

INFLUENCE OF SUBINHIBITORY CONCENTRATIONS OF ANTIMICROBIALS ON HYDROPHOBICITY, ADHERENCE AND ULTRA-STRUCTURE OF *FUSOBACTERIUM NUCLEATUM*

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ABSTRACT

Fusobacterium nucleatum is considered a bridge organism between earlier and later colonizers in dental biofilms and a putative periodontopathogen. In Dentistry, antimicrobial agents are used for treatment and control of infectious diseases associated with dental plaque. Antiseptics have been used in association with antibiotics to reduce infections after oral surgeries. In this study, the influence of subinhibitory concentrations (SC) of chlorhexidine, triclosan, penicillin G and metronidazole, on hydrophobicity, adherence to oral epithelial cells, and ultra-structure of *F. nucleatum* was examined. All isolates were susceptible to chlorhexidine, triclosan, and metronidazole; however, most of the isolates were susceptible to penicillin G, and all of them were hydrophilic when grown with or without antimicrobials. Adherence was decreased by all antimicrobials. Results suggest that adherence of *F. nucleatum* was influenced by adhesins because structures such as fimbriae or capsule were not observed by transmission electronic microscope.

Key words: *Fusobacterium nucleatum*, hydrophobicity, adherence, antimicrobial, ultra-structure.

INTRODUCTION

The American Association of Public Health Dentists, Subcommittee of Preventive Periodontology (2) describes the periodontal disease as the major cause of dental loss in adult population, which is considered a serious health problem worldwide.

Gingivitis and periodontitis are associated with the dental biofilm, which is mediated by intrinsic and extrinsic factors such as the use of antimicrobial drugs, host's susceptibility or microorganism-host interactions (4,13). The human periodontopathies are associated with Gram-negative anaerobic and aerobic rods such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens* and *Wollinella recta*, and these organisms are able to destroy periodontal tissues by using different virulence factors such as volatile compounds, enzymes, LPS or hemolysins (5,28).

Fusobacterium nucleatum is an important organism for its role as a bridge among earlier and later colonizers of the dental biofilm because of its ability of coaggregation with other microbial species (15,19,32).

Antimicrobial agents are used for treatment and control of infectious diseases associated to dental biofilm (17,29). Antiseptics have been used in association with antibiotics to reduce infections after oral surgeries and also to help in treating periodontal infections (27). Chlorhexidine is a cationic bis-guanide agent used as an active bacterial plaque inhibitor and active against Gram-positive and Gram-negative, aerobe or anaerobe organisms, yeasts and virus (2). Triclosan is an active bis-phenolic and non-cationic agent, and it has been added to dentifrices and colutories without side effects. However, this agent has a low substantivity on oral mucosa but an effective action against different oral organisms has been observed (16,23). Also, antibiotics are used for treatment of infectious processes (17).

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Considering *F. nucleatum* as a member of the human autochthonous microbiota and for the importance in oral and nonoral infections, and considering the action of different substances interfering with either bacterial virulence factors or with the host-bacteria interaction, the goal of this study was to evaluate the influence of subinhibitory concentrations of chlorhexidine, triclosan, penicillin G, and metronidazole, on cellular hydrophobicity, adherence on human oral epithelial cells, and ultra-structure of *F. nucleatum*.

MATERIALS AND METHODS

Patients and Organisms

Twenty periodontal patients (age ranged 18-40 years old) without sex or race distinction were selected from the Clinic of Periodontology, Dental School, University of São Paulo. All the patients showed periodontal pockets deeper than 5 mm, and the bone loss was observed by radiographic examination. None of them used antibiotic three months prior to samples collection. Subgingival samples were collected by using three sterilized paper points (EndoPoints Ind. Co. Ltd., São Paulo, SP, Brazil) introduced into periodontal pocket for 60 seconds and transported in 2.0 ml of Ringer-PRAS solution. After dilutions, aliquots of 0.1 ml were transferred to Omata and Disraely agar, and then, incubated in atmosphere of 90% N₂ + 10% CO₂, at 37°C, for 4 days. Twenty *F. nucleatum* isolates were identified by conventional biochemical tests (31). Reference strains *F. nucleatum* ATCC 10953 and ATCC 25586, were also included. In this study, one isolate from each patient was isolated.

