Evaluation of Anti-biofilm Activity of Silica nanoparticles against dental Streptococcus mutans: In-Vitro Study

Ayaat Nasser Wali[1], Zainab Nashaat AL-Saadi[1]

[1]*Department of Biology, College of Science, , Wasit University, Iraq*

KEYWORDS

Dental caries ,Streptococcus mutans, anti-biofilm activity , silica nanoparticles

ABSTRACT

Background: Dental caries is a prevalent chronic infection caused by cariogenic bacteria that cling to teeth, mainly Streptococcus mutans. These bacteria break down sugars into acid over time, demineralizing the tooth structure. Biofilm as an early colonizer and is the most important bacterium in the formation of dental caries . Materials and Methods: The present study concentrates on isolation and identification of the S.mutans pathogen from patients suffering from dental caries. On specific media, the isolates were cultivated. From human teeth, one hundred dental caries samples are gathered. Using MS-agar (Mitis Salivarius agar), only fifty samples are regarded as positive bacterial isolates to identify the S.mutans isolates, their morphological, cultural, biochemical, and VITEK features were examined. . The microtiter plate method was used to detect the development of biofilms by comparing the characteristics of the isolates. In this work, the median values of optical density (OD) at 630 nm were utilised to employ ELISA to distinguish between S. mutans isolates that developed biofilms and those that did not. To confirm the type of bacteria using molecular identification. Objectives: The purpose of this work is to determine the subminimum and minimum inhibitory concentrations of silica nanoparticles that function as anti-biofilm agents, as they have an impact on bacterial growth and details of silica nanoparticle characterization. Results: In this study considered to be S.mutans are twenty isolates the morphological, cultural, biochemical, and VITEK properties of the bacterial isolates were used to identify them . in this study molecular identification to more confirm of S.mutans the result of PCR showed that 20/20(100%) isolates were PCR positive for 16r RNA gene . in this investigation different isolates were evaluated for their capacity to create biofilm; nine (45%) were judged to be strong producers, seven (35%), to be moderate producers, and four (20%) to be weak producers. the sol-gel method was used in this study to synthesis silica nanoparticles, which were then characterised by XRD, FE-SEM, EDX, and TEM. In this study, Si-NPs were tested against isolates of S.mutans by using a resazurin mediated microtiter plate the results have shown that silica nanoparticles has inhibitory action (MIC) against S. mutans with concentration 0,78 mg/ml and sub-MIC with concentration 0.39 mg/ml. .The anti-biofilm effect of silica nanoparticles on isolates of S. mutans in the current investigation revealed that the biofilm mean of control (biofilm development without silica nanoparticles) was 0.583, whereas the findings for anti-biofilm utilizing sub-MIC of NPs, was 0.185 in microtiter plate method when the (p value) less than 0.05 this proves the NPs effected on the biofilm formation by S.murans and inhibition it . Conclusion: Silica nanoparticles' anti-biofilm action on S.mutans isolates demonstrated the importance of biofilm inhibition.

1. Introduction

Dental caries is the term for the localized breakdown of tooth tissues caused by bacterial fermentation of carbohydrates in the diet. Once the enamel is compromised, caries can move through the dentine and into the pulp. Cavities start off as tiny, demineralized spots beneath the surface of the enamel. Acids, especially lactic acid, which is created when microorganisms ferment dietary carbohydrates, demineralize enamel [1]. S. mutans is a component of the oral microbiota in humans. According to Peterson et al. (2011), there are roughly 100 cells per milliliter (cell/ml) of mutans streptococci in human saliva [2]. One of the main etiological agents of dental caries is thought to be S. mutans Grampositive, facultative anaerobic S. mutans cocci bacteria are visible in chains on gram stain. Dental caries arises from the frequent ingestion of sweets, which alters the biofilm microbiota that typically sits in the oral cavity in a homeostasis and becomes an acidogenic, aciduric, and cariogenic population [3]. Thus, it is believed that dental caries is a dietary-microbial illness that is caused by a cariogenic biofilm and is triggered by frequent consumption of fermentable carbohydrates (sucrose, glucose, fructose, and maltose). [4]. Streptococcus mutans which plays a key role in the formation of the dental plaque biofilm as an early colonizer, producing adhesins which attach the organism to the acquired

pellicle of the teeth, and is the most important bacterium in the formation of dental caries [5] .Silica-based nanoparticles (NPs) are significant in the field because of their tiny size, large surface area, low toxicity, low density, and adsorption capacity of nanotechnology. SiNPs are utilised in several areas of medical research to identify and treat genetic problems as well as regulate diseases [6]. Silica nanoparticles are used as biosensors, enzymatic support, and drug delivery systems. Dental filler, tooth polishing, and the treatment of dental hypersensitivity are among the uses of silica nanoparticles in dentistry. Silica nanoparticles have a deeper penetration into dental tissues and have a quicker influence on dental hypersensitivity [7]. Si PNs completely eliminated the S. mutans bacterium. Furthermore, osteoblasts showed no signs of cytotoxicity. According to these results, functionalising multiple nanoparticles may result in a material with strong mechanical qualities and strong antibacterial activity. In another study, the amount of S. mutans biofilms was decreased by adding silica nanoparticles to a dental adhesive [8]

Thus, the purpose of this work was to create silica nanoparticles and examine their antibacterial and anti-biofilm-forming properties. This would decrease the percentage of cariogenic species and inhibit planktonic bacteria and multispecies biofilm. This research concentrated on the application of silica. Therefore, the objectives of the study determine the minimum inhibitory concentration (MIC), sub-minimum inhibitory concentration (sub MIC) of nanoparticle , that concentration required them to inhibit the biofilm formation.

