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Inhibitory effect of carboxylated nanodiamond on oral pathogenic bacteria *Streptococcus mutans*

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Abstract

Background: Nanodiamonds (NDs) have been demonstrated to have bactericidal activity on several microorganisms and can be used in various kinds of dental materials. NDs are potential candidates for antibacterial dental materials. However, the possible inhibitory effect of NDs on oral pathogenic bacteria is largely unknown. This study was performed to investigate the inhibitory effects of carboxylated nanodiamond (cND) on *Streptococcus mutans*.

Methods: Fourier transform infrared spectroscopy was used to confirm carboxyl groups on the surface of commercial cND. The inhibitory effect of serially diluted cND on *S. mutans* was evaluated by spectrophotometry and plating methods. *Escherichia coli* was treated as a positive control in spectrophotometry. Chlorhexidine was used as a positive control in plating methods. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were employed to confirm the antibacterial activity of cND.

Results: The results showed that cND exhibited a significant inhibitory effect on *S. mutans*. For *S. mutans*, the minimum inhibitory concentration was 4 µg/ml and the minimum bactericidal concentration was 16 µg/ml. SEM and TEM results indicated that cND functioned as an antibacterial agent, likely due to its ability to disrupt the cell membrane of *S. mutans*.

Conclusion: In conclusion, these findings demonstrated an inhibitory effect of cND on *S. mutans* and suggest its use as a potential antibacterial dental material.

KEYWORDS
antibacterial activity, carboxylated nanodiamond, dental material, *Streptococcus mutans*

1 | INTRODUCTION

Dental caries and periodontitis are the most common oral diseases and contribute significantly to tooth loss worldwide.[1] Although these dental diseases are multifactorial in nature, some oral pathogenic bacteria such as *Streptococcus mutans* have been suggested to be associated with gingival inflammation, periodontal pocket depth, and alveolar bone loss.[2] As one of the most important oral pathogenic bacteria, *S. mutans* fermentatively produces strong concentrations of organic acids, which contribute directly to cavity formation.[3] This gram-positive organism acts as one of the initial colonizers, facilitating the establishment of

other oral pathogenic bacteria and dental plaque biofilms.[4] Thus, *S. mutans* is recognized as one of the primary causative agents of dental caries in human and is of great interest in the field of dental health. Currently, brushing, supragingival and subgingival scaling, and other mechanical methods are still the preferred methods for the removal of dental plaque.[5] Antibiotics have been one of the most effective methods in treating infectious diseases and limiting pathogen spread. However, pathogens often build up antibiotic resistance over time, leading to serious failure of the treatment.[6] The study and development of antibacterial materials demonstrating reduced side effects and less drug resistance represent a new field of interest in biomedical sciences.

Many antibacterial agents have been developed for oral health in recent decades, but there are also many side effects such as drug resistance and oral pigmentation.[7,8] Nanotechnology revolutionized the medical and dental fields by improving the mechanical and physical properties of materials. Nanomaterials are also promising candidates for antibacterial dental materials. Several kinds of inorganic antibacterial agents have been studied, including nanosilver[9] and zinc oxide,[10-12] while nanodiamonds (NDs) have not yet been studied as antibacterial agents in the dental field. NDs are becoming one of the most widely studied nanomaterials due to their unique properties such as hardness, thermal conductivity, and optical transparency, over a wide spectral range.[13] As dental materials, NDs can be used as fillers of various polymers used in dentistry,[14] fillers of scaffolds for guided tissue regeneration of periodontal bone, endodontic treatment and regenerative endodontics, modification of dental implants, etc.[15]

In the past several years, researchers have confirmed that 5-nm ND has a good inhibitory effect on some bacteria. For instance, 5-nm carboxylated nanodiamond (cND) exerted a significant degree of inhibition on gram-negative bacteria *Escherichia coli*.[16] Similarly, 5-nm partially oxidized ND showed good bactericidal activity against *E. coli* and the gram-positive bacterium *Bacillus subtilis*.[17] However, the application of antibacterial NDs in dentistry is still in its infancy. The antibacterial activity of NDs is considered to be related to the chemical groups on the surface of NDs.[16,17] Typically, many chemical groups are functionalized on the surface of ND particles synthesized by detonation technology. These functionalizations are comprised mainly of carboxyl, hydroxyl, ketone, and alkyl groups.[18-20] Although some studies have demonstrated that NDs can cause some adverse effects on cells in vitro,[21] their biocompatibility with microorganisms is still rarely studied. Currently, cND products are commercially available and homogeneously functionalized with carboxyl groups. In vivo studies conducted on zebrafish suggest that the safety of NDs is linked to the functional groups bound to them.[22] cND showed neither cytotoxic nor genotoxic effects on liver, kidney, intestine, and lung human cell lines in this study.[23] The biocompatibility, commercial availability, and potential antibacterial activity of cND make it more possible for dental applications. Thus, our study focused on the potential antibacterial properties of NDs for oral pathogens, and cND was chosen.

