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Investigation of Antibacterial Activities of Pristine Multi-Walled Carbon Nanotube Against Periodontitis-Associated Bacteria and Imaging by Transmission Electron Microscopy: An in vitro Study

Saf Çok Duvarlı Karbon Nanotüpün Periodontitis İlişkili Bakterilere Karşı Antibakteriyel Aktivitelerinin Araştırılması ve Transmisyon Elektron Mikroskobu ile Görüntüleme: Bir in vitro Çalışma

Yeşim Özkan Dağlıoğlu[1*], Mustafa Cihan Yavuz[2]

[1]Department of Molecular Biology and Genetics, Faculty of Arts and Science, Ordu University, Ordu, Turkiye.
[2]Department of Periodontology Dentistry, Faculty of Dentistry, İstanbul Medeniyet University, İstanbul, Turkiye.

ABSTRACT

It is very important to prevent periodontitis, which is a common oral disease all over the world, with the use of non-toxic and strong antibacterial activities. Recent years, nanomaterials are widely preferred in dentistry due to their biocompatible structure, durability, lightness and low toxicity. The aim of this study is to investigate the antibacterial activity of MWCNTs, with their strong antibacterial activity, low toxicity and high biocompatibility, against periodontitis-causing bacteria. In this in vitro study, the antibacterial activity of MWCNTs against *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*, oral pathogens that cause periodontitis, was evaluated by the agar well diffusion method, and ultrastructural changes were evaluated by TEM. In TEM analyses, images of cells internalizing MWCNT were observed in similar patterns under all incubation conditions. Untreated oral pathogen cells have a smooth surface. The nuclei of the cells are distinct and centrally located, their cytoplasm is regular, and the cell wall and cytoplasmic membrane structure are seen as a whole. Both pathogens indicated antibacterial activity at different concentrations and exposure times of MWCNTs. At low concentrations (5 and 10µl) and prolonged exposure time, it exhibited significant antibacterial activity in both pathogens ($P<0.05$). In both pathogens, the antibacterial effect decreased with increasing MWCNT concentration and longer exposure time. In TEM examinations, intense cell wall and membrane damage is observed in pathogenic cells. Lysis and ghost cell formations were observed in some cells.

Key Words

A. actinomycetemcomitans, MWCNT, antibacterial effect, TEM, periodontitis, *P. intermedia.*

ÖZ

Tüm dünyada yaygın ağız hastalığı olan periodontitisin toksik olmayan ve güçlü antibakteriyel aktivitelerin kullanımı ile önlenmesi çok önemlidir. Nanoteknolojinin hızla gelişmesiyle birlikte nanomalzemeler biyouyumlu yapıları, sağlamlıkları, hafiflikleri ve düşük toksisiteleri nedeniyle diş hekimliğinde oldukça fazla tercih edilmektedir. Bu çalışmanın amacı, çok duvarlı karbon nanotüpler (MWCNT'lerin), güçlü antibakteriyel aktiviteleri, düşük toksisiteleri ve yüksek biyouyumlulukları ile periodontitis etkeni bakterilere karşı karşı antibakteriyel aktivitesinin araştırılmasıdır. Bu in vitro çalışmada, MWCNT'lerin periodontitise neden olan oral patojenlerden *Prevotella intermedia* ve *Aggregatibacter actinomycetemcomitans*'a karşı antibakteriyel aktivitesi agar kuyu difüzyon yöntemi ile, ultrayapısal değişiklikler ise TEM ile değerlendirilmiştir. TEM analizlerinde, saf MWCNT'yi içselleştiren hücrelerin görüntüleri, tüm inkübasyon koşullarında benzer şekillerde gözlenmiştir. Tedavi edilmeyen oral patojen hücreleri pürüzsüz bir yüzeye sahiptir. Hücrelerin çekirdekleri belirgin ve merkezi yerleşimli, sitoplazmaları düzenli, hücre duvarı ve sitoplazmik membran yapısı bir bütün olarak görülmektedir. Her iki patojen de saf MWCNT'lerin farklı konsantrasyonlarında ve maruz kalma sürelerinde antibakteriyel aktivite göstermiştir. Düşük konsantrasyonlarda (5 ve 10µl) ve uzun maruz kalma süresinde (72 saat), her iki patojende de önemli antibakteriyel aktivite sergilemiştir ($P<0.05$). Her iki patojende de antibakteriyel etki, MWCNT konsantrasyonunun artması ve maruz kalma süresinin uzamasıyla azalmıştır. TEM incelemelerinde patojen hücrelerde yoğun hücre duvarı ve membran hasarı görülmektedir. Bazı hücrelerde lizis ve hayalet hücre oluşumları gözlenmiştir

Anahtar Kelimeler

A. actinomycetemcomitans, MWCNT, antibacterial effect, TEM; periodontitis, *P. intermedia.*

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Correspondence to: Y.Ş. Dağlıoğlu, Department of Molecular Biology and Genetics, Faculty of Arts and Science, Ordu University, Ordu, Turkiye.

