

Evaluation of Antibacterial Properties of Carbon Quantum Dots on *Streptococcus mutans*—In Vitro Study

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Abstract

Background: Carbon quantum dots (CQDs), also known as carbon dots, are extremely small (less than 10 nm), luminous carbon nanoparticles with some sort of surface passivation. These dots have a variety of qualities, including low cytotoxicity, antibacterial action mostly by bacterial cell wall destruction, no environmental risk, and an inexpensive price. In addition to different simple synthetic routes. **Objectives:** Testing the sensitivity of different concentrations of CQDs on oral *Streptococcus mutans* using agar well technique then comparing with 0.2% chlorhexidine and deionized water, to assess the antibacterial properties of CQDs. **Materials and Methods:** Agar well technique was used. No bacterial growth was measured when inhibition zones around each well were seen. No inhibition zone means a full resistance of the bacteria to the tested agent. **Results:** The results revealed that all the tested concentrations of CQDs exhibited good antibacterial activity against *S. mutans* with different inhibition zones when comparing them with chlorhexidine. **Conclusion:** Even low concentrations of CQDs have good antibacterial activity against *S. mutans*, with an easy method of synthesis and affordable price, and this could be a new effective material to be used in dentistry and in antibacterial application.

Keywords: Antibacterial effect, carbon quantum dots, inhibition zone, nanoparticles, *Streptococcus mutans*

INTRODUCTION

Dental caries, which is one of the most common oral diseases,^[1] is a multifactorial disease, that occurs when the acidogenic and aciduric bacteria proportion increases, and this leads to enamel demineralization, which occurs due to the fast metabolism of dietary sugars into acid, leading to a low salivary pH.^[2]

The human oral cavity is home to a large diversity of microorganisms, many of which have a substantial impact on oral and overall health. Numerous bacterial species are linked to various oral disorders, including dental caries.^[3]

Streptococcus mutans, one of the aciduric bacteria, is a Gram-positive anaerobic facultative bacterium, mostly found in our oral cavity, was found to be the predominant bacteria in the caries process^[4,5] as many studies showed the positive association between the presence of *S. mutans* and the initiation of carious lesions by demonstrating these bacterial species in the dental plaque adjacent to carious tooth surfaces.^[6,7]

With the spread of bacterial resistance, which is found to be one of the largest challenges to public health,^[8] nanotechnology has revealed promising possibilities for such problems because nanomaterials have many unique properties due to their highly small-sized particles, with a greater surface area. So it provides a new effective antibacterial agent with minimum toxicity, and cannot be affected by bacterial mechanisms that develop antibiotic resistance. These specific features can provide antibacterial activity with no antibiotic basis, and lead to their use in other branches such as biosensors, optical devices, and gene delivery; moreover, it is also used to improve drugs' therapeutic effectiveness.^[9]

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Nano-sized materials have numerous biological properties, including antimicrobial,^[10] antifungal, and antiviral, which are distinct and unique, which made it possible for nanomaterials to be used in different branches of dentistry.^[11-13] However, there are still some important concerns relating to their toxicological aspects in dentistry, such as silver nanoparticles' toxicity^[14] and other nanomaterials.^[15,16] On the other hand, the good antimicrobial activity with minimum toxicity of Carbon made it possible to anticipate that carbon nanoparticles would act as a good substitution for other materials.^[17] Carbon-based nanomaterials have introduced interest in dental practice, as they lead to refinement in composite materials, implants' strength, cell adhesion and proliferation, and increased antibacterial protection.^[18]

Quantum dots (QDs) also called "artificial atoms" are nano-sized particles with a size range of a few nanometers^[19] and better properties when compared to the same material in the bulk form. Moreover, QDs do not agglomerate, as other nanoparticles do.^[20]

Carbon quantum dots (CQDs) are nanoscale carbon particles that emit fluorescence and have a diameter of less than 10 nm. Despite being discovered at the beginning of the twenty-first century,^[21] CQDs have grown quickly into a potent, nontoxic, affordable, and environmentally friendly nanomaterial with a lot of potential. Numerous applications^[22-25] have been significantly and immediately impacted by their influence on the nanotechnology community. CQDs stand out among the large majority of known nanoscale phosphors for their exceptional physicochemical characteristics, including tunable photoluminescence, straightforward surface passivation and functionalization, and strong biocompatibility.^[26]

Different experimental techniques can be used to create CQDs from synthetic or natural carbon sources. The huge particles of carbon-rich materials will be broken down using "Top-down" methods, whilst small precursor molecules will be used to create CQDs using "Bottom-up" methods.^[27]

CQDs' primary antibacterial efficacy is caused by the oxidative stress that reactive oxygen species cause.^[28]

Up to this date, no other previous study has reviewed the antibacterial effect of CQDs suspension solution on *S. mutans* bacteria, so this study was conducted.

