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Detection of dental plaque with disclosing agents in the context of preventive oral hygiene training programs

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ABSTRACT

No studies have evaluated the relationship between the detection points for dental bacterial plaque (DBP or biofilm) and gender, age, socioeconomic status, body mass index (BMI), and oral health, hence the need to investigate and clarify their possible association. This study aimed to map out the occurrence of DBP, investigate and evaluate the factors affecting its localization, and design preventive interventions. The research was conducted on 588 public school children aged 4–18 years in a provincial area of Greece. The subjects' oral health status and anthropometric characteristics were examined by a dentist (A.F.) and a dietitian (E.P.), respectively. To identify DBP, chewable double-staining disclosing tablets were used. The results of the present study indicate the following: (1) Age and socioeconomic status seem to be associated with DBP development, particularly in the oral cavity. (2) Overweight schoolchildren show more DBP on the upper posterior occlusal and upper posterior buccal surfaces compared to normal-weight children. (3) Moderate caries disease is associated with DBP detection on almost all tooth surfaces and especially on the tongue and lower anterior labial surface. (4) Severe caries disease is most strongly associated with DBP in the upper posterior palatal, lower posterior buccal, and lower posterior lingual spaces, as well as on the tongue. (5) Sex is the only variable without a significant impact on DBP detection surfaces. In conclusion, DBP identification in specific areas of the mouth seems to be influenced by age, socioeconomic level, BMI, and oral health. Gender has no influence on DBP detection points. Disclosing agents can be used in oral health prevention programs, both for more effective guidance on the use of oral hygiene tools and for their evaluation.

1. Introduction

The strong and two-way relationship of oral health and the general health of the human body is now proven [1]. Safeguarding and promoting oral health contributes decisively to maintaining overall health and wellness and should thus be prioritized and improved. The role of public healthcare providers is crucial in this effort. Providing experiential education to the public in relation to oral hygiene at an early age proves to be particularly effective [2, 3]. An important part of this training is the detection and localization of dental bacterial plaque (DBP), which is the main cause of the most common oral diseases (caries and periodontal disease) [4, 5]. DBP is a thin, yellowish-white coating (thereafter referred to as biofilm) which adheres to various dental surfaces and consists of microbial colonies and products of oral microbial flora metabolism. Biofilm is defined as "bacterial communities that are embedded in a self-produced matrix of extracellular polymeric substances" [6, 7].

Removing biofilm from various areas of the oral cavity is crucial to oral disease prevention and is achieved through regular personal and professional removal [8]. To be effectively removed with teeth brushing [9], it must first be accurately detected. Biofilm can be accurately localized with special dyes [10], mainly iodine, gentian violet, erythrosine, basic fuchsin, fast green, food dyes, fluorescein, and two-tone disclosing agents in the form of tablets, solutions, wafers, lozenges, or mouth rinses. When taken, these agents color the areas of the oral cavity where biofilm is present; the intensity of the color depends on thickness of the plaque [11]. The use of these disclosing agents is very effective as it

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helps to (a) establish the level of the user's oral hygiene, (b) raise awareness of the need for biofilm removal, (c) provide personalized instructions and incentives for better oral hygiene, (d) facilitate user self-assessment, (e) measure oral hygiene effectiveness, (f) evaluate prevention and training programs for better oral hygiene, and (g) enable studies on biofilm identification. Moreover, their role in the implementation of preventive dentistry programs is particularly useful, especially in school-aged children. The live visualization of the colored surfaces of the teeth and tongues of the children participating in these programs facilitates one's understanding of the various theoretical concepts and medical terms associated with them [12]. Also, regarding the experiential education of school students, as far as the use of oral hygiene tools is concerned, biofilm staining allows for better and more effective guidance from healthcare instructors.

The most common locations of biofilm are those in which access to and management of oral hygiene tools are difficult. Also, biofilm is located where the tongue and saliva's natural self-cleaning process does not work effectively for a variety of reasons. Studies suggest that biofilm deposits accumulate in larger quantities on irregular areas, "attached gingiva," and the lateral surfaces of the tongue [13, 14].

The surface area of biofilm detection points depends directly on the level of oral hygiene knowledge and the use of appropriate tools for a cleaner mouth (toothbrush, toothpaste, tongue scrapers, floss, etc.). Epidemiological studies on biofilm detection and localization through the use of special dyes in the oral cavity are particularly useful and necessary, especially with schoolchildren as it is easier for them to incorporate and adopt oral hygiene habits and behaviors.

Based on the above, the purpose of this study was (a) to map the existing situation by recording biofilm detection points, (b) to investigate and evaluate the factors affecting biofilm, and (c) to set priorities for preventive intervention planning, as well as for control, systematic monitoring, and evaluation of all steps of the implementation stages.