As a potential antibacterial nanomaterial, the inhibitory effect of cND on oral pathogens such as *S. mutans* is still largely unknown. This study is a preliminary test of cND for its potential antibacterial properties against oral pathogens and focuses on whether cND can be used as a candidate dental antibacterial nanomaterial. We investigated the potential inhibitory effect of cND on *S. mutans*. The possible mechanisms of the antibacterial activity of cND were also preliminarily explored.

2 | MATERIALS AND METHODS

2.1 | Materials

Carboxylated nanodiamond samples were purchased from Carbodeon Company (UDIAMOND®VOX P). *Streptococcus mutans* (ATCC, UA159) and *E. coli* (ATCC 25922) as positive controls were purchased from the American Type Culture Collection. The characteristics of cND (UDIAMOND®VOX P) were described by the manufacturer as follows: ND crystal size 4.2 ± 0.5 nm, ND content ≥97 wt%, oxidizable carbon content ≤2 wt%, bulk density ~0.5 g/cm^3, pycnometric density 3.1–3.2 g/cm^3, specific surface area 330 m^2/g, crystal lattice constant 0.3573 ± 0.0005 nm, and moisture content typical 2%.

Streptococcus mutans and *E. coli* were stored at −80°C and cultured on brain heart infusion (BHI) nutrient broth and agar (Nanjing Oddofoni). All media and required materials were sterilized at 121°C for 15 min in a vertical pressure steam sterilizer (Shanghai Boxun). All water used for experiments was treated with a Depyrogen ultrapure water system (Millipore).

2.2 | Characterization of cND

The characteristics of cND were determined by Fourier transform infrared spectroscopy (FTIR). The compression method was used for sample preparation, wherein a mixture of cND (approximately 1.5 mg sample) was finely ground together with potassium bromide (1:100 g/g) until well ground and uniform. Subsequently, the sample was placed in a powder pressing machine and treated at 10 MPa for 3 min. The samples were then placed in the magnetic sample holder of a VERTEX70 Fourier Transform Infrared Spectrometer (BRUKER) and experimental parameter settings were as follows: resolution 4 cm^{-1}, 16 scans per sample, and scan range 4000–400 cm^{-1}. Samples were analyzed in triplicate.

2.3 | Determination of the minimum inhibitory concentration and minimum bactericidal concentration

To determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of cND on *S. mutans*, the

following experiments were performed. *Streptococcus mutans* strains maintained at −80°C were used as inoculators for streak plates of BHI culture agar medium and incubated in a chamber (Shanghai Boxun) at 37°C for 24 h. Subsequently, a monoclonal colony was inoculated into BHI broth and cultured for an additional 24 h, after which the bacterial solution was diluted to 10^5 colony-forming unit (CFU)/ml. Separately, a sterile cND suspension at a concentration of 5120 µg/ml was sequentially diluted using an equal amount of distilled sterile water to obtain cND suspensions of 2560, 1280, 640, 320, 160, 80, 40, and 20 µg/ml. Ten microliters of each cND dilution was added to 90 µl 10^5 CFU/ml bacterial culture solution in a 96-well plate in triplicate, which ultimately resulted in final concentrations of cND at 256, 128, 64, 32, 16, 8, 4, and 2 µg/ml. A BHI sterile culture solution of 100 µl with 10^5 CFU/ml bacteria was used as the negative control without cND added.

The MIC assay was performed similarly to the macrobroth dilution method in Mueller–Hinton broth according to CLSI (Clinical Laboratory Standardization Institute), as reported previously.[10,11]

To determine MIC, the 96-well plate was then incubated for up to 16 h in the Anaero Pack System (MGC), with optical density (OD) values determined at a wavelength of 600 nm using a microplate reader (Thermo Fisher) before and after microaerophilic culture. To determine the MBC, 100 µl bacterial culture solutions for each concentration were serially diluted 10-fold, and 100 µl of each treatment was cultured on BHI agar culture medium and incubated for 48 h at 37°C, with subsequent colony counts to determine bacterial inhibition in each treatment.