Antimicrobial Susceptibility

The minimal inhibitory concentration (MIC) was determined by using a macrodilution method (NCCLS, 1995) in brain heart infusion (BHI, Difco Laboratories, SP, Brazil) supplemented with 0.5% yeast extract (Difco). The cellular standardization (10⁸ cells/ml) was performed using a 0.5 McFarland scale. Two-fold serial dilutions of chlorhexidine (Sigma Chemical Co. USA), triclosan (Ciba-Geigy, SP, Brazil), penicillin G (Wyeth Laboratories, SP, Brazil) and metronidazole (Rhodia Farma Ltd., SP, Brazil) were used. 5.0 ml of BHI with and without antimicrobials were inoculated with bacteria, in duplicate, and incubated in anaerobiosis, at 37°C, for 48 h. The MIC was defined as the lowest concentration of the drug able to inhibit the bacterial growth. The subinhibitory concentration (SC) was determined taking 50% MIC values.

Cellular Hydrophobicity

The cellular hydrophobicity was performed using a modification of the Gibbons and Etherden (10) method. Briefly, 100 µl of each bacterial inoculum (10⁸ cells/ml) were grown in 10 ml of BHI with antimicrobial in SC. Also, BHI without drug was used as control. All the tubes were incubated in anaerobiosis at

37°C, 48 h. Then, cells were harvested, washed and resuspended with 3.0 ml of phosphate urea magnesium (PUM) buffer and then, 400 µl of n-hexadecane (Sigma Chemical Co., USA) were added. All the tubes were incubated at 37°C, for 10 minutes, and then, vortexed (twice for 30 seconds) and, two phases were observed. Hydrophobicity values were obtained using a spectrophotometer A_{550 nm} (Coleman Instruments Corporation, Mod. 6/35, USA). Isolates were considered hydrophobic when 50% cells were bounded to n-hexadecane, and hydrophilic when cells were observed in PUM buffer. All the tests were performed in triplicate.

Bacterial adherence

Adherence on oral epithelial cells (OEC) was performed by a Childs and Gibbons (3) method. Briefly, 100 µl of bacterial inoculum (10⁸ cells/ml) was transferred to 1.9 ml of BHI with or without antimicrobials in SC. All the tubes were added of 5 mCi/ml H³-methyl-thymidine (Amersham Life Science, USA) to complete 2 ml, and incubated in anaerobiosis, at 37°C, for 48 h. Then, bacterial were harvested, washed and resuspended in phosphate-buffered saline (PBS, 0.01 M) to give the final concentration of 10⁷ cells/ml (A_{550 nm}). OEC were obtained from jugal region from a donor male, healthy, and blood type A Rh-positive, by using a wood piece and resuspended in PBS and washed three times (1.000 x g, 5 minutes). Cellular suspension was adjusted to 2 x 10⁵ cells/ml using a Neubauer chamber. 75 µl of each bacterial suspension and 75 µl of OEC was mixed by rotation (6 rpm, 2 h, at room temperature) (Cole Palmer Instruments Co., Mod. 7637, USA). The mixture was transferred to Ultra-clear tubes (Beckman Instruments, USA) containing 4 ml of 50% percoll (Sigma Chemical Co., USA), and centrifuged at 31.000 x g, 4°C, for 20 minutes in a SW-50.1 rotor (Beckman Instruments, USA). The upper layer (1 ml) was removed and diluted in 3 ml of PBS and filtered in cellulose ester membrane (0.65 mm) (Millipore, USA). 75 µl of bacterial suspension without antimicrobials and bacteria grown in SC of antimicrobial plus 5 mCi/ml de H³-methyl-thymidine were used as control. Adherence values were obtained using a scintillation machine LS-5000 TD (Beckman Instruments, USA).