The research hypothesis focused on the possible correlation between biofilm and (1) sex, (2) age, (3) socioeconomic status, (4) body mass index, and (5) oral health status.

Hypothesis: The presence of biofilm is affected by age, socioeconomic level, body mass index, and/or oral health status.

Null hypothesis: The presence of biofilm is unaffected by age, socioeconomic level, body mass index, and/or oral health status.

2. Material and methods

2.1. Participants

Data were derived from a rural area in northern Greece. The study was conducted in 14 public schools during the school year 2017–2018 over 58 sessions involving students in all levels of primary and secondary education: two grades of preschool, six grades of primary school, three grades of middle school, and three grades of high school. To complete the program, 58 visits were conducted. In the study, 588 schoolchildren (295 boys and 293 girls) aged 4–18 years participated. Children with learning disabilities and special healthcare needs were excluded, as they are more vulnerable to a wide range of additional health problems than the general population [15, 16].

2.2. Ethics and morality

The study was conducted within the framework of a national preventive action program in schools based on the guidelines of good clinical practice (GCP) [17], defined as the international ethical standard of scientific quality for the design, conduct, performance, documentation, analyses, and reporting of clinical trials. The ethical standards of the study protocol were approved by the Ministry of Education.

The schools were informed by the concerned government authorities, which requested collaboration with the health professionals. Parents were informed about the program and authorized their children's participation in this clinical study by handwriting their name, surname, and signature, as well as the date, on the consent form. The study was conducted by health professionals (a dentist [A.F.] and a nutritionist-dietitian [E.P.]) specially trained in oral health disorder and obesity prevention, as well as oral hygiene education.

2.3. Evaluation of demographic characteristics

The demographic characteristics of sex, date of birth, school class, and nationality were provided by school directors. The exact ages were calculated from the dates of birth and examination. Nationality was used as a socioeconomic element, as foreign schoolchildren of this region often come from migrant families from neighboring countries looking for work in rural areas. The personal details of the students (name and surname) were not recorded.

2.4. Assessment of anthropometric characteristic

To determine anthropometric characteristics, a portable seca scale and height-measuring equipment were used. The weight and height of each participant were recorded, and, following standardized techniques, BMI was calculated as a ratio of weight to height squared (kg/m^2). For the BMI classification, child and adolescent BMI growth curves (according to the WHO) were used [18].

2.5. Assessment of oral health

Upon physical examination, general extraoral characteristics were evaluated, such as facial anthropometry (facial skeletal type, such as dolichofacial, brachyfacial, or mesofacial) and the temporomandibular joint (clicking, crepitation, limitation of mouth opening, lateral deviation, or pain). Intraoral characteristics were also evaluated, such as the relationship among dental arches, occlusion, transverse and anteroposterior molar relation, the presence of oral parafunction, and passive lip seal. Stomatological evaluation included observation of the mouth floor, lips, tongue, palate, vestibule, and oral mucosa, in addition to accurate intraoral inspection of the teeth [19].

The widely used decayed, missing, and filled teeth index (DMFT) was divided into three categories: (a) no caries disease (DMFT = 0), (b) moderate caries disease (DMFT = 1–5), and (c) severe caries disease (DMFT = 6–10).

Orthodontic disorders (OD) were divided into two categories: (a) absence and (b) presence, without further categorizations.

Periodontal diagnosis was formulated in two categories. The first, absence, indicates a healthy periodontium in which only the gingival tissues may be directly observed. Such tissues are described as being stippled, pale pink, or coral pink, with various degrees of pigmentation in other races and a lack of bleeding upon probing. Healthy gingival tissue is also tightly adhered to the underlying tissues with a knife-edge margin where it abuts the tooth. In the absence of pathology, the gingival margin is located at the cemento-enamel junction. The second category, presence, indicates bleeding on probing with either a loss or no loss of attachment [20, 21].

Dental examination was performed using sterile examination kits containing a mirror (LS456 480/5, Carl Martin GmbH, Solingen, Germany), dental explorer (LS1091/33, Carl Martin GmbH, Solingen, Germany), and perio-probe (LS973/80 WHO, Carl Martin GmbH, Solingen, Germany). Articulating paper, a head light, and sterile gloves were also used.

To dye the teeth, two-tone disclosing agents (Mira-2-Ton tablets, Hager & Werken, Duisburg, Germany) were used. Teeth and gingiva examination was carried out before the use of the disclosing agents, as the aforementioned dye stains the oral soft tissues and dental surfaces as well as dental plaque, leaving the color for several hours after use. For purposes of convenience sampling, the presence or absence of biofilm was recorded without disclosing its score or quantity [22].