2. Methodology . Sample collection

The patients with various dental caries (pit, fissure, and dental roots) had swabs taken from their mouths to gather samples. These samples were then placed in peptone water and streaked on mitis salivarius agar(MSA) (HIMEDIA-India) . At the Dental Specialist Center in Al-Kut City, those patients received care. For these cases, the doctor collected the following data: name, age, diabetes, and clinical manifestation.

2.2 Bacterial Isolation and Identification

Bacterial isolates from dental caries samples were streaked on a selective medium (Mitis Salivarius Agar) (HIMEDIA-India) in order to isolate S. mutans. After that, the plates were incubated for 48 hours at 37°C in a candle jar under anaerobic conditions. Until pure culture was obtained, this process was repeated. The VITIK-2 system (bioMerieux, France), biochemical tests, and morphological (microscopic) and cultural traits of the bacterial isolates were used to identify them.

2.3.Detection of Biofilm production • Microtiter plate method

According to [9], biofilm formation test was detected by microtiter plate method.

2.4.Polymerase chain reaction

Using the Easy Pure® Bacteria Genomic DNA Kit, a commercial purification technology, DNA was recovered from twenty clinical isolates of S. mutans. Genomic DNA was extracted in accordance with the company's production process. Using a Taq Readymaster mix kit and following the manufacturer's instructions, an aseptic PCR reaction mix was created using 7µl of DNA extract and a final reaction volume of 50µl. Taq Green Master Mix PCR Kit was used to multiplex PCR each primer. The oligonucleotide forward and reverse primer sequence were employed to identify 16SRNA.

Forward primer CCTACGGGAGGCAGCAGTAG and

Reverse primer CAACAGAGCTTTACGATCCGAAA

2.5. Synthesis silica nanoparticles by biological method

Preparation of silica nanoparticles

The SiNPs nanoparticles were prepared through sol-gel process. The procedure involves 20 ml TEOS was first added into a homogenous mixture of water (20 ml) and ethanol (80 ml) as a solvent. Followed by stirring and subsequent addition of 25 ml of urea (0.06 g dissolved in 25 ml of water) one drop every second with stirring, then leave it for one hour, then add 1N of NaOH until the solution turns foggy (35 ml to 50 ml). Then the precipitate is washed with a decantation several times, It is then dried and burned at a temperature of 800C°[10].

2.5. Silica nanoparticles Characterization

Transmission electron microscopy (TEM) (TEM, Zeiss-EM10C-100 KV). was used to confirm the nanoscale sizes and particle shapes of the Si-NPs powder [11].

Furthermore, the X-ray diffraction technique (XRD) (XRD, PW1730, Philips) was used to determine the crystallite size and crystallographic structure of the produced nanopowders. The crystallite sizes in Si-NPs were calculated using the Debye Scherrer equation [12] .

$D = k \lambda \ \beta COS$

θ

K is equal to 0.9 Scherrer constant, θ, is the angle found using 2θ values, which correspond to the maximum intensity peak at half maximum represented by (λ) and (β), and are the maximum intensity peak in the XRD. series.

Field-emission scanning electron microscopy (FESEM, MIRA III, Tescan) and corresponding energydispersive X-ray spectroscopy (EDX) were applied to analyze microstructures and compositions of the specimens [13] .

2.6.**Determination of the MIC and sub MIC of SiNPs**

The resazurin test was used to determine MIC and sub MIC.this method was based on previous research [12]. In the MHB medium, the components were dissolved at a concentration of 50 mg/mL. Columns 1 through 10 were then used to get the concentrations of each solution by serial dilution, which were 50, 25, 12.50, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, and 0.10 mg/mL, respectively. The concentration of silica nanoparticles was the highest in Column 1 and the lowest in Column 10. Column 11 was the positive control (bacterial suspensions only), and Column 12 was the negative control (media only). Each well contained a total of 200 µL. Following that, each sample was placed into a microtiter plate with 96 wells and incubated for 48 hours at 37 resazurin (CDH ,India) (107.5 mg in 50 ml of DW, manufactured, stored in the dark, and correctly mixed) was added in 10 µL increments to each well of the 96-well plates in order to determine the MIC and sub MIC. The color differences in the wells were then noticed when the plates were incubated for a further 4 hours at 37 °C. The blue, constant columns had concentrations that were either higher than or equal to the MIC value. A higher number of dead cells (MIC) is indicated by the color blue, whereas a higher number of active cells (sub MIC) is indicated by the color pink.

2.7 Effect of silica nanoparticles on biofilm production

- **Microtiter plate method**

  According to [14], [15] effect of silica nanoparticles on biofilm formation was determined by microtiter plate method. The inhibition rate (%) was read as following

  Inhibition rate (%) = OD of control - OD of treated / (OD of control) × 100

  The microtiter plate anti-biofilm assay calculates the proportion of reduced bacterial biofilm in comparison with the control wells where the concentration of silica nanoparticles was fixed at

100% to show their absence. In contrast, negative percentage data show that Si-NPs have no inhibitory effect on the formation of biofilms.