Ultra-structure

Bacteria were grown in 5 ml BHI with or without each antimicrobial in SC and incubated in anaerobiosis, at 37°C, for 48 h. After centrifugation, the pellet was fixed in 2% glutaraldehyde, 2.5% formaldehyde and 0.1 M sodium cacodilate buffer (pH 7.4) and maintained in rotation (6 x g, 2h, at room temperature). The mixture was post-fixed in 1% osmium tetroxide. Samples were treated with 70 % alcohol, at 4°C, overnight, and a resin/acetate mixture (3:1 and 1:1) was used. Finally, a pure resin was added and maintained in rotation (6 x g, overnight). Samples were included in Spurr resin (Electron Microscopy Sciences, Fort Washington, USA) and these blocks were cut by using

ultramicrotome Sorvall MT2-B with glass-knives and stained with 0.25% toluidin blue. Small pieces were placed on copper supports (200-300 meshes) and 2% uranyl acetate and 0.5% lead citrate solution was used as contrast. Samples were photographed with a transmission electronic microscope (TEM) JEOL 100 CX II (Japan).

Statistic Analysis

A non-parametric test was used. The null hypothesis value was fixed in 0.05 or 0.5% ($P=0.05$) for all the tests. A Wilcoxon test for two non-independents samples was used to compare the hydrophobicity and adhesion values, respectively, i.e., control group (bacteria plus PUM) with bacteria plus PUM plus n-hexadecane, for hydrophobicity and; bacteria plus H³-methyl-tymidine (control) with bacteria plus H³-methyl-tymidine plus OEC, for adherence assay. Also, the variance analysis of Kruskal-Wallis (30) to compare the hydrophobicity or adhesion values between both groups with and without antimicrobials was used.

RESULTS

Table 1 shows the MIC values to each antimicrobial agent. Chlorhexidine and triclosan showed MIC values of 2 to 8 µg/ml, penicillin G, < 0.03 to 512 µg/ml and, metronidazole, 0.25 µg/ml to 1 µg/ml. Isolates with MIC values to penicillin G < 0.03 µg/ml were not tested for hydrophobicity or adherence in SC of drugs.

Most of the tested isolates and reference strains were hydrophilic when grown without antimicrobial agents or in SC of chlorhexidine, triclosan, or metronidazole. On the other hand, isolates 1, 2 and 12, were hydrophobic ($\leq 50\%$ values) (Table 2). Isolates grown with or without antimicrobials were capable to adhere on oral epithelial cells. Mean values of adherence from control group and bacteria treated with antimicrobials are showed in Table 3. By variance analysis was observed that control group showed better adherence than bacteria treated with antimicrobials. Then, we can observe, for the adherence process in bacteria grown in SC of antimicrobial: bacteria plus chlorhexidine > Bacteria plus triclosan > bacteria plus penicillin G or metronidazole. However, nine bacteria treated with metronidazole increased their adherence on OEC. Also, seven isolates treated with triclosan and four with penicillin increased their adherence process (Table 3).

TEM showed that *F. nucleatum* grown without agents were 0.4 to 0.5 µm of diameter, round and with opacity-electron (Fig. 1). Bacteria treated with chlorhexidine or penicillin G showed an irregular cell wall, but no alteration in diameter was observed (Fig. 2), and isolates grown in SC of triclosan or metronidazole did not show any alteration in cell wall or diameter. In Fig. 3 can be observed the *F. nucleatum* grown in SC of chlorhexidine adhered to epithelial cells.

Table 1. MIC values of chlorhexidine, triclosan, penicillin G and metronidazole, to 20 *F. nucleatum* isolates and reference strains.