MATERIALS AND METHODS

The CQDs suspension solution is prepared according to a published procedure^[21] with little modification. The preparation method was based on the irradiation, with a 10 J/cm² laser fluence and 1064 nm wavelength, of bulk graphite immersed in pure water. The produced carbon nanostructures were re-irradiated with 532 nm from the

same laser to achieve quantum size. Picosecond Nd-YAG laser was used for the synthesis of CQDs.^[29]

In this experiment, the CQDs suspension solution effect was tested at the different concentrations on the viable counts of *S. mutans*.

Under standardized conditions, stimulated saliva samples were collected from 10 healthy participants to obtain 20 *S. mutans* isolates. All the participants were healthy-looking, with no history of systemic diseases, aged between 20 and 35 years old. After obtaining pure bacterial isolates, they were preserved in nutrient agar in the fridge until they were needed for the study.

Bacterial isolates were inoculated in 10 mL of nutrient broth (pH 7.0), then and they were incubated anaerobically in prepared anaerobic jars containing a range of atmospheric concentrations of CO₂ which was about 5% at 37°C for a specific period of time (18–24 h). This step was done for bacterial activation before the conduction of the experiment.^[30]

To test the sensitivity of *S. mutans* to different concentrations of CQDs suspension solutions, Agar well technique was applied. Then, results were compared to 0.2% chlorhexidine and deionized water as positive as negative controls, respectively.

According to the instructions of Hi-Media, Mueller Hinton Agar media was prepared and used.^[31] Different concentrations of CQDs suspension solutions were prepared according to the same method that was previously mentioned. The tested concentrations were (50, 25, 20, 15, 10, and 5 Ug/mL) of CQDs suspension solution.

In accordance with the Helsinki Declaration and its guiding principles,^[32] Ethical approval was carried out at the Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, following receipt of that approval (Ref. No. 564 on April 17, 2022).

RESULTS

The synthesized CQDs solution appears light yellow under daylight and cyan blue when subjected to UV-light emission in a dark room as shown in Figure 1.

As the QD particles are very tiny, their size and shape were tested using transmission electron microscopes (TEM) at high resolution as seen in Figure 2.

In this experiment, the CQDs suspension solution effect was tested at different concentration on the viable counts of *S. Mutans*, and results showed that there were different inhibition zones (clear zone) of no bacterial growth for different tested concentrations, even in very low concentrations. This indicates a high antibacterial activity of the agent used against the selected bacterial strains, as seen in Figures 3 and 4.

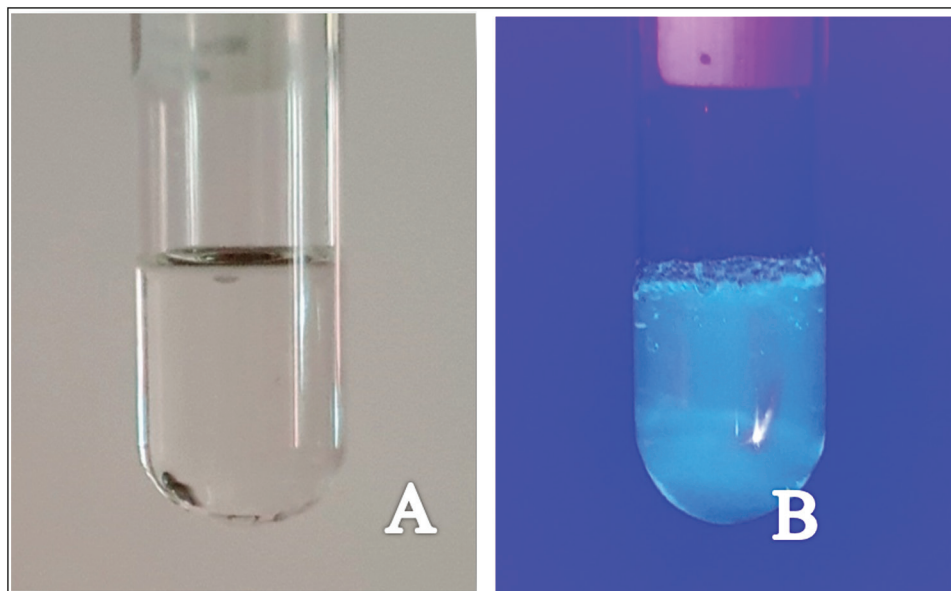


Figure 1: Optical images of CQDs dispersion in water. (A) CQDs under normal daylight exposure. (B) CQDs under UV-light exposure