2.8 Statistical analysis

Graph Pad Prism 8 and Microsoft Excel 2013 were used to examine the data results of this investigation for each biological replicate. The threshold for significance at P values less than ≤ 0.05 was employed

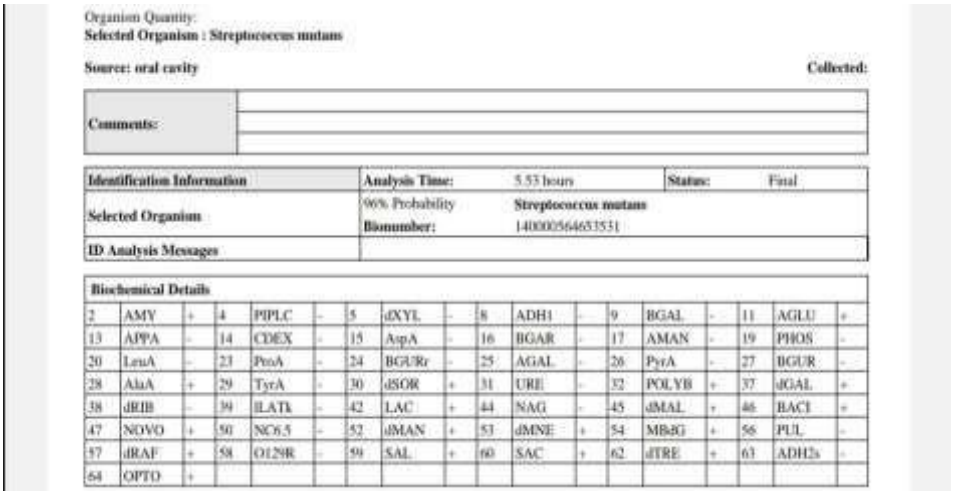
to determine a noteworthy distinction.

3. Result and Discussion 3.1.Collection of samples

In this study, one hundred samples were collected by taking swabs from patients suffering from dental caries at the Dental Specialist Center in Al-Kut City . Of these 100 samples, only fifty samples showed positive results.

3.2. Identification of bacterial isolates

In this study bacterial isolates grown on selective medium (MSA medium) and suspected to *S. mutans* were identified according to their morphological on MSA , cultural characteristics , biochemical test as they show catalase negative and gamma hemolysis on blood agar , and The identification of bacterial isolates as *Streptococcus mutans* was confirmed through examination using the automated VITEK-2 system. Results indicated that out of fifty positive samples, only twenty isolates were categorized as *S. mutans*. As shown on fig (1)



Organism Quantity:
Selected Organism : Streptococcus mutans

Source: oral cavity **Collected:**

Comments:	

Identification Information	**Analysis Time:** 5.53 hours	**Status:** Final
Selected Organism	96% Probability **Streptococcus mutans** **Biomumber:** 14000056465353 1	
ID Analysis Messages		

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	+
13	AIPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	-	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig (1) Results of the identification of *S. mutans* based on their biochemical characteristics were obtained through the utilization of the VITEK-2 identification system.

3.3. Biofilm Production Assay

The results of biofilm formation to *S. mutans* isolate (20 isolates) by microtiter plate method indicated that 9(45 %) were strong for biofilms formation, while 7 (35 %) were moderate and 4 (20 %) were demonstrated as a weak biofilm formation,(0%) were reported as non-biofilm producing isolates as show in fig (2).

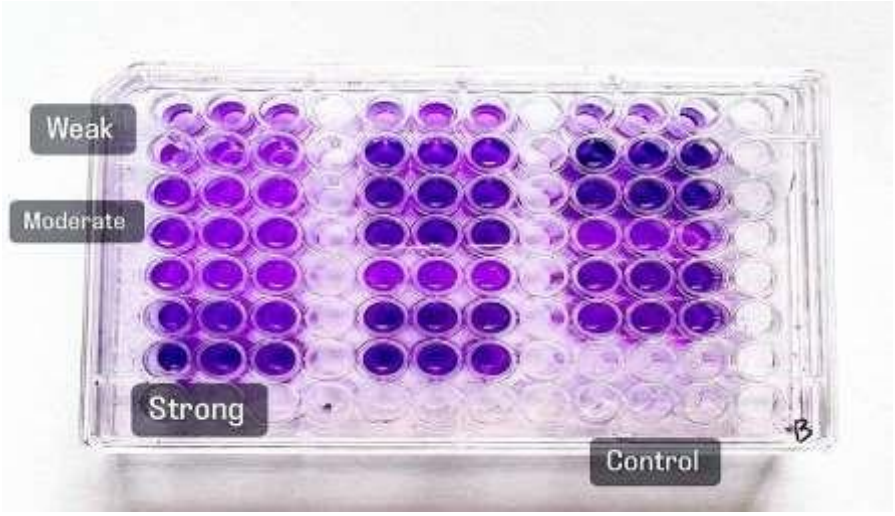


Fig (2) Microtiter plate test of biofilm porduction *Streptococcus mutans.*

3.4. Genotypic method

In this study, Polymerase Chain Reaction (PCR)Techniques amplification of 16S rRNA from 20 isolates was performed to confirm bacterial identification. Primers for conserved region of 16S rRNA were designed and used for amplification of DNA of *S. mutans* isolates by PCR ,then PCR products were separated on agarose gel Figure-3. The result demonstrated that 20/20 (100%) of *S. mutans* had 16S rRNA gene band with 100 bp. Identification of *S. mutans* isolates by using 16S rRNA is more accurate than bacteriological and biochemical assays as show in fig (3).