Isolate	Chlorhexidine (µg/ml)	Triclosan (µg/ml)	Penicillin G (µg/ml)	Metronidazole (µg/ml)
1	4	4	0.06	0.5
2	4	4	0.06	0.5
3	4	4	0.06	0.5
4	4	4	0.12	1
5	4	4	0.06	0.5
6	4	4	0.06	0.5
7	4	4	0.06	1
8	4	8	≤ 0.03	0.5
9	4	4	≤ 0.03	0.5
10	4	4	≤ 0.03	1
11	4	4	≤ 0.03	0.5
12	4	8	0.12	0.25
13	4	4	≤ 0.03	0.5
14	4	4	128	0.5
15	4	4	≤ 0.03	0.5
16	4	4	0.06	0.25
17	4	8	128	0.5
18	4	8	≤ 0.03	0.25
19	4	4	512	0.5
20	8	8	≤ 0.03	0.5
<i>F. nucleatum</i> ATCC 10953	2	2	≤ 0.03	0.25
<i>F. nucleatum</i> ATCC 25586	4	2	≤ 0.03	0.25

Breakpoint: Chlorhexidine and Triclosan, 4 µg/ml; Penicillin G and Metronidazole, 8 µg/ml.

DISCUSSION

Periodontal disease is a result of the bacterial virulence factors action in a susceptible host (13). Also, virulence factors such as hydrophobicity and adherence are considered an important prerequisite for colonization and subsequent infection (7,32). Chemical compounds such as chlorhexidine or triclosan, penicillin G or metronidazole, have been used in patients with difficulties to maintain a good oral clean or with refractory or rapid progression periodontopathies (11).

The antimicrobial inhibitory concentrations or SC may alter either the bacterial virulence factors or the microorganism-host relationship (6,14,18). Although, few antimicrobials in inhibitory concentrations are able to remain for long period into the oral cavity, because of diluents of the saliva and by cleansing action of the oral musculature (22). All the *F. nucleatum* isolates were susceptible to chlorhexidine or triclosan with MIC values ranged from 4 to 8 µg/ml. It is known that chlorhexidine shows a higher substantivity than triclosan on oral mucosa (1,23). However, both of agents showed similar action on tested isolates.

Table 2. Mean values of the hydrophobic or hydrophilic characteristic of *F. nucleatum*, measured in spectrophotometer $A_{550\text{ nm}}$

Isolate (No.)	WA		CH		TR		PE		MZ	
	C	H	C	H	C	H	C	H	C	H
1	0.33	0.33	0.47	0.42	0.38	0.33	0.54	0.19	0.32	0.25
2	0.32	0.27	0.31	0.25	0.24	0.22	0.58	0.25	0.23	0.19
3	0.31	0.24	0.23	0.21	0.24	0.22	0.47	0.46	0.24	0.21
4	0.41	0.34	0.55	0.51	0.27	0.20	0.32	0.26	0.39	0.32
5	0.38	0.27	0.53	0.49	0.38	0.33	0.51	0.53	0.33	0.26
6	0.22	0.20	0.49	0.33	0.48	0.45	0.55	0.35	0.22	0.20
7	0.25	0.24	0.47	0.42	0.40	0.39	0.54	0.43	0.20	0.1
8	0.34	0.28	0.48	0.41	0.35	0.35	NT	NT	0.34	0.29
9	0.25	0.23	0.31	0.21	0.30	0.27	NT	NT	0.20	0.17
10	0.40	0.33	0.52	0.49	0.46	0.42	NT	NT	0.18	0.14
11	0.21	0.23	0.36	0.36	0.43	0.42	NT	NT	0.20	0.21
12	0.56	0.54	0.44	0.45	0.43	0.41	0.42	0.21	0.48	0.48
13	0.60	0.55	0.54	0.52	0.37	0.35	NT	NT	0.55	0.50
14	0.48	0.48	0.38	0.39	0.44	0.44	0.56	0.55	0.51	0.46
15	0.38	0.35	0.38	0.35	0.46	0.44	NT	NT	0.30	0.27
16	0.59	0.57	0.56	0.45	0.30	0.27	0.51	0.51	0.55	0.49
17	0.53	0.51	0.40	0.38	0.31	0.30	0.50	0.48	0.49	0.47
18	0.60	0.59	0.57	0.51	0.28	0.25	NT	NT	0.56	0.55
19	0.39	0.31	0.30	0.23	0.25	0.21	0.50	0.46	0.39	0.33
20	0.45	0.41	0.48	0.47	0.39	0.38	NT	NT	0.50	0.48
<i>F. n-1</i>	0.46	0.43	0.53	0.47	0.33	0.30	NT	NT	0.46	0.43
<i>F. n-2</i>	0.45	0.37	0.49	0.44	0.50	0.45	NT	NT	0.44	0.36
<i>B. f.</i>	0.34	0.30	0.36	0.36	0.32	0.32	0.56	0.55	0.30	0.24
Mean	0.402	0.364	0.441	0.397	0.361	0.336	0.505	0.402	0.364	0.326