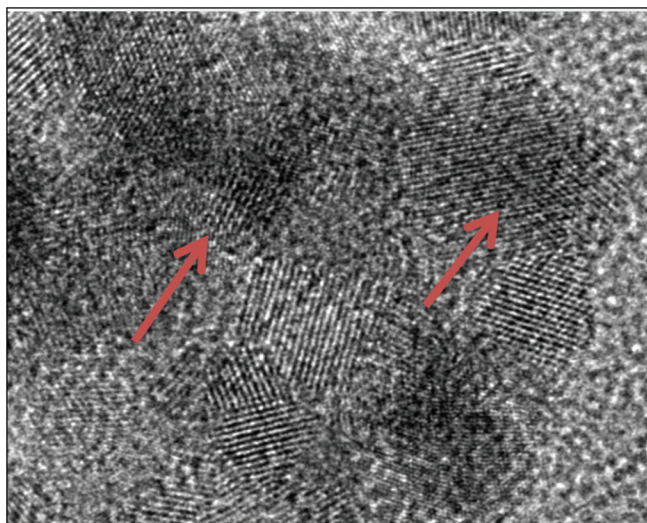


Figure 2: TEM-image of synthesized CQDs at high resolution (2 nm)

All of the tested concentrations of CQDs suspension showed obvious inhibition zones, with less clear zones appearing with lower concentrations, which means that bacterial inhibition diameter increased respectively with increasing the concentrations of the tested solution. Meanwhile, no inhibition zone was found with DW (the negative control), while Chlorohexidine (CHX) which is a gold antibacterial standard *S.mutans* (the positive control) showed a specific inhibition zone that was almost equal to the lowest tested concentration of CQDs, as shown in Figures 5 and 6.

Results revealed that all tested concentrations of CQDs showed different mean values, with different inhibition zones. With increasing the concentration, respective increases in mean values were recorded as seen in Table 1. ANOVA analysis revealed a statistically significant difference between the groups.

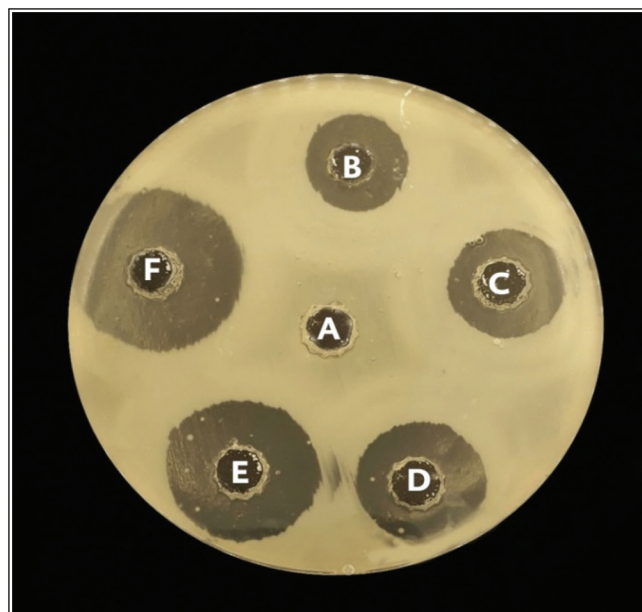


Figure 3: Agar well diffusion method for the sensitivity of different concentrations of CQDs suspension solutions against *S. mutans*. (A) Control negative water. (B) 5 Ug/mL CQDs. (C) 10 Ug/mL CQDs. (D) 15 Ug/mL CQDs. (E) 25 Ug/mL CQDs. (F) 50 Ug/mL CQDs

The multiple comparisons of CQDs inhibition zones among the groups showed that the inhibition zone of 50 Ug/mL was greater than the other lower concentrations, and the difference was highly significant ($P < 0.01$).

All Inhibition zones followed a certain rhythm, as they were increasing with increasing the concentration, moving from 5 Ug/mL until reaching the final tested concentration (50 Ug/mL), and the difference was statistically significant ($P < 0.05$).