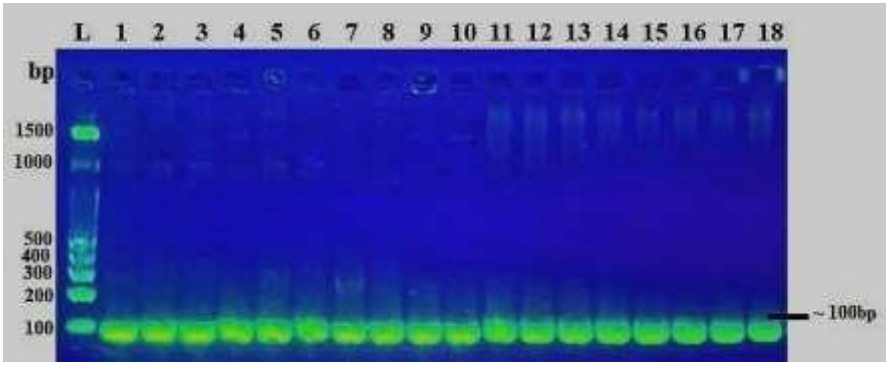


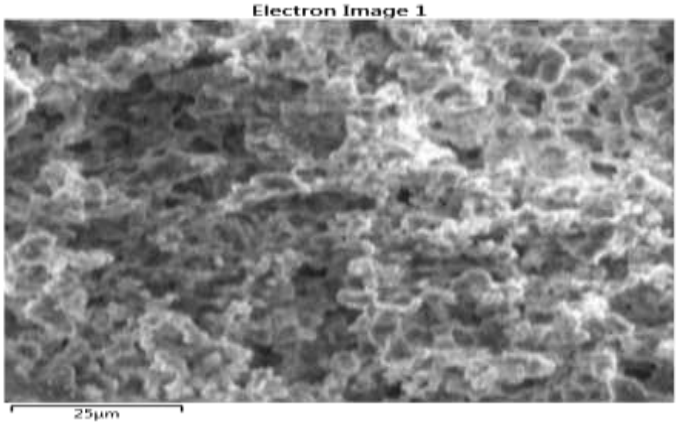
Fig 3-Amlification of a 100-bp 16s rRNA gene of S.mutans isolates on agarose gel (2%) electrophoresed in 75 volt for 1 h, The Lane (M): DNA marker (100-1000 bp)

3.5.Characterization of silica nanoparticles 1-EDX and FE-SEM analysis

In this study, the morphological traits and elemental compositions of the extracted powder during the calcination process were determined using the FE-SEM and EDX techniques. Fig (4a) displays the SiNPs FE-SEM picture. The structures consist of clusters of semi-spherical nanoparticles with sizes ranging of 35.22 nm . The SiNPs particles are shown to be relatively homogenous by this study. The silica nanoparticles' EDX spectra are seen in Fig (4b) . Si and O were discovered to be the basis elements, as the EDX result revealed. The EDS spectrum also shows a trace quantity of Au in addition

to Si and O. Si and O atom contents were approximately 50.43 and 49.57 weight percent, respectively.

a



b

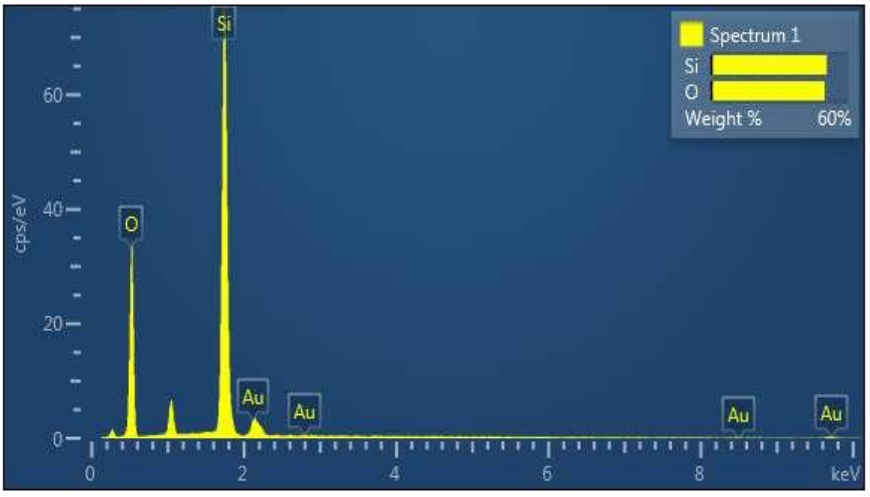


Figure 4: shows (a) the field emission scanning electron microscope (FESEM) image and (b) the energy-dispersive X-ray spectroscopy (EDX) spectrum of silica nanoparticles .

2- X-ray analysis

In this investigation, the XRD is a popular technique for figuring out the size and structure of the particles. The XRD of the several NPs made using the sol-gel is displayed in Fig.(5).The dimensions of the SiO2 NPs were calculated using the Debye-Scherrer equation, where D is the crystal volume, λ is the wavelength of the X-ray radiation (λ = 0.15406 nm) and β is the line width at half-maximum height. The SiO2 NPs had peaks that correspond to the planes 2θ = 21.83 , 2θ = 28.34 , 2θ = 31.36, 2θ = 36.18 , and 2θ = 42.69. 2θ = 44.77, 2θ =46.98, 2θ =48.63, 2θ =57.31, 2θ =60.39 , 2θ =62.14, 2θ =65.30 , 2θ =69.19, 2θ =74.25. The XRD spectra show that the sol-gel-made SiO2 NPs were crystalline. The estimated crystal diameters using Scherrer's formula were around 28.93 nm.