WA: without antimicrobial

CH: chlorhexidine

TR: triclosan

PE: penicillin G

MZ: metronidazole

C: control

H: hydrophobicity

NT: not tested

F. n-1: *F. nucleatum* ATCC 10953;*F. n-2*: *F. nucleatum* ATCC 25586*B. f.*: *B. fragilis* ATCC 23745.

Wilcoxon test: (C x H); $Z_{\text{critic}} = 1.96$; Control: $Z = 3.74^*$ (C.C > H.C); Chlorhexidine: $Z = 3.88^*$ (C.CH > H.CH); Triclosan: $Z = 3.92^*$ (C.TR > H.TR); Penicillin G: $Z = 2.75^*$ (C.PE > H.PE); Metronidazole: $Z = 4.06^*$ (C.MZ > H.MZ).

Variance analysis of Kruskal-Wallis: (C x CH x TR x PE x ME); $H_{\text{critic}} = 9.49$; Control: $H = 19.69^*$; Hydrophobicity: $H = 7.35$;
* Significant values.

Multiples comparisons test: TR group > PE or MZ group; $P = 0.05$.

Penicillins are still considered drugs of choice in dentistry for treatment of different oral infections, but recently, an amoxicillin/metronidazole association has been observed in literature (9,21). Most of *F. nucleatum* isolates were susceptible to penicillin G, however three isolates (15%) were resistant (MIC ranged from 128 to 512 $\mu\text{g/ml}$) (Table 1) in accordance with Kononen *et al.* (20). The susceptibility to metronidazole is a characteristic of the genus *Fusobacterium* and all the tested isolates were susceptible to this drug (MIC ranged from 0.25 to 1 $\mu\text{g/ml}$) in accordance with Feres *et al.* (19).

It is known that cellular hydrophobicity is closely associated with bacterial virulence and, microorganisms adsorbing to the

n-hexadecane are also able to adhere to the acquired dental pellicle, epithelia or prosthetic denture (7,10). All the *F. nucleatum* grown without antimicrobials and the most of the isolates grown in SC showed a hydrophilic character. However, three-tested *F. nucleatum* grown in SC of penicillin G were hydrophobic, and it suggests that any factor might have altered the surface. In addition, it is known that chlorhexidine alter the outer membrane and cytoplasm of Gram-negative bacteria (7). In this study, chlorhexidine was not able of modifying the cellular hydrophobicity of *F. nucleatum*.