E-Mail: yozkan52@gmail.com

INTRODUCTION

Periodontitis is a chronic inflammatory disease characterized by the destruction of the periodontium, the tissues surrounding and supporting the tooth, including the periodontal membrane, alveolar bone, cementum layer and gingiva, as a result of bacterial induction[1]. This disease is one of the most common causes of tooth loss [1]. There is a close relationship between periodontitis and oral pathogenic bacteria. *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* are the most notable pathogens in periodontitis. *A. actinomycetemcomitans*, which is a gram-negative bacterium, is found in the oral cavity of most people [2]. The prevalence of this pathogenic bacterium varies greatly according to the region of residence, age and lifestyle (especially diet) [3]. Prevotella species are anaerobic gram-negative bacteria of the phylum Bacteroidetes, which also includes the clinically important genera Bacteroides and Porphyromonas [4]. The strains of the genus Prevotella are considered among the commensal bacteria because they are frequently found in healthy humans and rarely cause infection. *P. intermedia* species, which is the most common pathogen in infections of periodontic and endodontic origin. This pathogen is the most isolated species of the genus prevotella from dental plaque, and its role in infections such as periodontitis is still unclear. Some reports have noted a linear relationship between *P intermedia* pathogen and progressive periodontitis [5].

With the development of nanotechnology, the branch of nanomedicine has penetrated into our daily lives. As in many medical fields, various nanoparticles are used in dentistry due to their biocompatible and antibacterial properties. The antibacterial/antimicrobial properties of these nanoparticles are investigated before use [6-9].

In the early 2000s, as a result of the rapid progress of nanotechnology, the search for improving human health also manifests itself in dentistry. In particular, antibiotic resistance is one of the biggest problems in medicine in recent years. For this reason, the development and application of alternative products to antibiotics has attracted a lot of attention. For this purpose, it has brought to mind the question of whether MWCNTs, which are materials with high biocompatibility and low toxicity, can be applied in dentistry. MWCNTs unusual chemical, structural, electronic and optical properties make them useful for medical applications [10]. MWCNTs are used in many applications with their high aspect ratio and large surface area properties thanks to their nano-sized diameters and micro-sized lengths.

In this study was to evaluate the *in vitro* antibacterial effect of multi-walled carbon nanotubes in the 5-10 nm size range on oral pathogens (*P. intermedia* and *A. actinomycetemcomitans*) and the morphological properties (cellular deformations caused by bacteria) of bacterial cells. Interactions between MWCNT and pathogenic bacteria were visualized by transmission electron microscopy (TEM).

MATERIAL and METHODS

MWCNT materials

In this study, MWCNTs 90+% purity, 5-10 nm nanopowders was purchased from Nanography (Ankara, Turkey). Different concentrations of MWCNT solutions were prepared with pure water. Before experiments, these solutions were mixed in an ultrasonic water bath (Bandelin Sonorex, Super RK510H) for 15 minutes. However, this study was conducted in accordance with the principles of the Declaration of Helsinki.

Preparation of Cultures for Freeze-Drying

The lyophilized gram negative anaerobic species (*Aggregatibacter actinomycetemcomitans* DSM 11123 and *Prevotella intermedia* DSM 20706) used in this research were obtained from German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Braunschweig, Germany). Each inactive bacteria for reactivation were grown under anaerobic conditions and then stored in a bacterial suspension. Briefly, the *A. actinomycetemcomitans* strain were incubated in CaSo Bouillon (Carl Roth) for 24-48h at 37°C in a 5 % CO_2 medium under anaerobic standard conditions. After incubation, the *A. actinomycetemcomitans* were suspended in Schaedler liquid medium to provide a turbidity equivalent to the 10^8 CFU/mL^{-1} McFarland standard. *P. intermedia* strain was cultured under anaerobic conditions 10% CO_2, 5% H_2 and 85% N_2 on Columbia blood agar plates containing 5% sheep blood and 0.5% K vitamin at 37°C for at least 48 hours.

Toxicity Evaluations

MWCNT was tested for antimicrobial activity against *Aggregatibacter actinomycetemcomitans* and *Prevotel-*

la intermedia using the agar well diffusion method. Bacteria were grown under microaerophilic conditions at 37°C in a jars. Bacterial colonies grown on plates were suspended in sterile distilled water and the density was adjusted to 0.5 McFarland standards. A sterile drigalski spatula was used to evenly graft the surface on Columbia Blood Agar (Liofilchem, Italy) plates. A sterile 6 mm cork drill was used to make wells on each plate. To prepare MWCNT solutions, 0.1 mg/mL MNCT was prepared in deionized water and sonicated in an ultrasonic bath (Elma/S30, Singen, Germany) for 10 minutes at 100 W. MWCNT experimental solution in the range of 10-50 µl at different concentrations was prepared from the sonicated stock solution were poured into each well. At the end of the incubation period, the mean diameters of the bacterial inhibition growth zones formed around the wells in the petri dishes were measured. For each bacterial strain exposed to MWCNT, mean and standard deviation values from six replicates were obtained.

Cell culture TEM analysis

In our study, TEM was used to evaluate the ultrastructural morphological changes of MWCNT on *A. actinomycetemcomitans* and *P. intermedia* species. For this purpose, the strains used as standard reference microorganism were exposed to MWCNT as 10 mL cell suspensions by incubation at 37°C for 48 hours. Cell suspensions were centrifuged in sterile plastic centrifuge tubes at 5000 g for 15 minutes and then washed three times for 10 minutes with phosphate buffered saline (PBS). Afterwards, the supernatant was discarded and the pelleted cells were left for primary fixation in PBS buffer containing 2.5% glutaraldehyde overnight at +4°C. After this process, the fixed cells were dissolved in PBS buffer and washed three times for 15 minutes in PBS after 2 hours of incubation period for secondary fixation by taking in 1% osmium tetroxide. Fixed cells were embedded in 5% agar and block staining with 1% uranyl acetate. Then, each was dehydrated by passing through 40%, 60%, 75%, 80% and 95% ethanol for 15 minutes each. Final dehydration was carried out in 100% ethanol for 1 hour, alternating every 30 minutes. The dehydrated samples were polymerized by embedding in Epoxy resin at 60°C for 2 days. Fully thin sections of 60 nm thickness taken from the blocked samples were taken on copper grids with the help of an ultramicrotome (Leica Ultracut R). Afterwards, the sections were stained with uranyl acetate and lead citrate. Sectioned samples were analyzed for ultra-structural changes with a JEOL JEM 1220 brand/model transmission electron microscope.