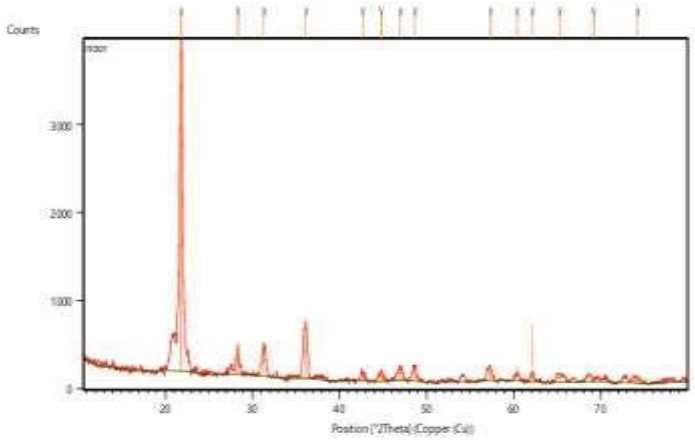
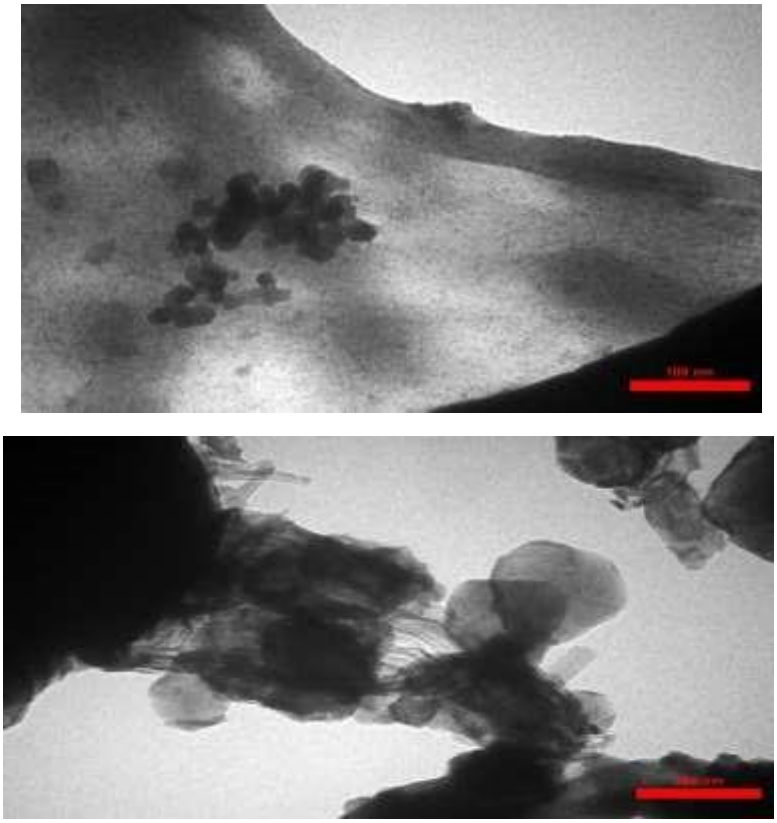


Fig (5) : The X-ray diffraction profile of Si NPs synthesized using the sol-gel method.

TEM analysis

In this study , more thorough understanding of the internal structure of NPs can be gained from TEM pictures. TEM picture of the SiO2 NPs produced using sol gel is displayed in Fig(6 a ,b). The enhanced two-dimensional image of the NPs indicates that the circular structures are composed of separate, unique NPs that are created differently. TEM imaging measures the size of the generated NP, which is approximately less than 100 nm. This size matches the amount determined using the XRD pattern.



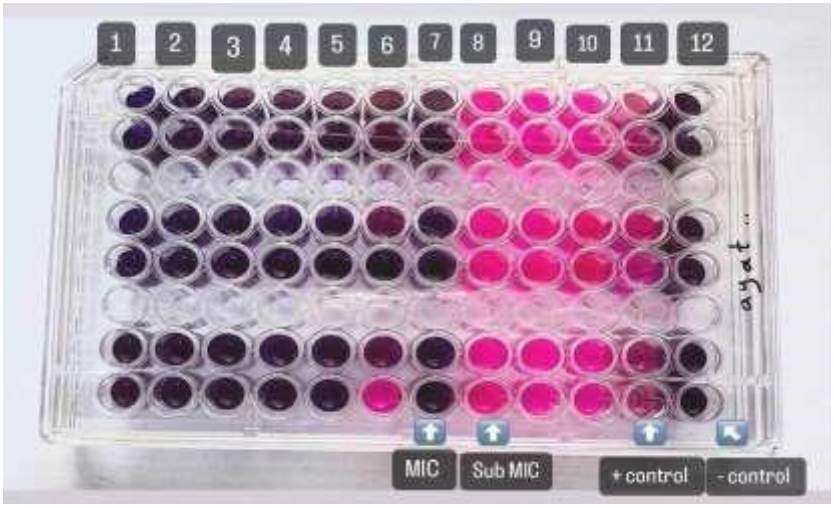
a

b

Fig a,b (6) :TEM micrograph of SiNPs nanoparticles synthesized by sol-gel

3.6.Determination MIC and Sub-MIC of Silica nanoparticles

In this study, Si-NPs were tested against 20 isolates of *S.mutans* by using a resazurin mediated microtiter plate. The color change of the resazurin indicator was used to visually reflect the inhibitory action. The results have shown that silica nanoparticles have inhibitory action (MIC) against *S. mutans* with concentration 0,78 mg/ml and sub-MIC with concentration 0.39 mg/ml. Determination of MIC is important for bacteria to determine the lowest concentration of SiNPs that is necessary for inhibition of visible bacterial growth and anti-biofilm after incubation period at 37°C for 48 h as shown in fig (7).