The hydrophobicity in *F. nucleatum* was not associated to adhesion on host's oral cells, in accordance with Okuda *et al.*

Table 3. Adherence mean values in *F. nucleatum* grown in different antimicrobial agents

Isolate (No.)	WA		CH		TR		PE		MZ	
	C	A	C	A	C	A	C	A	C	A
1	78.74	46.24	61.33	21	87.66	40	101	51.33	126	31
2	94.07	30.58	53.33	26	86.66	27.33	122.66	22.33	71.33	55
3	84.58	34.66	45.33	26	52	27	155	73.66	76.33	35
4	14.08	33.58	97	26	108.33	21.33	149.66	36	123.33	32
5	13.24	33.83	80.66	20	84.33	18.66	216	163	156.66	51
6	81.58	22.83	57.33	18	72.66	31.33	123	23	102.33	33.33
7	90.99	28.16	81	18	45.66	42.66	113.66	23	69.33	29
8	104.66	32.77	58	25	126	23.66	NT	NT	129.66	38.66
9	75.77	20.88	62	15.66	159	36.33	NT	NT	83.33	33.33
10	72.32	23.22	57.33	18.33	55	24	NT	NT	133.66	38
11	85.33	29.66	56.66	17	95.66	60.33	NT	NT	109.33	36.33
12	106.08	31.49	50.66	21.33	92.33	22.33	94.66	52.66	166.33	25.66
13	99.66	22.10	67.33	21.33	121.66	22.66	NT	NT	112	30.66
14	97.66	22.55	71.66	18.33	85.66	21	109.33	60.33	76.66	20.33
15	96.44	29	74.33	16.66	55.33	16	NT	NT	110.33	25
16	99.49	22.99	95.33	17.66	141	27.33	119	26	93.33	26
17	90.83	23.24	100.66	17	68.66	21.33	98.33	19.66	85.66	19.33
18	94.86	23.21	69.33	23	124.33	27	NT	NT	76.66	39.33
19	65.41	19.25	77.33	20.33	56.66	24	84	23.33	71.66	18
20	62.33	20.99	93.33	17	42	19.66	NT	NT	73.33	19.66
<i>F. n-1</i>	121.55	30.55	130	32.33	102.66	22.66	NT	NT	97	25
<i>F. n-2</i>	74.22	28.10	52.66	25.66	80	23.33	NT	NT	76	34
<i>B. f.</i>	26876.53	14601.53	9031.66	5285.33	29789.95	6315	19574.36	14779.56	4381.66	622
Mean	1257.888	661.366	461.924	249.868	1379.704	300.649	1620.051	1181.066	287.040	57.288

WA: without antimicrobial

CH: chlorhexidine

TR: triclosan

PE: penicillin G

MZ: metronidazole

C: control

A: adherence

NT: not tested

F. n-1: *F. nucleatum* ATCC 10953*F. n-2*: *F. nucleatum* ATCC 25586*B. f.*: *B. fragilis* ATCC 23745.

Wilcoxon test: (C x A); Z critic= 1.96; Control: Z=4.20* (C.C > A.C); Chlorhexidine: Z=4.20* (C.CH > A.CH); Triclosan: Z=4.20* (C.TR > A.TR); Penicillin G: Z=3.18* (C.PE > A.PE); Metronidazole: Z=4.20* (C.MZ > A.MZ).

Variance analysis of Kruskal-Wallis: (C x CH x TR x PE x MZ); H critic= 9.49; Control: H = 22.08*; Adhesion: H = 22.10*;

* Significant values. **Multiples comparisons test:** CH group > PE or MZ group; TR group > PE group. **Multiples comparisons test:** Ch group > PE or MZ group; C group > CH group; P = 0.05.

(26). All the tested isolates adhered to oral epithelial cells, but when bacteria were grown in SC of antimicrobials a decreasing or increasing in adherence values was observed in accordance with Kadry *et al.* (18). Xie *et al.* (33) showed that several adhesins are present in *F. nucleatum*, suggesting that the adherence can be increased or decreased by influence of different chemical substances. Our results suggest that bacterial adhesion mechanism in *F. nucleatum* was influenced by adhesins localized in cell wall or outer membrane, because structures such as fimbriae or capsule were not observed by TEM, in accordance with Falkler Jr. and Hawley (8) and Ellepola and Samaranyake (7).