Characterization

Pristine MWCNT was purchased from Nanografi (Ankara, Turkey), one of Turkey's main suppliers in the field of nanotechnology. Briefly, in the TEM analysis, the average diameter of MWCNT was given as 5-10 nm and the carbon purity was 95+%. To prepare suspensions with nominal concentrations of 5, 10, 20, 40, 80 and 160 µg mL^{-1}, first the stock MWCNT solution was prepared in ultrapure water (Milli-Q) and sonicated for 60 minutes in an ultrasonic water bath (Bandelin, SonoRex).

MWCNT was characterized by several methods. Surface plasmon resonance Ultraviolet–visible spectroscopy (SHIMADZU, Japan) an alysis was examined in the range of 200 nm to 800 nm. To determine the functional groups contained in pristine MWCNT, FT-IR analyzes were carried out with IR Affinity-I, Model No. SHIMADZU-A213748 device in the wavelength range of 600-4000 cm-1. The morphology of pristine MWCNT was characterized by SEM (Hitachi SU-1510, Japan).

Energy Dispersive X-Ray Spectroscopy (EDX) results were used to show the relative weight percentage of elements in the pristine MWCNT. EDX measurement was performed using the Oxford instrument X-Max combined with a SEM.

RESULTS

Characterization of pristine MWCNTs

In the TEM analysis, the average diameter of MWCNT was given as 5-10 nm and the carbon purity was 95+%. To demonstrate the homogeneous distribution of pristine MWCNT in solutions, MWCNT at different experimental concentrations dissolved in pure water were examined by UV–vis scanning spectrophotometer. Figure 1 shows the effect of MWCNT concentration on the UV–vis spectra of MWCNT suspensions. It was observed that all MWCNT suspensions displayed a characteristic peak in the UV–vis spectrum at approximately 364 nm wavelength. However, it is indicated that the higher the concentration, the higher the intensity of the characteristic peak, while the intensity of the measurement progressively decline from UV to near IR.

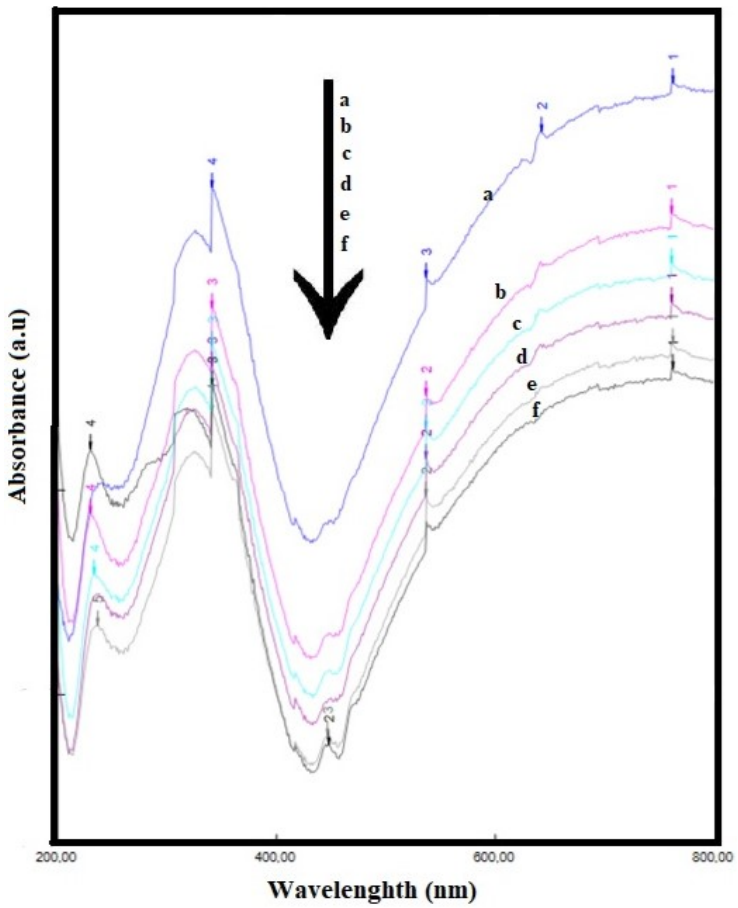

Figure 1. UV/visible absorption spectrum spectra of pristine MWCNT in aqueous solution. (a.5, b.10, c.20, d.40, e.80 ve f.160 µg mL^{-1}).

The bond structure and functional groups between intact MWCNT molecules were examined by FTIR spectroscopy analysis. In Figure 2, the spectra appeared in the range of 1700–1400 cm^{-1}. The peaks at 1697, 1651 and 1519 cm^{-1} can be assigned to C=C groups in aromatic rings, in accordance with the literature. These bands may represent surface defects on the surface of MWCNTs [11]. In the SEM image in Figure 3, it can be seen that pure MWCNTs are in aggregate form and in EDX they are made of only C element.