Fig(7) : Microtiter plate showing MIC and sub MIC detection by Micro-broth dilution method using resazurin dye .

3.5 Testing the Effects of Silica Nanoparticle on Biofilm Formations

3.5.1 Microtiter Plate Methods

In this study ,Table (1) shows the average inhibition rates against *S. mutans* isolates that were triggered by each of the Si-NPs doses. Si-NPs dramatically reduce the formation of biofilms in a concentrationdependent way (Fig. 8). The concentration of Si-NPs at 0.39 mg/ml exhibited the most pronounced inhibitory effect on biofilm formation in microtiter plate method when the (p value) less than 0.05 .This proves the NPs effected on the biofilm formation by *S.mutans* and inhibition it .

Table (1):Determination of biofilm and anti-biofilm in *Streptococcus mutans* using silica nanoparticles

NO.	Biofilm production measured by (OD) at 630 nm	Nanoparticles with biofilm measured by(OD)at 630 nm	Mean
1	0.425	0.155	0.290
2	0.739	0.156	0.448
3	0.497	0.185	0.341
4	0.990	0.129	0.560
5	0.915	0.290	0.603

6	**0.781**	**0.289**	**0.535**
7	**0.468**	**0.105**	**0.287**
8	**0.167**	**0.123**	**0.145**
9	**0.860**	**0.265**	**0.563**
10	**0.168**	**0.124**	**0.146**
11	**0.405**	**0.159**	**0.282**
12	**1.055**	**0.263**	**0.659**
13	**0.126**	**0.125**	**0.126**
14	**0.390**	**0.174**	**0.282**
15	**1.043**	**0.287**	**0.665**
16	**0.355**	**0.148**	**0.252**
17	**0.781**	**0.171**	**0.476**
18	**0.926**	**0.264**	**0.595**
19	**0.115**	**0.139**	**0.127**
20	**0.445**	**0.157**	**0.301**
Mean	**0.583**	**0.185**	**0.384**
P-Value	**0.002**		

Fig(8) : Silica nanoparticles effect on biofilm formation by *S.mutans*

Discussion :

As the most common disease in the world, dental caries is a serious healthcare issue. Dental caries affects nearly all adult individuals. For this reason, it is thought that dental caries is a microbiological illness caused by food, and it requires a cariogenic biofilm and frequent consumption of carbohydrates that ferment, such as sucrose [16]. The most significant etiological bacterium for tooth caries is thought

to be *Streptococcus mutans*. *S. mutans* is an acidogenic and aciduric Gram-positive facultative anaerobic bacteria [17].

In this investigation, specimens collected from the oral cavity of individuals with dental caries were plated on MSA. The composition of this medium promotes the proliferation of Streptococci Identification of bacterial strains was carried out based on their distinctive colony characteristics observed on MSA medium .The findings of this study were consistent with the observations made by [18] in Baghdad City. In this study when subjected to gram staining, these colonies manifest as Grampositive cocci, exhibiting a spherical shape and arranged in pairs or short chains. consistent with the observations made by James and Natalie [19] while the Identification according to the VITEK2 system results, and only twenty isolates (40%) were identified as *Streptococcus mutans*. The findings presented in this study are consistent with the results reported by [20] in AL Kufa city, used in this investigation to assess the test isolates of (20) *S. mutans* more thoroughly for biofilm formation. The current investigation utilized [ELISA] to distinguish between *S. mutans* isolates that produced biofilms and those that did not. with the median optical density (OD) values at 630 nm. Following the evaluation of twenty different isolates for biofilm development, it was determined that 9 (45%) exhibited high biofilm production, 7 (35%) moderate biofilm production, and 4 (20%) low biofilm production . This finding is consistent with the study conducted by [21] Their research indicated that 35 Streptococcus test isolates obtained from dental plaque (43.75%) demonstrated high biofilm production,

In this study, the major subunit of the 16S rRNA gene is encoded by a portion of the gene, which is amplified using particular primer sequences, to facilitate the molecular identification of *S. mutans*. The discovered 20/ 20 has a yield of 100%. The Vitek 2 system's results and the isolate's 100 bp 16sRNA gene of *S. mutans* were in agreement. The percentage of *S. mutans* found by PCR in this investigation is consistent with a study conducted in Baghdad using molecular techniques for 16S rRNA gene PCR-based bacterial identification [22]. while disagree with study in kut done by [23].