The morphological alterations produced for chlorhexidine or penicillin G (Fig. 2) suggests an alteration in the adhesin production decreasing the bacterial adhesion on OEC. Grenier (12) showed that *P. gingivalis* grown with chlorhexidine there was a decreasing of the adhesion process. In addition, Novaes Jr. *et al.* (25) showed that metronidazole did not produce any ultra-structural modifications in *P. gingivalis* or *P. intermedia*. Similarly, triclosan and metronidazole did not produce any cell alteration in the tested *F. nucleatum* but these agents were able to decrease the adherence values. On the other hand, several antimicrobials can modify any virulence factors in *F. nucleatum* but the knowledge about the host-fusobacteria relationship is still unclear.

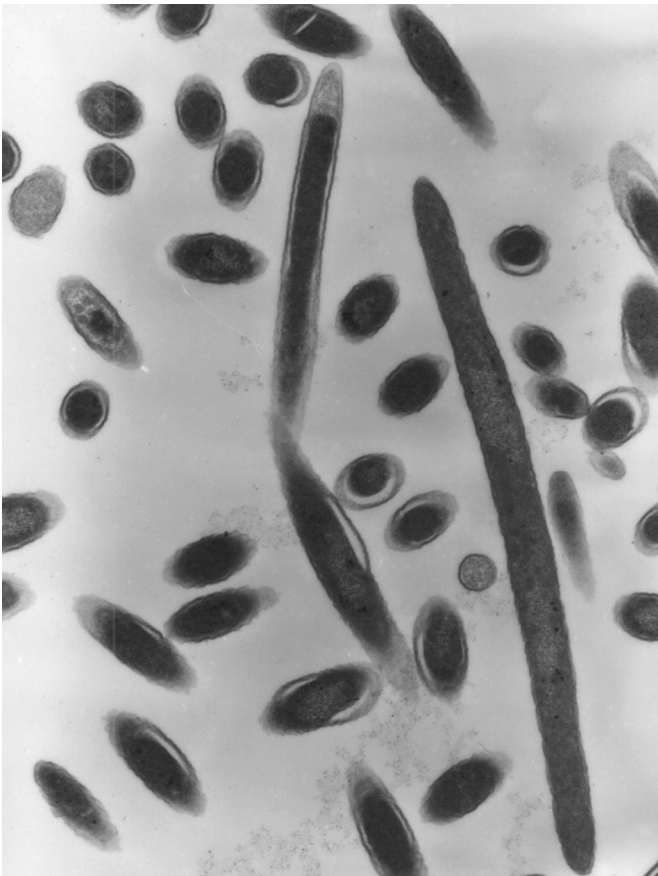


Figure 1. *F. nucleatum* grown in BHI without antimicrobials. Cells with 0.4 to 0.5 mm of diameter, round and opacity-electron (28,000 X).