Antimicrobial Activity of pristine MWCNT

Negatively charged bacteria show the ability to bind to activated carbon particles with high affinity through strong Lifshitz-van der Waals forces, despite the electrostatic repulsion between their cells and carbon surfaces [12]. Furthermore, the contact between bacteria and CNT has bactericidal properties depending on the bacterial cell surface charge, hydrophobicity of the interacting surfaces, and incubation conditions. Here, toxicity was tested in gram-negative *A. actinomycetemcomitans* and *P. intermedia* bacterial cells (10^8 cfu/mL) to investigate how MWCNT distribution and incubation condition affected the bacteria. As seen in Table 1, no matter what concentration or incubation time is used, the loss of viability caused by MWCNTs is more pronounced in *P. intermedia*. This shows that MWCNT has a better antibacterial activity on *P. intermedia*.

Table 1 indicates the antibacterial effect of MWCNTs treated at different concentrations on *A. actinomycetemcomitans* and *P. intermedia*. Both pathogens demonstrated the highest antibacterial effect at 24 and 72h exposure to 10 µL of MWCNT. The antibacterial effect of *A. actinomycetemcomitans* decreased rapidly after 20 µL and for *P. intermedia* after 40 µL. In addition to, the antibacterial activity of *P. intermedia* is slightly higher than that of *A. actinomycetemcomitans*. According to the statistical results, there are significant differences at low concentrations of MWCNT ($P<0.05$).

Antimicrobial Mechanism of pristine MWCNT

To better understand the antimicrobial mechanisms, TEM microscopic studies were performed to visualize

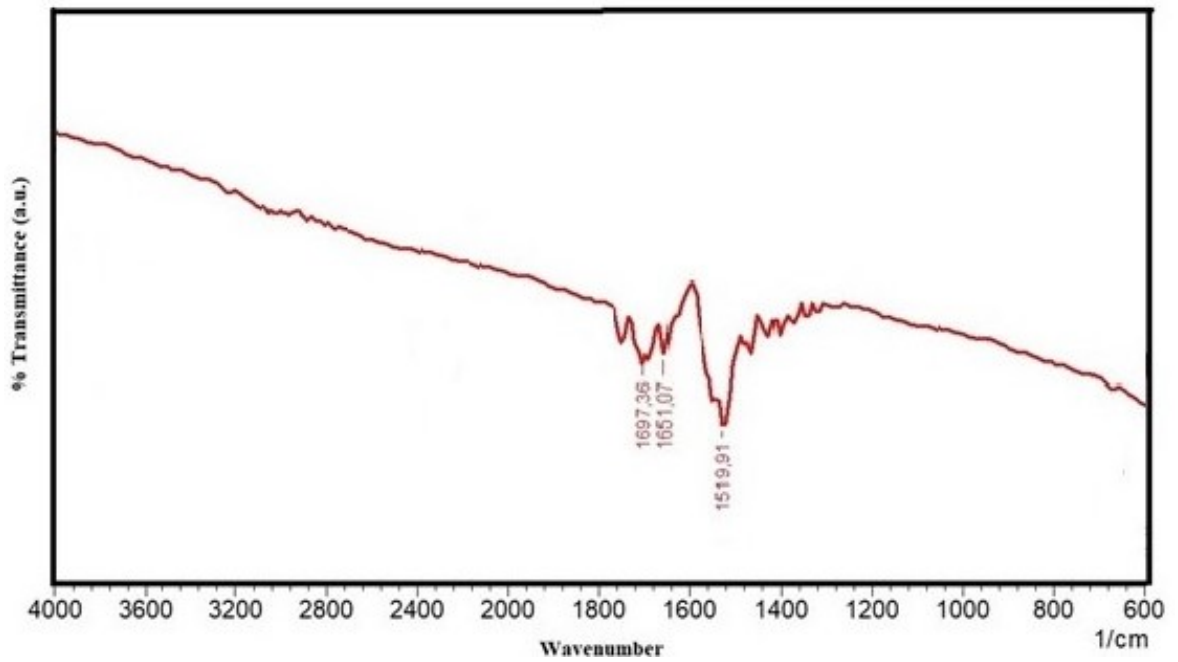

Figure 2. FT-IR spectra of pristine MWCNT.