In this study, Synthesized silica NPs by sol-gel method and It has been determined characterized of silica nanoparticles by XRD, FE-SEM and EDX and TEM. The EDX and FE-SEM analysi , morphological features and elemental compositions were examined the SNPs' SEM picture. The formations consist of clustered, nanoscale, semi-spherical particles with sizes ranging from 35.22 nm . Si and O were discovered to be the basis elements, as the EDX result revealed. The EDX spectrum also shows a trace quantity of Au in addition to Si and O. The content of Si and O atoms was around 50.43 and 49.57 wt %, respectively. These results agree with study done by [24] The FE -SEM image of SNPs After being calcined, the structures become agglomerated semi-spherical nanoparticles with sizes ranging from 60 to 135 nm. the calcined silica powder's EDX spectrum. Si and O were discovered to be the basis elements, as the EDX test revealed. The EDX spectrum also shows trace amounts of Na and Cu in addition to Si and O. Si and O atom contents were around 52.2 and 27.1 weight percent, respectively . Even though The XRD spectra show that the sol-gol-made SiO2 NPs were crystalline. Tetragonl silica is the crystal phase of this kind. The estimated crystal diameters using Scherrer's formula were around 28.93 nm. our study matches with the reference [25] as their results showed the XRD was used to analyse the sample's structural properties. Their findings tetragonal crystals make up this kind of silica. The computation indicates that the crystallite size of crystalline SiO2 is around 28 nm. More information about the internal structure of NPs can be seen in the most recent TEM photos. TEM imaging indicates that the generated NP is roughly less than 100 nm in size. These results are related to study by [26] through their results were each produced NP has a size of about less than 100 nm, as measured by the TEM imaging .

In current study anti-biofilm effect of silica nanoparticles on 20 isolates of *S.mutans* showed that the biofilm mean of control (biofilm formation without silica nanoparticles) was 0.583 ,while it was 0.185 as a results for anti-biofilm using sub-MIC of NPs, in microtiter plate method when the (p

value) less than 0.05 . This proves the NPs effected on the biofilm formation by *S.murans* and inhibition it . the NPs affected and inhibited *S. murans'* ability to produce biofilms. The current work produced Si.NP and showed that it could inhibit the formation of oral biofilms. By lowering the production of lactic acid and extracellular polymer (EPS), the Si.NP considerably reduced the proliferation of oral planktonic bacteria and multispecies biofilm. It also lessened the cariogenicity of biofilms. As a result, the Si.NP shows promise for a variety of clinical anti-caries applications. This result agree with [27] Where it was explained SiCial was the most successful of the two new silicacore based NPs that were suggested as a potential surface-active anti-biofilm filler for resin-based dental materials. When compared to the thoroughly researched NPs, the addition of SiCial NPs has anti-biofilm effect . In our study, the lowest possible concentration was used that had a significant effect on inhibiting bacterial growth or as an anti-biofilm this result Compatible with [28]. In vitro studies ,more reports have suggested the use of low over the high concentrations . Higher concentrations caused extensive agglomeration, whereas not observed with lower concentrations, indicating that low concentrations are prone to a more homogenous distribution of the NPs in the polymer . The uniform particle dispersion and impregnation in the matrix is crucial to avoid the development of stress concentration areas, impairing the mechanical properties of the resin. When the data are analyzed in terms of bacterial eradication, it is evident that finer surfaces polished with silica nanoparticles are easier to remove *S. mutans* bacteria from than rougher surfaces. This may be useful in shielding teeth against cariogenic bacteria-induced damage. The results of this study show promise for usage as additive constituents in toothpastes, mouthwashes, gels, varnishes, resin composites, etc. that comply with [29] The results presented here demonstrate an advantage of using silica nanoparticles as abrasives for the polishing of dental surfaces to preserve the enamel surfaces on teeth, polishing is frequently done. Shown that the use of silica nanoparticles in the polishing process results in a significant reduction in the roughness of the polished surface. Therefore, by reducing tooth cavities, polishing acts as a primary line of defense against the cariogenic bacteria

4. Conclusion and future scope

We came to the conclusion in our investigation that the clinical isolates of S. mutans produce biofilms, which are important for both pathogenicity and antibiotic resistance. In this study, sol-gel was used to synthesize Si-NPs, which were shown to have good biosafety when compared to common clinical antimicrobials. Si-NPs also changed the species composition of biofilms, lowering the percentage of cariogenic species. Finally, Si-NPs inhibited the proliferation of planktonic and biofilms . Si-NPs therefore show promise for a variety of anti-caries applications.

Conflict of interest: No known conflict of interest correlated with this publication.

Funding: This research did not receive any grant from agencies in the public, commercial, or not-forprofit sectors

Availability of data and materials: The data used and/ or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interest: The authors declared that they have no competing interests

Reference

[1] Marsh, P. D., Lewis, M. A., Rogers, H., Williams, D., & Wilson, M. (2016). Marsh and Martin's Oral MicrobiologyE-Book: Marsh and Martin's Oral Microbiology-E-Book. Elsevier health sciences.

[2] Peterson SN, Snesrud E, Schork NJ & Bretz WA (2011) Dental caries pathogenicity: a genomic and metagenomic perspective. International dental journal 61: 11-22.

[3] Todar K. The Normal Bacterial Flora of Humans .Online textbook of Bacteriology. Accessed on 8th April.2015. http:/textbook of bacteriology. net/ normal flora_3.html.

[4] Rajendran R, Sivapathasundaram B. Shafer's Textbook of Oral Pathology. 7th ed. Elsevier 2014.El Sherbiny GM (2014) Control of growth Streptococcus mutans isolated from saliva and dental caries. Int.J.Curr.Microbiol.App.Sci 3(10): 110.

[5] Clarke JK. 1924. On the bacterial factor in the etiology of dental caries. Br J Exp Pathol 5:141–147.

[6] Halas, N. J. (2008). Nanoscience under glass: the versatile chemistry of silica nanostructures. ACS nano, 2(2), 179183.