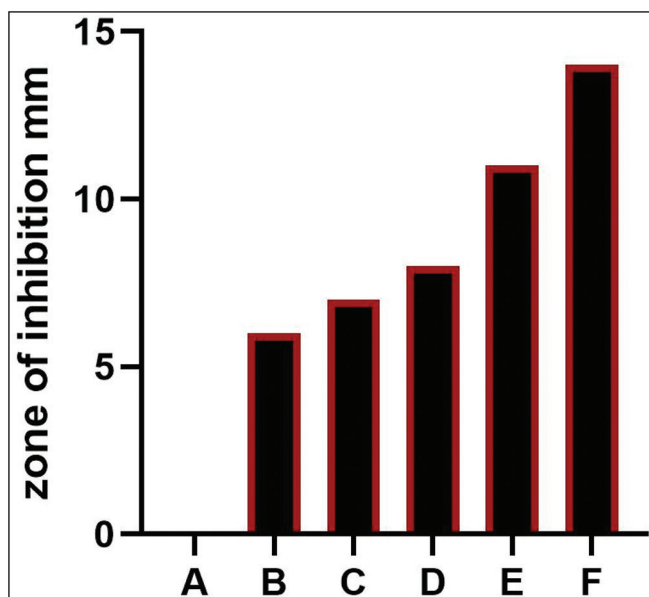


Figure 4: Different concentrations of CQDs suspension solutions against *S. mutans*. (A) Control negative deionized water. (B) 5 Ug/mL CQDs. (C) 10 Ug/mL CQDs. (D) 15 Ug/mL CQDs. (E) 25 Ug/mL CQDs. (F) 50 Ug/mL CQDs

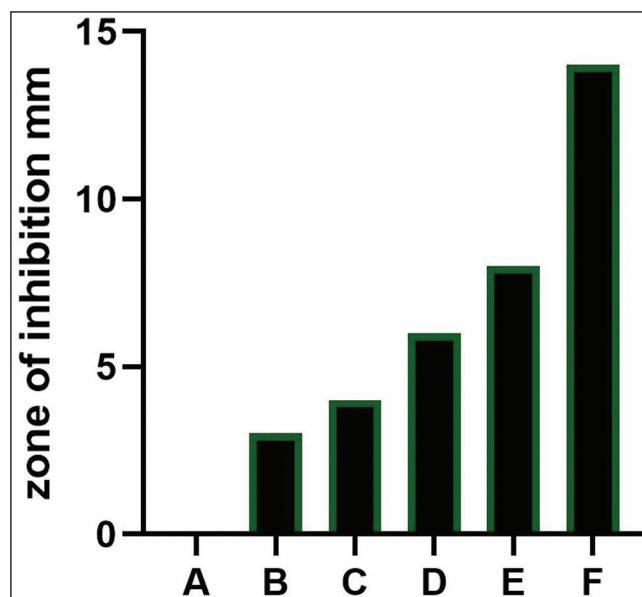


Figure 6: Different concentrations of CQDs suspension solutions against *S. mutans*. (A) control negative (deionized water). (B) Control positive chlorohexidine. (C) 10 Ug/mL CQDs. (D) 15 Ug/mL CQDs. (E) 20 Ug/mL CQDs. (F) 25 Ug/mL CQDs

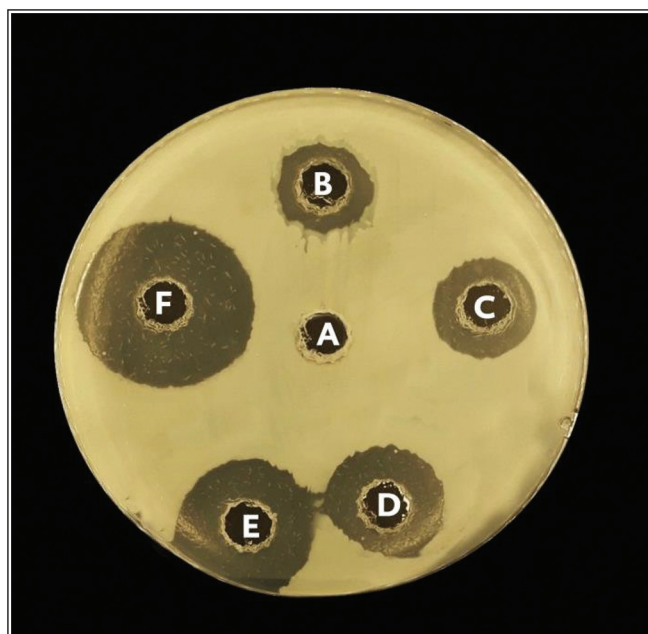


Figure 5: Agar well diffusion method for different concentrations of CQDs suspension solutions against *S. mutans*. (A) control negative (deionized water). (B) Control positive, (chlorohexidine). (C) 10 Ug/mL CQDs. (D) 15 Ug/mL CQDs. (E) 20 Ug/mL CQDs. (F) 25 Ug/mL CQDs

As shown in Table 2, there is a highly statistically significant difference ($P < 0.01$) between deionized water, chlorhexidine, and CQDs.