In the assay, MIC was defined as the lowest concentration of the antibacterial agent, where the resultant increase in OD value was <0.05, while MBC was defined as the minimum concentration of antimicrobial agent necessary to reduce the number of surviving bacteria by more than 99.9%. Data shown in this study represent the means of triplicate tests for MIC and MBC values of cND. *Escherichia coli*, as a positive control, was treated with the above methods and partially with reference to Vassallo et al.[24] In addition, 0.2 wt% chlorhexidine was used as a positive control in the determination of MBC for its antibacterial effects, as reported previously.[25,26]

2.4 | Scanning electron microscopy

Single clone of *S. mutans* was inoculated in BHI liquid medium and grown to logarithmic phase under microaerophilic conditions at 37°C. cND at a concentration of 4× MIC (16 µg/ml) was used to treat *S. mutans* in a 37°C microaerophilic incubator for 16 h. Then, the bacterial samples were centrifuged at 4000 rpm for 2 min at room temperature. The supernatant was removed, and the pellets were rinsed 2 times with phosphate buffer solution (PBS). One more centrifugation was performed to remove excess supernatant, and samples were sent for scanning electron microscopy (SEM) analysis. Measurements were made, and photomicrographs were taken with a scanning electron microscope (S-3000N/H; HITACHI), with reference to Maa et al.[27]
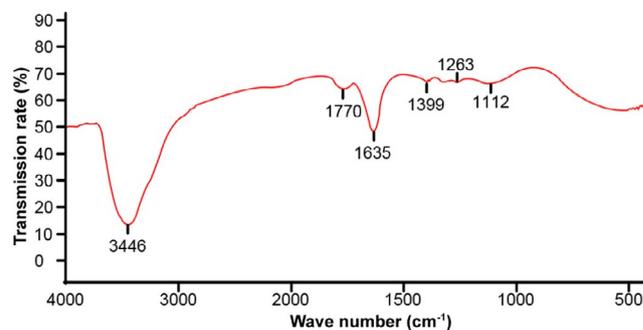
FIGURE 1 Fourier transform infrared spectroscopy revealed that carboxylated nanodiamond (cND) samples were homogeneously functionalized with carboxyl groups. The samples used in this study contained carboxyl groups on their surface. The absorption band at approximately 1770 cm^{-1} was attributed to acid anhydride groups, which may contribute to the antibacterial activity of cND

2.5 | Transmission electron microscopy

Bacterial samples were prepared following the same procedures for SEM. Once the OD600 values of bacterial cultures were approximately 0.8, samples were centrifuged at 1800 *g* for 10 min. After discarding the supernatant, the remaining pellet was retained and put into a microcentrifuge tube and centrifuged at 11,000 *g* for another round. The fixative solution was added slowly along the wall of the tube containing the sample pellet. Care was taken to not disturb the bacterial pellet, and samples were stored in a 4°C refrigerator prior to transmission electron microscopy (TEM) observation (JEM-1400plus; JEOL Ltd.), with reference to Alomary et al.[28]

2.6 | Statistical analysis

The statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc.). The results are expressed as the mean ± standard deviation.

3 | RESULTS

3.1 | Confirmation of carboxylation of cND by FTIR

The chemical groups were validated on the ND samples, which were supposed to be cND. The carboxylation of ND was confirmed using FTIR, determined by characterizing well-resolved absorption bands of C=O stretching and O-H bending of carboxyl groups on the cND surface. As shown in Figure 1, the wavenumber of 1635 cm^{-1} was the main carbonyl C=O stretch, whereas the peak at 1770 cm^{-1} was the C=O stretch of anhydride. While 1403 cm^{-1} was the aromatic C=C stretch, the acidic O-H stretch occurred at 3446 cm^{-1}. Therefore, the results indicated that the cND samples in the experiments were homogeneously functionalized with carboxyl groups.

3.2 | MIC of the cND for *S. mutans* was 4 μg/ml

The OD of the bacterial culture was used to measure the growth of *S. mutans* and determine the minimal contention of cND for inhibiting *S. mutans*. The cND itself in the culture medium could contribute to OD, as it was observed that the OD values increased as the concentrations of cND increased even without culturing (Figure 2), clearly suggesting that the changes in OD values were correlated with the amounts of cND in the assay. At 16 h after culturing, the OD values of the bacterial cultures also increased as the concentrations of cND increased (Figure 2). Compared with the control group, the data indicated that the bacteria grew at different speeds and that the growth of bacteria was inhibited by certain concentrations of cND. To be more visual on showing the growth inhibition, the real OD values were transformed to the differences of OD values by subtracting the average OD value of controls (Figure 3) and by subtracting the average OD value of initial exposure (Figure 4). An apparent cessation of growth was observed when cND was used at a concentration approaching 4 μg/ml (delta OD value <0.05) (Figure 4), which indicated that the MIC of cND for *S. mutans* was 4 μg/ml in the assay. As a positive control, cND also showed evident inhibition of *E. coli*, and the MIC was also 4 μg/ml.