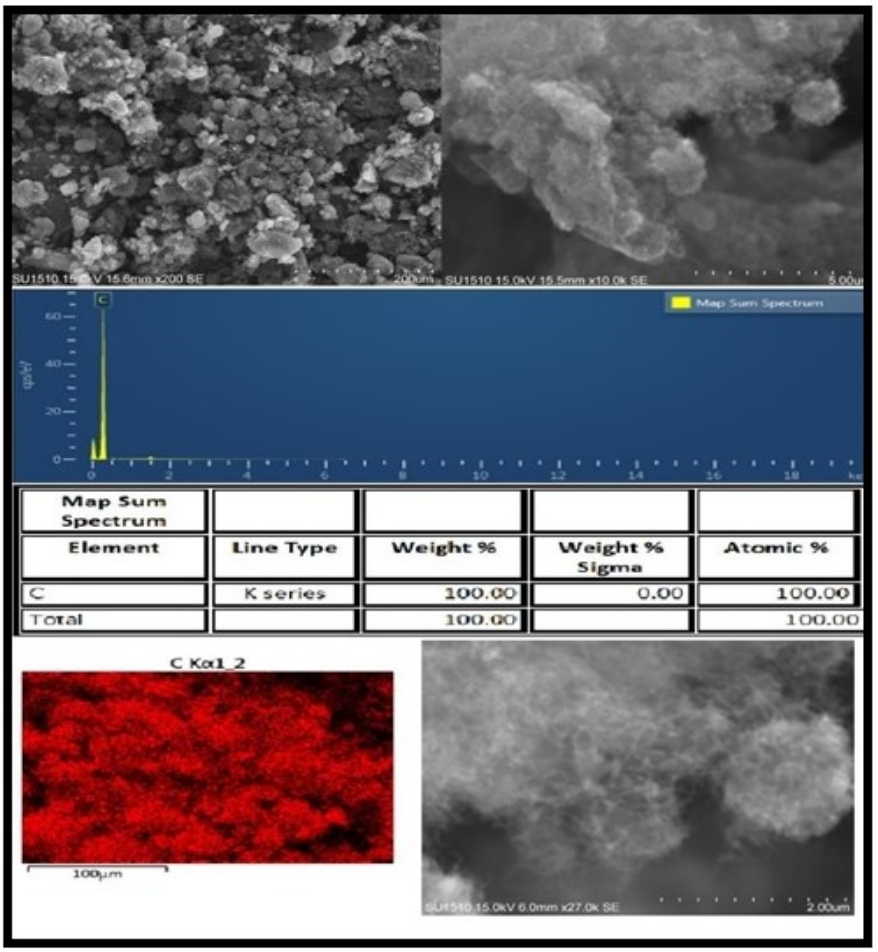

Figure 3. SEM of image EDX spectra of pristine MWCNT.

Table 1. Mean values of the inhibition zone of oral pathogenic bacteria treated with pristine MWCNT for 24 and 72 hours measured by the agar well method.

MWCNT Concentration ($\mu g\ mL^{-1}$)	Zone of inhibition (mm in diameter)			
	A. actinomycetemcomitans		*P. intermedia*	
	24-h	72-h	24-h	72-h
5	17.8 ± 1.45*	19.2 ± 1.19*	22.1 ± 0.30*	23.4 ± 0.31*
10	21.4 ± 0.48*	22.6 ± 0.52*	24.0 ± 0.36**	24.5 ± 0.36**
20	14.0 ± 1.10	14.5 ± 1.15	19.8 ± 0.91*	20.1 ± 0.90*
40	8.55 ± 0.45	8.00 ± 0.33	12.6 ± 0.11	11.9 ± 0.11
80	3.67 ± 0.46	3.50 ± 0.46	7.9 ± 0.15	6.19 ± 0.14
160	1.18 ± 0.67	1.18 ± 0.67	1.5 ± 0.25	1.7 ± 0.24

Note: *p < 0.05; **p < 0.01 indicates significant differences in groups treated with different concentrations of MWCNT.

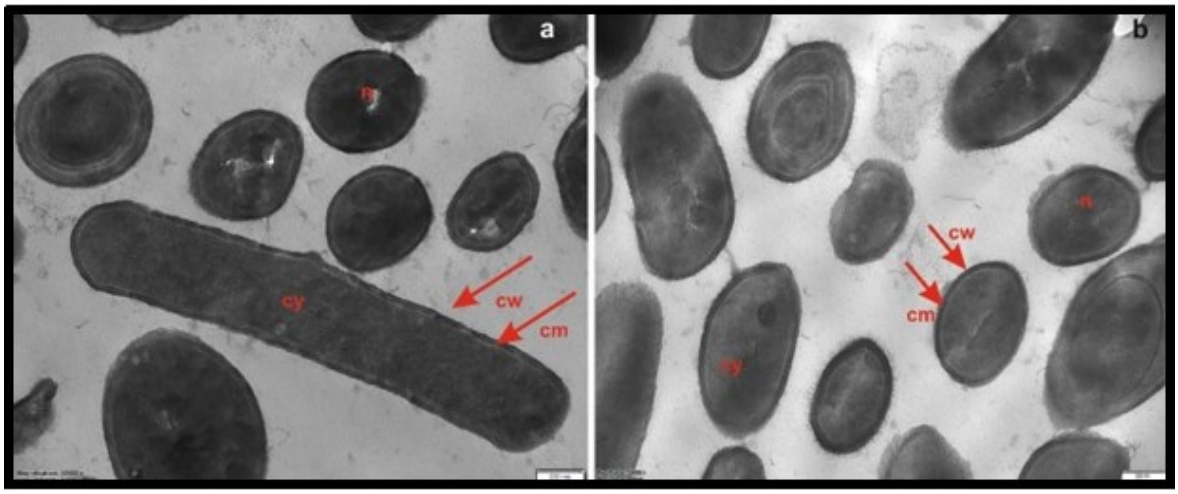

Figure 4. TEM micrographs of oral pathogenic bacteria not exposed to pristine MWCNT A) P. intermedia, B) A. Actinomycetemcomitans. (cy: cytoplasm, cm: cell membrane, cw: cell wall, n: nucleus).

the interaction of pristine MWCNT with oral pathogenic bacterial cells. Images of cells internalizing MWCNT were observed in similar shapes under all incubation conditions. As can be seen in Fig. 4 TEM micrographs, untreated oral pathogen cells have a smooth surface. The nuclei of the cells are clearly and centrally located, the cytoplasm is regular, and the cell wall and cytoplasmic membrane structure are seen as a whole (Fig. 3 (a and b).

TEM micrographs in Fig. 5-6 show *P. intermedia* and *A. actinomycetemcomitans* bacteria incubated with MWCNT under a shaking speed of 5000 g for 15 min. Compared with untreated cells, bacterial cell morphologies treated with MWCNT showed significantly different morphological features. MWCNT aggregates appear to localize next to and inside the bacteria (Fig. 5 (a3 and a4)).