Multiple comparisons of the inhibition area of chlorhexidine between each concentration of CQDs in Table 2 revealed highly significant differences ($P < 0.01$), except at (5, 10, and 15 Ug/mL) of CQDs which were not

significant. A highly significant difference ($P < 0.01$) was recorded when comparing the inhibition zone of all CQDs concentrations with deionized water.

As shown in Figure 7, The Zone of inhibition of chlorhexidine was a little higher than the lowest tested concentration of CQDs (5 Ug/mL), and it was similar to the second tested concentration of CQDs (10 Ug/mL). However, all higher tested concentrations (15, 20, 25, and 50 Ug/mL) recorded a greater zone of inhibition than CHX, while in comparisons with DW, the inhibition zone of deionized water was zero.

DISCUSSION

Results showed that the mean values of inhibition zones for all the tested concentrations of CQDs suspension solution used in the study were not zero; this indicates a clear antibacterial activity of the agent against the tested bacteria. Regarding the concentrations that were used in the study, the inhibition zones and their mean values were increased with the increase in the concentration, and the maximum value was at the higher concentration tested (50 Ug/mL), and this could be attributed directly to the antibacterial properties of CQDs, which are primarily attributed to the production of oxidative stress brought on by reactive oxygen species.^[28]

When comparing the mean values and the relative effect size of inhibition zones between chlorhexidine with each concentration of CQDs, the (5, 10, and 15 Ug/mL) CQDs concentrations showed no significant difference with CHX, and this could be explained as both agents have approximated antimicrobial activity at

Table 1: Descriptive and statistical analysis (mean and standard deviation) of *S. mutans* among groups. Levene *P* value = 0.002, DF= 7

			Mean	±SD	<i>F</i>	<i>P</i> -value
<i>S. mutans</i>	DW		0.000	0.000	159.547	0.000**
	CQDs	5 Ug/mL	6.500	1.354		
		10 Ug/mL	7.800	1.033		
		15 Ug/mL	9.100	0.994		
		20 Ug/mL	10.200	1.033		
		25 Ug/mL	11.600	0.843		
		50 Ug/mL	13.300	0.949		
	CHX		7.8000	1.229		

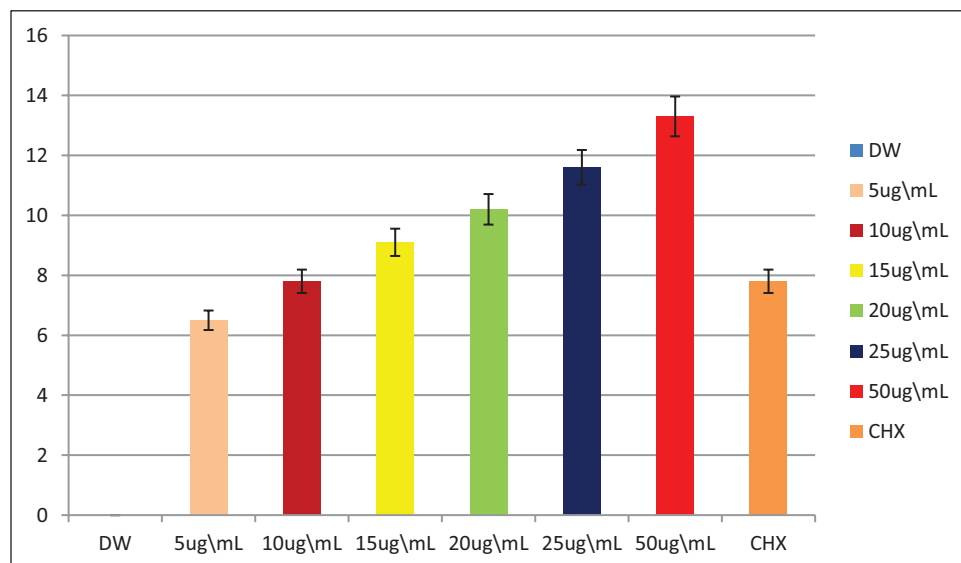
**Highly Significant

Table 2: Multiple pairwise comparisons of *S. mutans* between groups using Dunnett's T3