[7] Kakoty, V., Sarathlal, K. C., Pandey, M., Dubey, S. K., Kesharwani, P., & Taliyan, R. (2022). Biological toxicity of nanoparticles. In Nanoparticle Therapeutics (pp. 603-628). Academic Press.

[8] Mokeem, L., Balhaddad, A. A., Garcia, I. M., Collares, F. M., & Melo, M. A. S. (2022). Nanoparticle-based antimicrobial for dental restorative materials. In Emerging Nanomaterials and Nano-Based Drug Delivery Approaches to Combat Antimicrobial Resistance (pp. 661-700). Elsevier.

[9] Alquraishi FE, AL-Saadi ZN, Al-Azzawi JA. Detection of Biofilm Formation Among the Clinical Isolates of Klebsiella pneumoniae: Phenotypic and Genotypic Methods.

[10] Guo, Q., Huang, D., Kou, X., Cao, W., Li, L., Ge, L., & Li, J. (2017). Synthesis of disperse amorphous SiO2 nanoparticles via sol–gel process. Ceramics International, 43(1), 192-196.

[11] Hussain, D., Rheima, A.M., Jaber, S., Kadhim, M., 2020. Cadmium ions pollution treatments in aqueous solution using electrochemically synthesised gamma aluminum oxide nanoparticles with DFT study. Egypt. J. Chem. 63, 417–424. https://doi.org/10.21608/ejchem.2019.16882.2026

[12] Al-Saadi, Z. N. (2020). Bactericidal activity of new silver nanoparticles biosynthesized from methicillin-resistant Staphylococcus aureus against clinical MRSA and mssa isolates. Biochemical & Cellular Archives, 20(2).

[13] Jaya, R. P. (2020). Porous concrete pavement containing nanosilica from black rice husk ash. In New Materials in Civil Engineering (pp. 493-527). Butterworth-Heinemann

[14] Namasivayam SKR, Preethi M, Bharani A, Robin G, Latha B. Biofilm inhibitory effect of silver nanoparticles coated catheter against Staphylococcus aureus and evaluation of its synergistic effects with antibiotics. Int J Biol Pharm Res. 2012;3(2):259-65.

[15] Mahdi, H. T. (2024). African Journal of Biological Sciences

[16] Schwendicke F, Dörfer CE, Schlattmann P, Foster Page L, Thomson WM, Paris S. Socioeconomic inequality and caries: a systematic review and meta-analysis. J Dent Res. 2015 Jan;94(1):10-8. [PubMed] [Reference list]

[17] Steinberg, D.; Friedman, M. Sustained-release drug delivery of antimicrobials in controlling of supragingival oral biofilms. Expert Opin. Drug Deliv. 2017, 14, 571–581

[18] Salh, A., Risan, M., & Jasim, H. (2022). Biochemical characteristics and antibiotics susceptibility of Streptococcus mutans isolates from dental caries in baghdad city. International Journal of Advanced Biological and Biomedical Research, 10, 32

[19] James ,C. and Natalie, S .(2014) . Microbiology. A laboratory manual. Pearson Education .

[20] Israa A. and Mahdi H. (2015). Investigation of Streptococcus mutans isolated from dental caries patients. Magazin of Al-Kufa University for Biology.7(1), 2446-2452

[21] Zayed, S. M., Aboulwafa, M. M., Hashem, A. M., & Saleh, S. E. (2021). Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. AMB Express, 11(1), 73.

[22] Flayyih, A. S., Hassani, H. H., & Wali, M. H. (2016). Identification of Streptococcus mutans from human dental plaque and dental caries using 16srrna gene. Iraqi Journal of Science, 552-557.

[23] Al-Emarah, M. A. J., Al-Ruaby, K. J., & Touhali, I. S. (2021). Isolation and Molecular Detection of Virulence Factor (Gtfb) and (Gtfc) to Streptococcus Mutans Associated with Dental Caries in Kut City of Iraq. Annals of the Romanian Society for Cell Biology, 5767-5779.

[24] Larkunthod, P., Boonlakhorn, J., Pansarakham, P., Pongdontri, P., Thongbai, P., & Theerakulpisut, P. (2022). Synthesis and characterization of silica nanoparticles from rice husk and their effects on physiology of rice under salt stress. Chilean journal of agricultural research, 82(3), 412-425.

[25] Joni, I. M., Nulhakim, L., Vanitha, M., & Panatarani, C. (2018, August). Characteristics of crystalline silica (SiO2) particles prepared by simple solution method using sodium silicate (Na2SiO3) precursor. In Journal of Physics: Conference Series (Vol. 1080, p. 012006). IOP Publishing.

[26] Eissa, D., Hegab, R. H., Abou-Shady, A., & Kotp, Y. H. (2022). Green synthesis of ZnO, MgO and SiO2 nanoparticles and its effect on irrigation water, soil properties, and Origanum majorana productivity. Scientific Reports, 12(1), 5780.

[27] Zaltsman, N., Ionescu, A. C., Weiss, E. I., Brambilla, E., Beyth, S., & Beyth, N. (2017). Surface-modified nanoparticles as anti-biofilm filler for dental polymers. PloS one, 12(12), e0189397.

[28] Balos, S., Pilic, B., Markovic, D., Pavlicevic, J., & Luzanin, O. (2014). Poly (methyl-methacrylate) nanocomposites with low silica addition. The Journal of prosthetic dentistry, 111(4), 327-334

[29] Gaikwad, R. M., & Sokolov, I. (2008). Silica nanoparticles to polish tooth surfaces for caries prevention. Journal of dental research, 87(10), 98.