3.3 | MBC of the cND for *S. mutans* was 16 μg/ml

Colony-forming units were used to estimate the number of viable bacteria after culturing in the presence of various concentrations of cND. As shown in Figures 5 and 6A–I, cND at a concentration of 16 μg/ml inactivated more than 99.9% of *S. mutans*. Figure 6J
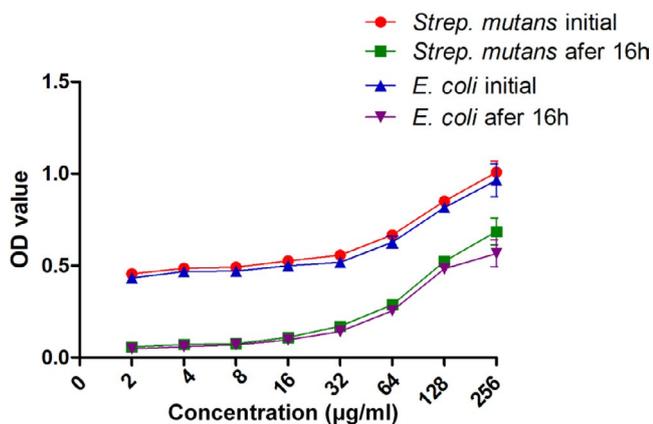
FIGURE 2 Original results of optical density (OD) values measured before and after culturing of *Streptococcus mutans* in the presence of various concentrations of carboxylated nanodiamond (cND) for 16 h. Red and blue dots (initial exposure) represent the OD values taken just after the addition of the indicated concentrations of nanodiamond. Green and purple dots (16 h after exposure) represent the OD values taken 16 h after adding the indicated concentrations of cND and anaerobic cultivation. Three independent repeated tests were conducted. The data were expressed as the mean ± standard deviation

showed the result of positive control, which confirmed the well-known antibacterial effect of 0.2 wt% chlorhexidine. Therefore, according to the definition of MBC, the MBC of cND for *S. mutans* was 16 μg/ml in the assay.

3.4 | SEM results indicated that cND destroyed the cell membrane of *S. mutans*

To study the mechanisms underlying the antibacterial ability of cND, morphological changes in the cell membrane and ultrastructural changes of *S. mutans* after cND treatment were observed by SEM and TEM, respectively. Without cND treatment, the cell membrane of *S. mutans* showed a spherical look with a bright and smooth surface but without obvious cell debris or cell lysis (Figure 7A). However, the cell membrane and the whole structure of the bacteria were evidently changed after treatment with 16 μg/ml cND. After 16 h of cND treatment, the ball-shaped *S. mutans* was dramatically changed into rough granules with debris and irregular shapes. Although some bacteria still had intact cell membranes, slight swelling and obvious cavity formation were noticed on the surfaces of the cell membrane for most of the bacteria (Figure 7B). Therefore, cND at a concentration of 16 μg/ml was able to substantially break the cell membrane of *S. mutans* to cause cell fragmentation even at 16 h after treatment.

3.5 | TEM results demonstrated significant cell membrane injury in *S. mutans* treated with cND

The changes in the ultrastructure of the bacteria after cND treatments were observed by TEM. The cell membrane of the *S. mutans* without cND treatment showed a smooth surface and no obvious cell lysis or cell debris formation was found (Figure 8A). When the bacteria were treated with 16 μg/ml cND for 16 h, the cell membrane and the whole shape of *S. mutans* were significantly changed. The cell membrane and the whole shape of *S. mutans* showed apparent cell membrane injury. While the cell membrane ruptured, more visible cell debris, as well as outflow of cell contents from the gap in the cell membranes and penetration of the cell wall, was observed in most cND-treated *S. mutans* (Figure 8B–D).