(I) Groups			(J) Groups						
			5 Ug/mL	10 Ug/mL	15 Ug/mL	20 Ug/mL	25 Ug/mL	50 Ug/mL	CHX
DW	MD		-6.500	-7.800	-9.100	-10.200	-11.600	-13.300	-7.800
		<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CQDs	5 Ug/mL	MD		-1.300	-2.600	-3.700	-5.100	-6.800	-1.300
		<i>P</i>		0.434	0.004	0.000	0.000	0.000	0.540
	10 Ug/mL	MD			-1.300	-2.400	-3.800	-5.500	0.000
		<i>P</i>			0.204	0.002	0.000	0.000	1.000
	15 Ug/mL	MD				-1.100	-2.500	-4.200	1.300
		<i>P</i>				0.423	0.000	0.000	0.326
	20 Ug/mL	MD					-1.400	-3.100	2.400
		<i>P</i>					0.088	0.000	0.005
	25 Ug/mL	MD						-1.700	3.800
		<i>P</i>						0.013	0.000
	50 Ug/mL	MD							5.500
		<i>P</i>							0.000

(I) Group (Tested Agents) – (J) Group (Tested Concentration)

The bold Value referred to (Highly Significant)


Figure 7: Graph of inhibition zones of different concentrations of CQDs in comparison with distilled water and CHX

these concentrations. However, at higher concentrations (20, 25, and 50 U μ g/mL) of CQDs, the results showed a highly significant difference with CHX and this could be attributed to the better antibacterial effectivity of CQDs at higher concentrations.

At low concentrations of CQDs, when a pathogen challenge occurs, for instance, reactive oxygen species function as signaling molecules within the cells. Oxidative stress will cause oxidative damage to proteins, lipids, and nucleotides, and this will result in DNA damage and lipid peroxidation, leading to destroy the cells function physiologically, and eventually bacterial cells death of. Also, it can cause direct lipid peroxidation through free radicals found on the surface of CQDs, thereby destroying cell membranes and causing bacterial cell death.^[33,34]

In addition to ROS, Carbon Dots have other antibacterial mechanisms including DNA binding, photocatalysis, membrane destabilization, physical and mechanical damage, and inhibition of metabolic pathways of the bacteria.

Various research reports have described the importance of CD size and shape for antimicrobial function.^[35] Because bacteria are microscopic microorganisms, and the bacterial membrane pores are nanoscale, small carbon dots can penetrate the cell wall of the bacteria, and the intracellular components of the bacteria can leak through their activities.

Zhang *et al.*^[36] concluded that the bactericidal effect increased with increasing size, and this did not agree with our study results. However, it agreed with this study's results that showed that the antibacterial activity was found to be concentration-dependent.

In 2019, Zhao *et al.*^[37] studied the antibacterial activity of nitrogen-doped CQD against different bacterial species that concluded that positively charged N-CQDs bind to negatively charged bacteria, leading to cell membrane rupture, and it has broad antibacterial activity against different forms of bacteria.

According to Li *et al.* 2020,^[38] the electrostatic interaction between positively charged nanoparticles and negatively charged bacteria results in bacterial membrane rupture, and the CQDs have high inhibitory effects for certain bacterial species (*E. coli* and *S. aureus*).

Also, Malmir *et al.* 2020^[39] found that the antibacterial activity of CQDTiO₂ against *E. coli* was less than *S. aureus*, using the MIC test and characterization of bacterial death.

In 2021, Sun *et al.*^[32] concentrates on the role of non-ROS pathways. Their research provided the size effect's first experimental demonstration. When compared to the other sizes, they discovered that the smaller CGCDs

in these particles significantly increased antibacterial activity. This difference in antibacterial activity may be related to differences in cellular absorption and plasma membrane distribution.

CONCLUSION

Carbon, with its good antimicrobial activity and minimum toxicity, made it possible for CQDs to be a potential antibacterial agent with high antibacterial activity against different bacterial species with the possibility of being introduced in the dentistry field. CQDs revealed a good antibacterial activity against *S. mutans*; therefore, it can be used in oral health programs as an effective preventive material against dental caries.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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