences Europe, 24*, 1-13.
19. Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology, 7*, 1831.
20. Dhami, N. K., Reddy, M. S., & Mukherjee, A. (2014). Application of calcifying bacteria for remediation of stones and cultural heritages. *Frontiers in Microbiology, 5*, 304.

21. Du, B., Pan, L., Zheng, M., Zhang, B., & Hu, Y. (2024). Preparation of AgNPs/oregano essential oil composite film and its antibacterial application in the conservation of paper relics. *Inorganic Chemistry Communications, 160*, 112008.
22. El-Banna, A. H., Youssef, F. S., Youssef Elzorba, H., Soliman, A. M., Mohamed, G. G., Ismail, S. H., . . . Osman, A. S. (2022). Evaluation of the wound healing effect of neomycin-silver nano-composite gel in rats. *International Journal of Immunopathology and Pharmacology, 36*, 03946320221113486.
23. El-Ramady, H. R., El-Marsafawy, S. M., & Lewis, L. N. (2013). Sustainable agriculture and climate changes in Egypt. *Sustainable Agriculture Reviews: Volume 12*, 41-95.
24. El-Taher, H. (2022). Community Media and Development in Upper Egypt. *Mass Communication in the Modern Arab World: Ongoing Agents of Change Following the Arab Spring*, 139.
25. Faisal, M., Kandil, D., & Maher, R. A. Reviving Qena's Forgotten Heritage: A Glimpse into Prince Youssef Kamal's Palace at Naj'Ḥammādī.
26. Ferrari, C., Santunione, G., Libbra, A., Muscio, A., Sgarbi, E., Siligardi, C., & Barozzi, G. S. (2015). Review on the influence of biological deterioration on the surface properties of building materials: organisms, materials, and methods. *International Journal of Design & Nature and Ecodynamics, 10*(1), 21-39.
27. Fistos, T., Fierascu, I., & Fierascu, R. C. (2022). Recent developments in the application of inorganic nanomaterials and nanosystems for the protection of cultural heritage organic artifacts. *Nanomaterials, 12*(2), 207.
28. Fouda, A., Abdel-Nasser, M., Eid, A. M., Hassan, S. E.-D., Abdel-Nasser, A., Alharbi, N. K., . . . Abdel-Maksoud, G. (2023). An Eco-friendly approach utilizing green synthesized titanium dioxide nanoparticles for leather conservation against a fungal strain, Penicillium expansum AL1, involved in the biodeterioration of a historical manuscript. *Biology, 12*(7), 1025.
29. Franco-Castillo, I., Hierro, L., de la Fuente, J. M., Seral-Ascaso, A., & Mitchell, S. G. (2021). Perspectives for antimicrobial nanomaterials in cultural heritage conservation. *Chem, 7*(3), 629-669.
30. Gambino, M., Ahmed, M. A.-a. A., Villa, F., & Cappitelli, F. (2017). Zinc oxide nanoparticles hinder fungal biofilm development in an ancient Egyptian tomb. *International Biodeterioration & Biodegradation, 122*, 92-99.
31. Gutarowska, B., Skora, J., Zduniak, K., & Rembisz, D. (2012). Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. *International Biodeterioration & Biodegradation, 68*, 7-17.