P. intermedia cells treated with MWCNT were severely damaged, which caused the cells to become fused with each other, the cytoplasm condensed and cell wall membrane to rupture (Fig. 5 (a1-a4)). It was observed that some cells were ruptured, cell contents leaked out, and membrane and wall damage occurred in some cells. In a small number of cells, bubble formations were observed protruding from the membrane (Fig. 4 (a3, a4)). Morphological deformities and outward bending of the

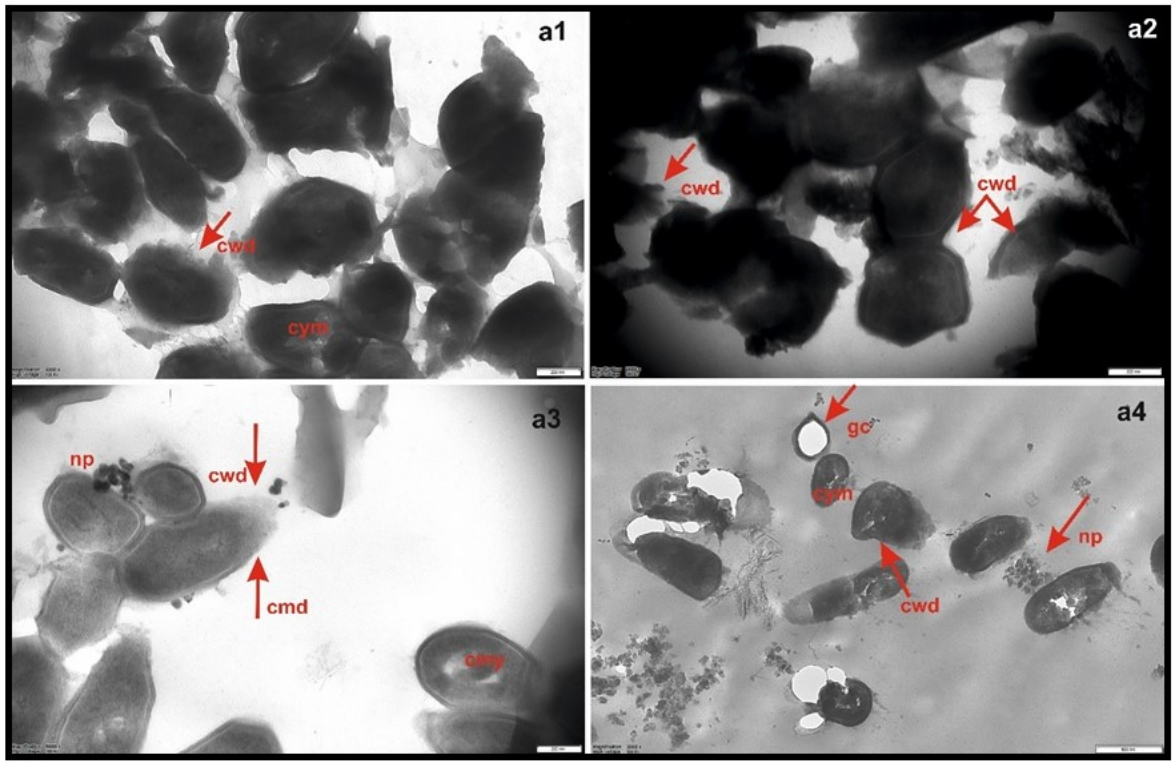

Figure 5. TEM micrographs of P. intermedia exposed to pristine MWCNT. (cym: cytoplasm melting, cmd: cell membrane damage, cwd: cell wall damage, gc: ghost cell, Scale bars: 200nm).

cell membrane and wall structure were observed in the cells that came into contact with MWCNT. There are also a few ghost cell residue that have all their contents emptied (Fig. 5 (a4)).

Fig. 6 indicates TEM micrographs that MWCNT are packed inside and outside the cell. The wall and membrane structures of pathogen cells, which generally have a regular oval appearance, were relatively preserved with a homogeneous cytoplasm appearance, but damages in the form of wall and membrane melting was observed in the cells contacted by MWCNT (Fig. 6 (a1, a3)).It has been determined that MWCNTs cause condensation of the cytoplasm in some cells, and in some cells, the cytoplasmic contents leak out as a result of the separation of the membrane from the cytoplasm, and bleb formations are observed in some cells (Fig. 6 (a2, a4)). Particularly in the apical parts of the cells, membrane cytoplasm separations are evident (Fig. 6 (a4)). However, there are also a few ghost cell views with empty content (Fig. 6(a2)).

DISCUSSION

In 2007, the antimicrobial activity of carbon nanotubes (CNTs) was reported for the first time [13]. MWCNTs have better biocompatibility and greater dispersibility compared to other CNTs, making their antibacterial effect stronger [14]. Physical characterization of pristine MWCNT provides information about the morphology, size and purity of the sample. Figure 1 shows the effect on the UV–vis spectra of pristine MWCNT suspensions prepared at different concentrations. All MWCNT suspensions showed a characteristic peak in the UV–vis spectrum at a wavelength of approximately 364 nm. However, the intensity of the peak of high concentration MWCNT suspensions is high. Our UV–vis results are consistent with literature findings showing that the characteristic peaks of MWCNTs are around 260 nm [15]. In one study, UV–vis was used to evaluate the dispersion quality of aqueous suspensions of CNT sonication time. It was observed that the characteristic peak intensity was proportional to the sonication time and that all suspensions had a characteristic peak at 300 nm [16]. Another study reported that UV–vis spectroscopy