4 | DISCUSSION

Nanodiamonds are a class of carbon-based nanoparticles that are rapidly gaining attention, particularly for biomedical applications. However, to date, there have been no studies on the inhibitory effect of NDs on oral pathogens. This study focused on the antibacterial activity of cND against *S. mutans*, one of the most important oral pathogenic bacteria. The study demonstrated that cND had an inhibitory effect on the growth and viability of *S. mutans*, with a detected MIC of 4 μg/ml and MBC of 16 μg/ml in the assays. Morphological changes in the cell membrane and the ultrastructural
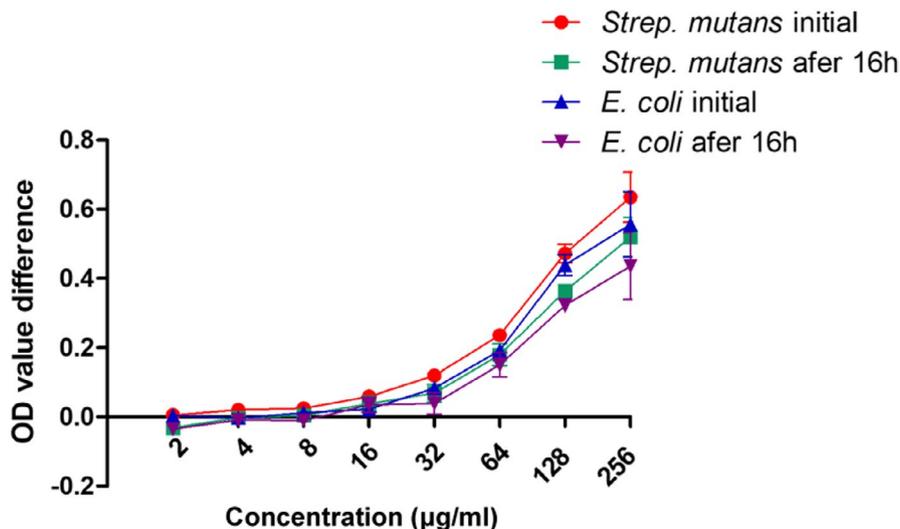
FIGURE 3 Changes in optical density (OD) values by subtracting the average OD value of controls. Red and blue dots (initial exposure) represent differences between the average OD values measured after adding the indicated concentrations of carboxylated nanodiamond (cND) and the average OD values of the corresponding controls. Green and purple dots (16 h after exposure) represent differences between the average OD values measured at 16 h after adding the indicated concentrations of cND and the average OD values of the corresponding controls. Three independent repeated tests were conducted. The data are expressed as the mean ± standard deviation
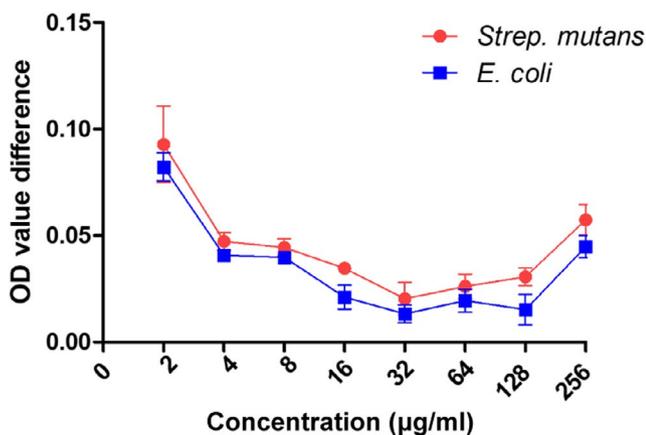
FIGURE 4 Changes in optical density (OD) values by subtracting the average OD value of the initial exposure. Dots represent differences between the average OD values measured at initial exposure and the average OD values at 16 h after adding the indicated concentrations of carboxylated nanodiamond (cND). Three independent repeated tests were conducted. The data were expressed as the mean ± standard deviation
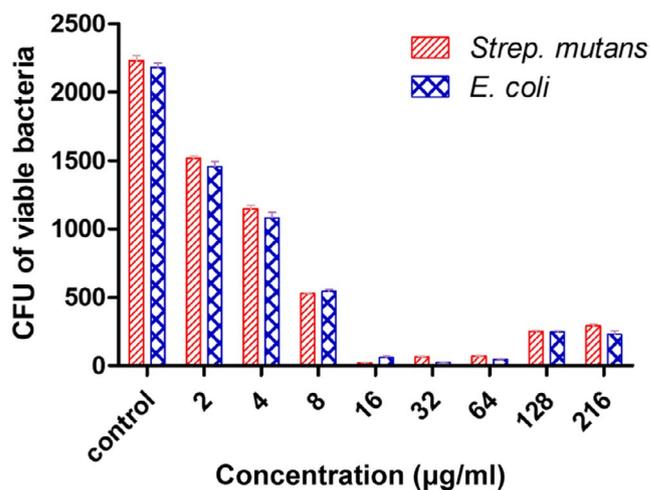
FIGURE 5 Colony-forming unit of viable bacteria after adding the indicated concentrations of carboxylated nanodiamonds. Three independent repeated tests were conducted. The data were expressed as the mean ± standard deviation

changes determined by electron microscopy studies suggested that cND inhibited *S. mutans* by disrupting the cell membrane and inhibiting DNA synthesis. The findings describe the exciting property of cND and suggest it as a potent antibacterial agent against the critical oral pathogenic bacteria *S. mutans*.