32. Horve, P. F., Lloyd, S., Mhuireach, G. A., Dietz, L., Fretz, M., MacCrone, G., . . . Ishaq, S. L. (2020). Building upon current knowledge and techniques of indoor microbiology to construct the next era of theory into microorganisms, health, and the built environment. *Journal of exposure science & environmental epidemiology, 30*(2), 219-235.
33. Husain, S., Nandi, A., Simnani, F. Z., Saha, U., Ghosh, A., Sinha, A., . . . Verma, S. K. (2023). Emerging trends in advanced translational applications of silver nanoparticles: a progressing dawn of nanotechnology. *Journal of Functional Biomaterials, 14*(1), 47.
34. Jadhav, K., Dhamecha, D., Bhattacharya, D., & Patil, M. (2016). Green and ecofriendly synthesis of silver nanoparticles: characterization, biocompatibility studies and gel formulation for treatment of infections in burns. *Journal of Photochemistry and Photobiology B: Biology, 155*, 109-115.
35. Kabeerdass, N., Al Otaibi, A., Rajendran, M., Manikandan, A., Kashmery, H. A., Rahman, M. M., . . . Mathanmohun, M. (2021). Bacillus-mediated silver nanoparticle synthesis and its antagonistic activity against bacterial and fungal pathogens. *Antibiotics, 10*(11), 1334.
36. Koestler, R. J. (2002). Art, Biology, and Conservation 2002: Biodeterioration of Works of Art. Abstract Booklet.
37. Lak, M., Mohammadi, P., & Ghadam, P. (2024). The engineered in situ silver nanocomposite as a surface protective coating with antimicrobial activity used in stony cultural heritage. *Polymer Bulletin*, 1-16.
38. Liao, C., Li, Y., & Tjong, S. C. (2019). Bactericidal and cytotoxic properties of silver nanoparticles. *International journal of molecular sciences, 20*(2), 449.
39. Mesquita, N. (2014). *Identification and control of fungal contamination in ancient heritage documents.*
40. Mostafa, A. M., Hamed, S. A. E.-K. M., Afifi, H., & Mohamady, S. (2019). A comparative study on the color change of pigments due to the consolidation of conventional spectroscopic techniques and laser-induced breakdown spectroscopy. *Applied Physics A, 125*(8), 559.
41. Ogunsona, E. O., Muthuraj, R., Ojogbo, E., Valerio, O., & Mekonnen, T. H. (2020). Engineered nanomaterials for antimicrobial applications: A review. *Applied Materials Today, 18*, 100473.
42. Pavić, A., Ilić-Tomić, T., Pačevski, A., Nedeljković, T., Vasiljević, B., & Morić, I. (2015). Diversity and biodeteriorative potential of bacterial isolates from deteriorated modern combined-technique canvas painting. *International Biodeterioration & Biodegradation, 97*, 40-50.
43. Pei, S., Wu, F., Chen, Y., Ma, W., He, D., Zhang, Q., . . . Feng, H. (2023). Mechanisms of lead-containing pigment discoloration caused by Naumannella cuiyingiana AFT2T isolated from 1500 years tomb wall

painting of China. *International Biodeterioration & Biodegradation, 185*, 105689.

44. Petersen, K., & Klocke, J. (2020). Understanding the deterioration of paintings by microorganisms and insects *Conservation of easel paintings* (pp. 710-730): Routledge.
45. Piñar, G., & Sterflinger, K. (2021). Natural sciences at the service of art and cultural heritage: an interdisciplinary area in development and important challenges. *Microbial Biotechnology, 14*(3), 806-809.
46. Rahman, A. Elite's Hunting in Egypt under the Reign of Mohamed Ali's Family (1805-1952). *Minia Journal of Tourism and Hospitality Research Vol, 3*.
47. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances, 27*(1), 76-83.
48. Reidy, B., Haase, A., Luch, A., Dawson, K. A., & Lynch, I. (2013). Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. *Materials, 6*(6), 2295-2350.
49. Stanaszek-Tomal, E. (2020). Environmental factors causing the development of microorganisms on the surfaces of national cultural monuments made of mineral building materials. *Coatings, 10*(12), 1203.
50. Strzelczyk, A. B. (2004). Observations on aesthetic and structural changes induced in Polish historic objects by microorganisms. *International Biodeterioration & Biodegradation, 53*(3), 151-156.
51. Sulymon, N., Bello, T., Nwaigwe, D., & Bello, A. Bio Deterioration of Building Materials As A Result of Microbial Action: A Case Study of Lagos, Nigeria.
52. Tessema, B., Gonfa, G., & Hailegiorgis, S. M. (2024). Synthesis of Modified Silica Gel Supported Silver Nanoparticles for the Application of Drinking Water Disinfection: A Review. *Results in Engineering*, 102261.
53. Tripathi, N., & Goshisht, M. K. (2022). Recent advances and mechanistic insights into antibacterial activity, antibiofilm activity, and cytotoxicity of silver nanoparticles. *ACS Applied Bio Materials, 5*(4), 1391-1463.
54. Wu, F., Gu, J.-D., Li, J., Feng, H., & Wang, W. (2022). Microbial colonization and protective management of wall paintings. *Cultural heritage microbiology: recent developments, 1*, 57-84.