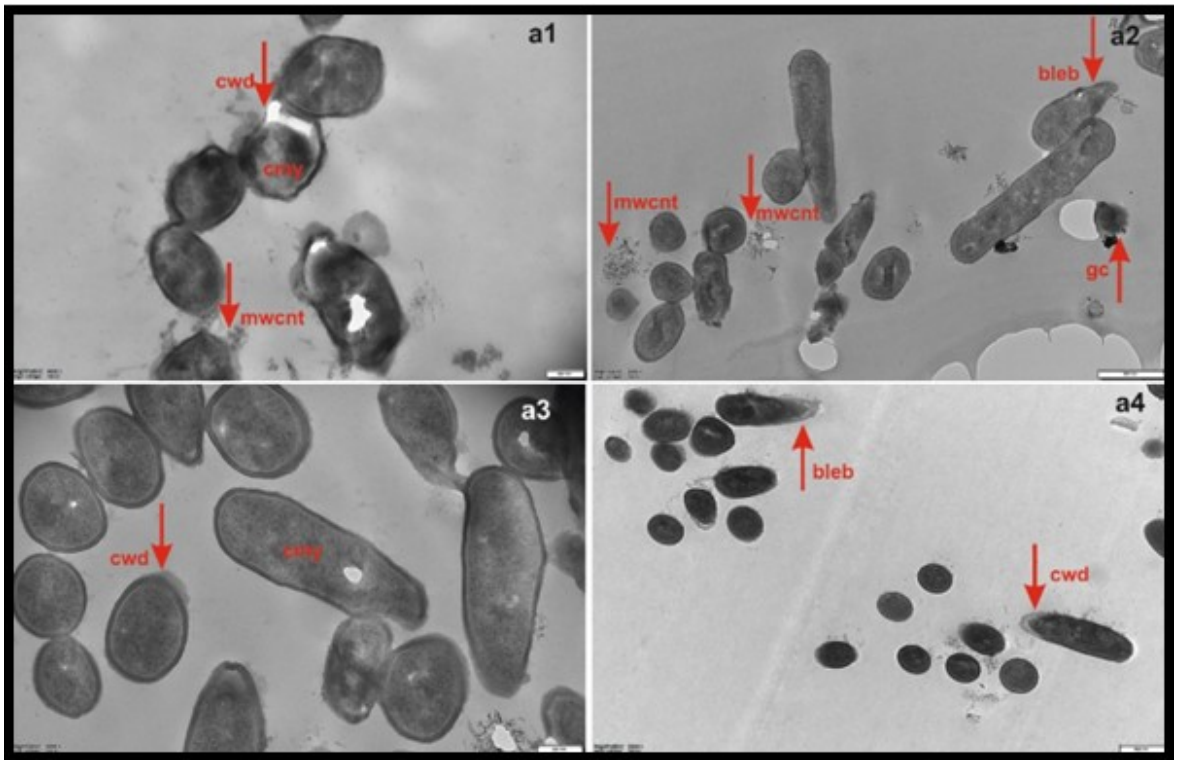

Figure 6. TEM micrographs of A. actinomycetemcomitans exposed to pristine MWCNT. (cym: cytoplasm melting, cmd: cell membrane damage, cwd: cell wall damage, gc: ghost cell, Scale bars: 200nm).

analysis can be used to monitor the dispersion process of CNTs and will be helpful in determining the optimal sonication time [17]. UV-vis spectrophotometry of CNTs is a useful method to detect the concentration of CNTs. Thus, it is possible to establish a relationship between the amounts of CNTs individually dispersed in the suspension and the intensity of the corresponding UV–vis absorption spectrums [18]. FT-IR was performed to confirm the presence of functional groups in MWCNTs. It showed the C=C bond structure between MWCNT molecules. Consistent with the literature, pristine MWCNT showed peaks in the same regions [19].

The current study is one of the first studies in which the antibacterial effect and cellular damage of MWCNT, which has potential use in dentistry, on periodontitis-causing bacteria were examined in detail using TEM. The antibacterial effect of MWCNT is known, but its toxicity mechanisms have not been fully revealed. In the current literature, it is stated that MWCNTs act by via the formation of free radicals, accumulation of peroxidative products and depletion of cell antioxidants [20]. However, the very different physicochemical properties, production methods, size, shape and functionalization of MWCNT greatly change the toxicity mechanism. Some studies have reported that nanoparticles larger than 10 nm accumulate in cell membranes, change cell membrane permeability, disrupt cell homeostasis, and cause leakage of intracellular components and ultimately cell death due to the inability to regulate substance migration [21.22]. In this study, it is seen in the TEM micrographs in Figures 4-5 that pristine MWCNT of 5-10 nm size in the range of 5-20 μg mL^{-1} for 72-h exposure times showed a strong antibacterial effect on the treated pathogens. In treated cells, it is observed that the cell content leaks out as a result of severe membrane and wall damage, especially by distorting the cell shape. In some cells, ghost cells were formed as the cell contents completely came out and bleb formations were observed, which caused severe damage.

Conclusions

It has been observed that the antibacterial effects of bacteria exposed to intact MWCNT are different. Additionally, this effect varied depending on the bacterial species. It is seen both in the zone diameter measurement in the antibacterial susceptibility test and in TEM micrographs that intact MWCNT causes more toxicity in *P. intermedia* than in *A. actinomycetemcomitans*. TEM analysis suggested that the main source of toxicity was

membrane damage, which played a role in the antibacterial effect of MWCNT.

In TEM micrographs, it has been shown that MWCNT is internalized and as a result, MWCNT damages the bacterial cell and wall, disrupting cell integrity, and this mechanism has an antibacterial effect.

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Conflict of interest
The authors of this article do not have any conflict of interest with any institution or person.