The cND used in this study is a kind of high-quality powder with carboxylated, hydrophilic, Zeta-negative surface chemistry and a crystal size of 4.2 ± 0.5 nm (http://www.carbodeon.net). The cND has one main kind of chemical group on the surface (i.e., the carboxyl group), and it demonstrates good physicochemical

properties such as physical adsorption, photostability, and biocompatibility, which will improve permeability, increase stability, and minimize safety issues.[29] Wehling et al[17] and Hees et al[30] performed complex surface modification of NDs in their studies, which may have resulted in many oxygen groups on the surface, providing great convenience for the potentially broad applications of cND.

Minimal inhibitory concentration experiments showed that cND can also be used as an antibacterial agent for grampositive bacteria. There are several kinds of inorganic antibacterial agents that have been studied, including nanosilver, TiO_2,
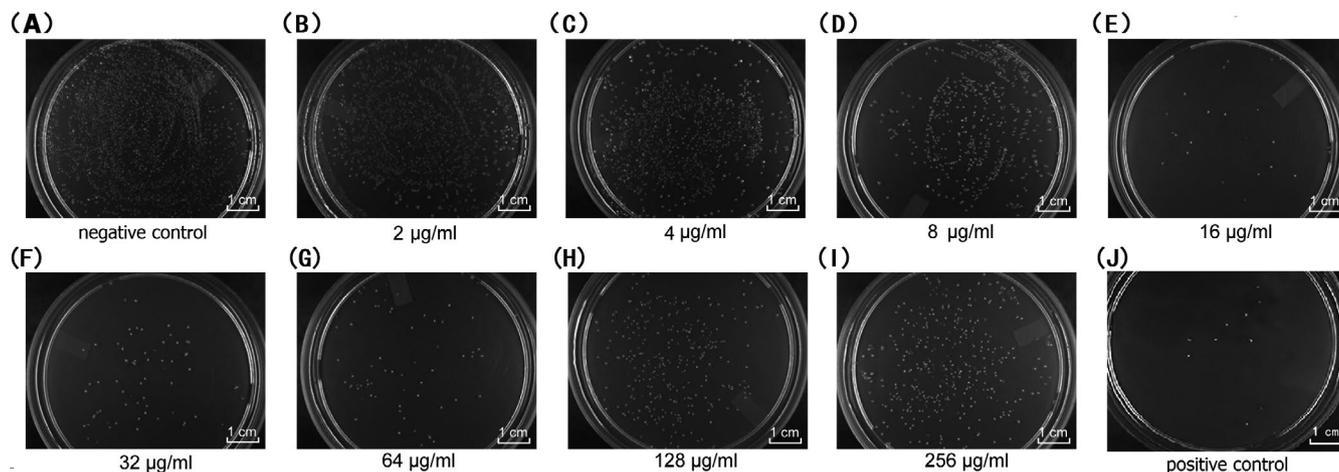
FIGURE 6 Images of *Streptococcus mutans* cultured on brain heart infusion (BHI) agar under the indicated concentrations of carboxylated nanodiamond (cND). Representative images of plates of cultured *S. mutans* in the presence of cND at concentrations of (A) 0 µg/ml (negative control), (B) 2 µg/ml, (C) 4 µg/ml, (D) 8 µg/ml, (E) 16 µg/ml, (F) 32 µg/ml, (G) 64 µg/ml, (H) 128 µg/ml, (I) 256 µg/ml, and (J) 0.2 wt% chlorhexidine (positive control)
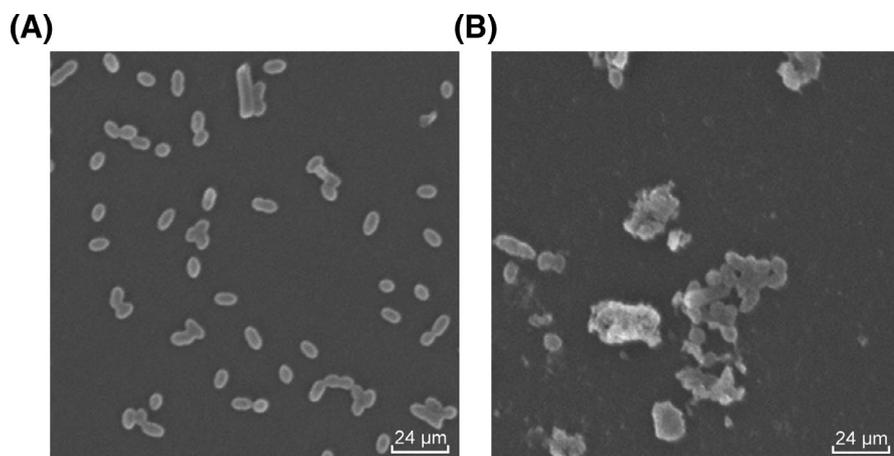
FIGURE 7 Scanning electron microscopy (SEM) observations showed the destruction of the cell membrane for *Streptococcus mutans* treated with carboxylated nanodiamond (cND). Representative SEM image of (A) normal *S. mutans* and (B) *S. mutans* after treatment with 16 µg/ml cND for 16 h. The cell fragments, rupture of the cell wall, and slight swelling of the cell membrane surface after cND treatment was shown
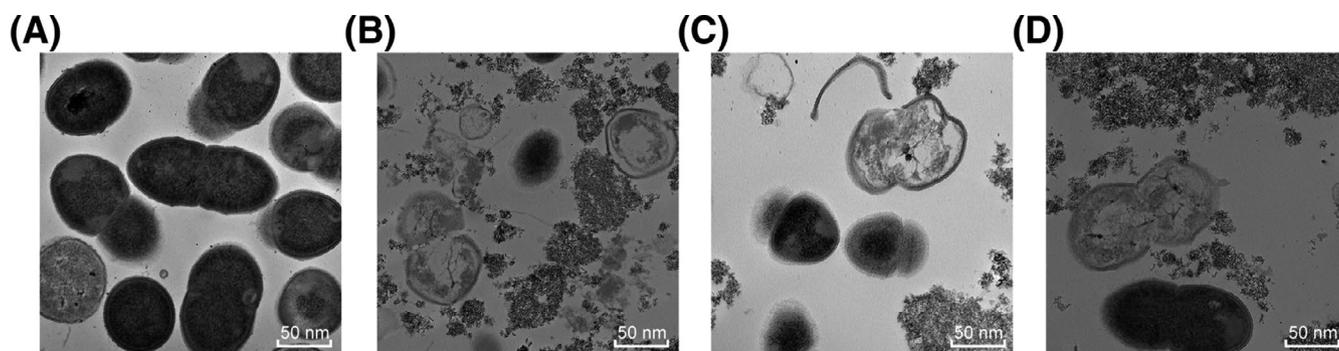
FIGURE 8 Transmission electron microscopy (TEM) observations showed cell membrane destruction and DNA synthesis inhibition in *Streptococcus mutans* after carboxylated nanodiamond (cND) treatment. Representative TEM image of (A) untreated *S. mutans* with regular shape and intact surface and (B–D) *S. mutans* after 16 µg/ml cND treatment for 16 h. More visible cell debris, destruction of cells during the mitotic phase, formation of voids and cytoplasmic compounds outside the cell, and penetration of the cell wall were observed in cND-treated bacteria