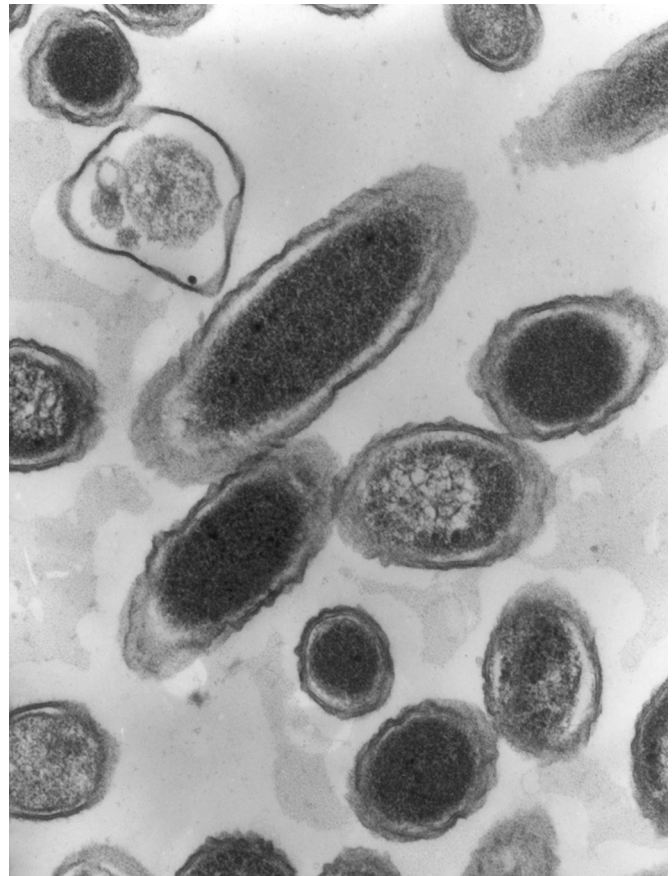


Figure 2. Morphologic alterations of *F. nucleatum* treated with chlorhexidine or penicillin G. Arrows show an irregular cell wall (44,360 X).

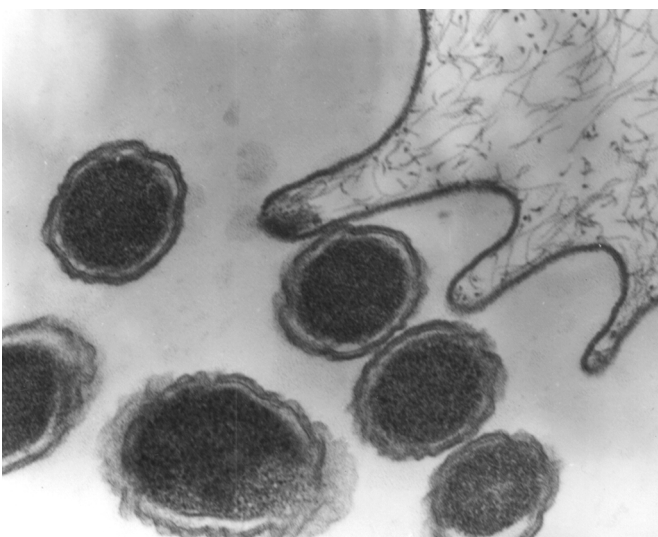


Figure 3. *F. nucleatum* grown in SC of chlorhexidine adhered on epithelial cells of (68,570 X).

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RESUMO

Influência de concentrações subinibitoriais de antimicrobianos na hidrofobicidade, aderência e ultraestrutura de *Fusobacterium nucleatum*

Fusobacterium nucleatum é um organismo que cumpre o papel de ponte de ligação entre os colonizadores iniciais e tardios no biofilme dentário, e é considerado um possível periodontopatôgeno. Em Odontologia, agentes antimicrobianos são usados para o tratamento e controle de doenças infecciosas associadas com a placa dental. Anti-sépticos têm sido usados em associação com antibióticos para reduzir infecções após

cirurgias orais. Neste estudo, foi avaliada a influência de concentrações subinibitórias de clorexidina, triclosan, penicilina G e metronidazol, sobre a hidrofobicidade, aderência às células epiteliais bucais, e a ultra-estrutura de *F. nucleatum*. Todos os isolados foram susceptíveis a clorexidina, triclosan, e metronidazol. A maioria deles foi sensível à penicilina G. Também, a maioria dos isolados, foi hidrofílica quando cresceram com ou sem antimicrobianos. A aderência foi diminuída pela ação dos antimicrobianos usados. Os resultados sugerem que a aderência de *F. nucleatum* foi influenciada por adesinas devido a que estruturas como fimbrias ou cápsula não foram observadas pela microscopia eletrônica de transmissão.

Palavras-chave: *Fusobacterium nucleatum*, hidrofobicidade, aderência, antimicrobianos, ultra-estrutura.

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