Authorship Contributions
Yeşim ÖZKAN DAĞLIOĞLU: Investigation, Validation, Methodology, Data curation, Writing – original draft. Mustafa Cihan YAVUZ: Investigation, Methodology.

References

1. R.P. Darveau, Periodontitis: a polymicrobial disruption of host homeostasis, Nat. Rev. Microbiol., 8 (2010) 481-490.
2. B. Henderson, J.M. Ward, D. Ready, Aggregatibacter (Actinobacillus) actinomycetemcomitans: a triple A* periodontopathogen?, Periodontol., 54 (2010) 78-105.
3. D. Haubek, The highly leukotoxic JP2 clone of Aggregatibacter actinomycetemcomitans: evolutionary aspects, epidemiology and etiological role in aggressive periodontitis, APMIS. Supplementum, 130 (2010) 1-53.
4. H.R. Jousimies-Somer, Update on the taxonomy and the clinical and laboratory characteristics of pigmented anaerobic gram-negative rods, Clin. Infect. Dis., 20 (1995) 187-191.
5. J. Slots, L. Bragd, M. Wikström, G. Dahlén, The occurrence of Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius in destructive periodontal disease in adults, J. Clin. Periodontol., 13 (1986) 570-577.
6. Y. Dağlıoğlu, M.S. Çelebi, The evaluation of the acute toxic effects of Polyvinylferrocenium supported platinum nanoparticles on Artemia salina Brine shrimp, Biological Diversity and Conservation, 8 (2015) 304-312.
7. Y. Dağlıoğlu, B. Yılmaz Öztürk, A novel intracellular synthesis of silver nanoparticles using Desmodesmus sp. (Scenedesmaceae): different methods of pigment change, Rendiconti Lincei. Scienze Fisiche e Naturali, 30 (2019) 611-621.
8. Y. Dağlıoğlu, M.A. Açıkgöz, M.M. Özcan, Ş.M. Kara, Impact of application of alumina oxide nanoparticles on callus induction, pigment content, cell damage and antioxidant enzyme activities in Ocimum basilicum. JIEAS., 17 (2022) 22-33.
9. Y. Dağlıoğlu, M.S. Çelebi, Ş. Önalan, Determination of acute toxic effects of poly (vinylferrocenium) supported palladium nanoparticle (Pd/PVF+) on Artemia salina. Pakistan J. of Zool., 48 (2016) 187-193.
10. R. Alshehri, A.M. Ilyas, A. Hasan, A. Arnaout, F. Ahmed, A. Memic, A, Carbon nanotubes in biomedical applications: factors, mechanisms, and remedies of toxicity: miniperspective. J. Med. Chem, 59 (2016) 8149-8167.
11. L. Stobinski, B. Lesiak, L. Kövér, J. Tóth, S. Biniak, G.Trykowski, J. Judek, Multiwall carbon nanotubes purification and oxidation by nitric acid studied by the FTIR and electron spectroscopy methods, J. Alloys Compd., 501 (2010) 77-84.
12. H.J. Busscher, R.J. Dijkstra, D.E. Langworthy, D.I. Collias, D.W. Bjorkquist, M.D. Mitchell, H.C. Van der Mei, Interaction forces between waterborne bacteria and activated carbon particles, J. Colloid Interface Sci., 322 (2008) 351-357.
13. S. Kang, M. Pinault, L.D. Pfefferle, M. Elimelech, Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir, 23 (2007) 8670-8673.
14. X. Li, L. Wang, Y. Fan, Q. Feng, F.Z, Cui, Biocompatibility and toxicity of nanoparticles and nanotubes, J. Nanomater., 1 (2012) 548389.
15. A. Sobolkina, V. Mechtcherine, V. Khavrus, D. Maier, M. Mende, M. Ritschel, A. Leonhardt, Dispersion of carbon nanotubes and its influence on the mechanical properties of the cement matrix. Cem. Concr. Compos., 34 (2012) 1104-1113.
16. P. Alafogianni, K. Dassios, S. Farmaki, S.K. Antiohos, T.E. Matikas, N.M. Barkoula, On the efficiency of UV–vis spectroscopy in assessing the dispersion quality in sonicated aqueous suspensions of carbon nanotubes. Colloids. Surf. A: Physicochem. Eng. Asp., 495 (2016) 118-124.
17. J. Yu, N. Grossiord, C.E. Koning, J. Loos, Controlling the dispersion of multi-wall carbon nanotubes in aqueous surfactant solution. Carbon, 45 (2007) 618-623.
18. Y. Ye, S. Cai, M. Yan, T. Chen, T. Guo, T, Concentration detection of carbon nanotubes in electrophoretic suspension with UV–vis spectrophotometry for application in field emission devices. Appl. Surf. Sci., 284 (2013) 107-112.
19. J. Tao, S.A. Cao, Flexible high dielectric thin films based on cellulose nanofibrils and acid oxidized multi-walled carbon nanotubes. RSC advances, 10 (2020) 10799-10805.
20. C. Ventura, J.F., Pereira, P. Matos, B. Marques, P. Jordan, A. Sousa-Uva, M.J. Silva, Cytotoxicity and genotoxicity of MWCNT-7 and crocidolite: Assessment in alveolar epithelial cells versus their coculture with monocyte-derived macrophages. Nanotoxicology, 14 (2020) 479-503.
21. V.K. Sharma, R.A. Yngard, Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci, 145 (2009) 83-96.
22. İ. Dağ, Kardamom yağının standart ve klinik Candida izolatlarına karşı antifungal etkinliklerinin değerlendirilmesi. BioDiCon., 11 (2018) 31-37.