and zinc oxide,[6,9-11,31,32] while cND has not yet been incorporated into applications in the dental field. There are four theories on the bactericidal mechanism of silver ions: electrostatic effects, metal dissolution, photocatalytic effects, and contact inhibition.[31] NanoTiO$_2$ is an N-type semiconductor material with strong reductive and oxidative effects, achieving cell inactivation via photocatalytic activity while also degrading the toxic components released by inactivated bacteria.[32] The antibacterial

mechanism of ZnO is unclear, and there are several hypotheses. One theory focuses on reactive oxygen species formation as an antibacterial mechanism, wherein generated hydrogen peroxide penetrates bacteria and damages the cell structure, causing cell lysis and bacterial inactivation.[33] In a similar manner, the antibacterial mechanism of cND has yet to be elucidated, though its activity is clear.

It is possible that the high surface-to-volume ratio and energy absorbance properties of cND are responsible for these antimicrobial effects. The bactericidal activity of partially oxidized NDs may be due to the interaction of the oxygen functional groups on the active surface and the cell components,[17] while the anisotropy of the surface charge distribution on the surface of NDs facilitates surface changes of bacterial membranes. The interaction between cND and *E. coli* might be due to the highly active surface, which leads to the destruction of the outer cell membrane, causing cell death. While *E. coli* is a gram-negative bacterium, *S. mutans* is gram positive, and thus, the antibacterial effects may be exerted through different means, as the cell surface properties can differ between these groups of bacteria. Wehling et al[17] demonstrated that partially oxidized NDs have antibacterial effects on *B. subtilis*, another gram-negative bacterium, which also underscores the crucial roles of oxidation groups on NDs in conferring bacterial inhibition.

Minimum bactericidal concentration refers to the lowest concentration of a given agent, which can kill 99.9% of bacteria in tests. The results showed that the MBC of cND was 16 μg/ml, which was nearly 4 times the MIC of cND, suggesting that cND in the study is a potent biocidal agent. Interestingly, the bactericidal activity did not depend closely on the concentration. In comparison with the concentrations between 4 and 64 μg/ml, the bactericidal activity outside this range decreased slowly. Theoretically, the higher the concentration is, the stronger the antibacterial activity of the agent. For example, 6 nm or 25 nm NDs ranging from 0 to 200 μg/ml can kill extracellular and intracellular uropathogenic *E. coli* in a dose-dependent manner.[34] However, in the results, the antibacterial activity did not become stronger as the concentration increased, which might be attributed to certain physical–chemical properties of cND.

Ultimately, any of these physicochemical properties could be responsible for the decreased biocidal activity observed for cND of >64 μg/ml. To clarify which properties are responsible for this activity, further studies are needed to determine if aggregation of cND occurs in suspensions at higher concentrations, but it is confirmed here that the bactericidal activity will decrease as the concentration increases. According to recent studies, the antibacterial activity of cND is mainly due to its functionalized surface, but the protein in the culture medium might modify its activity.[16,17] Therefore, we hypothesized that there was more aggregation during the coculture as the concentration of cND increased, which led to reduced surface interactions with *S. mutans*. Moreover, further factors such as the protein content in the culture medium might also modify the antibacterial activity of NDs.[16,17]

Many antimicrobial agents rely on the voltage of the bacterial membrane surface to achieve antibacterial effects.[35,36] However, these mechanisms can vary. The antibacterial action of cND on *E. coli* has been reported to be achieved through its attachment to the cell membrane surface and its ability to change protein structures on the bacterial wall.[16] Wehling et al[17] found that the antibacterial activity of ND was linked to the presence of partially oxidized and negatively charged surfaces, especially those containing acid anhydride groups. Because the oxygen-containing groups on the surface of partially oxidized NDs and other types of NDs are variable,[37] it is difficult to determine which chemical groups play a role in the bactericidal activity. From our results, cND at a concentration of 16 μg/ml not only inhibits the reproduction of bacteria, but also causes the cell membrane to be detached from polarization quickly, thus inhibiting the synthesis of cell membrane surface macromolecules and ultimately leading to cell death. It is also possible that cND can destroy nuclear materials when it is attached to the DNA of bacteria.

The results of SEM and TEM studies showed that the cell membrane of *S. mutans* was ruptured after cND treatment, and the surface of the bacteria was pore forming. However, it could not be determined whether cell death occurred before or after cell membrane damage. TEM images showed that cND could penetrate into the cell wall, but whether this penetration could cause the destruction of cell nuclei is still unknown. Gram-positive bacteria are destroyed by cND mainly through the disruption of the cell membrane, that is, the formation of transmembrane pores and the rupture of the cell membrane, which ultimately leads to bacterial cell lysis. In addition, mass cell death produced a large amount of cell debris after cND treatment, but some cells still retained their normal shape, suggesting that cells in the growth phase may affect the susceptibility of bacteria to antimicrobial agents. Furthermore, cND seems to be able to penetrate into the cell and cause the destruction of cell nuclei. Similarly, Cloete et al[18] found that carboxylic acid groups in acid anhydride forms were highly reactive toward nucleophilic additions, whereas Xing et al[21] found that NDs could cause DNA damage. However, the exact mechanisms of cND against gram-positive bacteria such as the precise location of the action sites between cND and bacterial cells still need to be deeply studied.

In summary, the data presented herein demonstrate that commercial cND exhibited a significant inhibitory effect on *S. mutans*. The MIC of cND on *S. mutans* was 4 μg/ml and the MBC was 16 μg/ml, suggesting that this material is a potent antibacterial agent. A preliminary exploration of the mechanisms of bactericidal activity suggests that cND may exert its effects by disrupting the cell membrane and interacting with the nucleus of *S. mutans*. This study is of great significance for the research and development of nanosized antibacterial dental materials such as fillers for various polymers for filling of dental caries, scaffolds for guided tissue regeneration of periodontal bone, and modification of dental implants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are included in this article.

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