2.6. Process of capturing DBP

The children were asked to brush their teeth on the day of the visit and to bring their toothbrushes with them. Before the clinical examination, the children were instructed to rinse their teeth to remove any existing food residues. In addition, a water-based lubricant was applied to their lips so that the stain could not color them.

Instructions were then given on how to use the tablet. Children were asked to chew the tablet for 30–60 seconds, making sure to transfer it to all parts of the teeth using the tongue, and then remove it. The administered tablets were slightly flavored to encourage their use.

2.7. Interpretation of results

On the clean surfaces of the teeth, the disclosing agents were not absorbed. On surfaces with a relatively thin biofilm (recent bacterial plaque), the staining was light-colored (red biofilm), and where there was a thicker biofilm, indicating older and more resistant plaque, the staining was darker and more opaque (blue biofilm).

To accurately read the tooth points where the biofilm was imprinted, each jaw was separated for recording purposes into anterior and posterior regions. The anterior region was divided into buccal and lingual areas, and the posterior region was divided into buccal, lingual, and occlusal areas. In addition, the tongue surface was recorded.

2.8. Statistical analysis

Biofilm detection by plaque-disclosing tablets was tabulated as multiple responses against demographic (sex, nationality, education level), body (BMI classes), and dental (DMFT ranks) variables. Additionally, orthodontic and periodontal effects were also investigated. A likelihood ratio chi-square test was conducted to determine whether the rate of each response differs across grouping levels, and individual tests for significance between observed and expected results were further estimated, with 0.05 as the reference level. Minitab® 18.1 (Minitab Inc.) and JMP 13.2 (SAS Institute Inc.) software were used for the statistical analysis.

3. Results

Gender was the unique variable with no real effect on the biofilm detection surfaces (Table 1), which means that all detection surfaces were equally deployed between males and females (see p-values in Table 1).

Non-Greek students showed a higher occurrence of dental plaque on the upper anterior labial ($p = 0.014$) and lingual surfaces ($p = 0.017$, 12.1% each, Table 2) and on the upper posterior palatal ($p < 0.001$) and lower posterior buccal surfaces ($p = 0.0001$, 29.3% each). Meanwhile, Greek students exhibited a higher percentage of dental plaque detected on the lower anterior labial surface ($p = 0.013$, 47.4%) compared with that of other nationalities (25.9%).

Educational grade was related to a greater presence of biofilm in four areas of the mouth at preschool age (Table 3). In fact, biofilm presence on the upper posterior palatal ($p < 0.001$) and lower posterior buccal surfaces ($p < 0.001$) predominated in that period against all other education levels. For the same surfaces, primary education predominated against middle school.

Upper anterior lingual surface biofilm was more frequently observed in preschool than in primary school ($p < 0.001$). Also, biofilm on the lower anterior labial surface ($p < 0.001$) occurred more often in the preschool and middle school students than in the high school students.

Preobese and obese students displayed higher amounts of biofilm on the upper posterior occlusal ($p = 0.024$) and buccal surfaces ($p = 0.001$) compared with people with normal weight (Table 4). The same result was also found for the lower posterior occlusal surface ($p = 0.018$).

Interesting results were drawn from the dental caries effects (Table 5). Mild dental caries (DMFT: 1–5) was associated with DBP on almost all

Table 1
Cross-tabulated numerical and percentage frequencies of oral disorders according to sex.

			Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Sex	Female	A	13	14	234	209	29	138	196	29	4	232	274	1372	293
			0.9%	1.0%	17.1%	15.2%	2.1%	10.1%	14.3%	2.1%	0.3%	16.9%	20.0%		
			4.4%	4.8%	79.9%	71.3%	9.9%	47.1%	66.9%	9.9%	1.4%	79.2%	93.5%		
Sex	Male	B	13	13	248	232	35	127	200	35	7	248	290	1448	294
			0.9%	0.9%	17.1%	16.0%	2.4%	8.8%	13.8%	2.4%	0.5%	17.1%	20.0%		
			4.4%	4.4%	84.4%	78.9%	11.9%	43.2%	68.0%	11.9%	2.4%	84.4%	98.6%		

Response	Chi Square	Prob > ChiSq
Upper posterior buccal	1,1230	0,2893
Lower posterior lingual	0,8184	0,3656
Upper posterior palatal	0,5431	0,4612
Lower posterior buccal	0,5431	0,4612
Lower anterior labial	0,4950	0,4817
Lower posterio occlusal	0,4803	0,4883
Tongue	0,4011	0,5265
Upper posterior occlusal	0,3604	0,5483
Upper anterior lingual	0,0405	0,8404
Lower anterior lingual	0,0279	0,8673
Upper anterior labial	0,0001	0,9931

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

Table 2
Cross-tabulated numerical and percentage frequencies of oral disorders according to nationality.

	Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Nationality	Greek	A	19 0.8% 3.6%	20 0.8% 3.8%	430 17.2% 81.1%	392 15.7% 74.0%	47 1.9% 8.9%	251 10.0% 47.4% **B**	349 14.0% 65.8%	47 1.9% 8.9%	9 0.4% 1.7%	428 17.1% 80.8%	507 20.3% 95.7%	2499	530
Nationality	Other	B	7 2.1% 12.1% **A**	7 2.1% 12.1% **A**	53 16.3% 91.4%	50 15.3% 86.2%	17 5.2% 29.3% **A**	15 4.6% 25.9%	47 14.4% 81.0%	17 5.2% 29.3% **A**	2 0.6% 3.4%	53 16.3% 91.4%	58 17.8% 100.0%	326	58

Response	ChiSquare	Prob > ChiSq
Upper anterior labial	6.0847	0.0136
Upper anterior lingual	5.6788	0.0172
Upper posterior palatal	14.4216	0.0001
Lower anterior labial	6.2200	0.0126
Lower posterior buccal	14.4216	0.0001

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

Table 3
Cross-tabulated numerical and percentage frequencies of oral disorders according to education Level.

Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Preschool	A	26 6.2% 36.1%	26 6.2% 36.1% **B**	55 13.1% 76.4%	47 11.2% 65.3%	26 6.2% 36.1% **B.C.D**	40 9.5% 55.6% **D**	49 11.7% 68.1%	26 6.2% 36.1% **B.C.D**	0 0.0% 0.0%	54 12.9% 75.0%	71 16.9% 98.6%	420	72
Primary	B	0 0.0% 0.0%	1 0.1% 0.3%	262 17.6% 81.1%	242 16.2% 74.9%	30 2.0% 9.3% **C**	136 9.1% 42.1%	202 13.5% 62.5%	30 2.0% 9.3% **C**	11 0.7% 3.4%	262 17.6% 81.1%	315 21.1% 97.5%	1491	323
Middle	C	0 0.0% 0.0%	0 0.0% 0.0%	134 18.1% 85.9%	122 16.5% 78.2%	5 0.7% 3.2%	80 10.8% 51.3% **D**	116 15.7% 74.4%	5 0.7% 3.2%	0 0.0% 0.0%	133 18.0% 85.3%	145 19.6% 92.9%	740	156
High	D	0 0.0% 0.0%	0 0.0% 0.0%	32 18.4% 86.5%	31 17.8% 83.8%	3 1.7% 8.1%	10 5.7% 27.0%	29 16.7% 78.4%	3 1.7% 8.1%	0 0.0% 0.0%	32 18.4% 86.5%	34 19.5% 91.9%	174	37

Response	**ChiSquare**	**Prob > ChiSq**
Upper anterior labial	109.203	<0.0001
Upper anterior lingual	101.847	<0.0001
Upper posterior palatal	38.8531	<0.0001
Lower posterior buccal	38.8531	<0.0001
Lower posterior lingual	13.1796	0.0043

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

Table 4
Cross-tabulated numerical and percentage frequencies of oral disorders according to BMI.

Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Underweight	A	3	3	24	20	4	8	22	4	0	24	29	141	29
		2.1%	2.1%	17.0%	14.2%	2.8%	5.7%	15.6%	2.8%	0.0%	17.0%	20.6%		
		10.3%	10.3%	82.8%	69.0%	13.8%	27.6%	75.9%	13.8%	0.0%	82.8%	100.0%		
Normal	B	12	12	225	197	39	142	198	39	6	223	293	1386	314
		0.9%	0.9%	16.2%	14.2%	2.8%	10.2%	14.3%	2.8%	0.4%	16.1%	21.1%		
		3.8%	3.8%	71.7%	62.7%	12.4%	45.2%	63.1%	12.4%	1.9%	71.0%	93.3%		
Overweight	C	8	9	156	148	15	77	116	15	3	156	162	865	163
		0.9%	1.0%	18.0%	17.1%	1.7%	8.9%	13.4%	1.7%	0.3%	18.0%	18.7%		
		4.9%	5.5%	95.7%	**B**	9.2%	47.2%	71.2%	9.2%	1.8%	95.7%	99.4%		
Obesity	D	3	3	78	77	6	39	60	6	2	78	81	433	82
		0.7%	0.7%	18.0%	17.8%	1.4%	9.0%	13.9%	1.4%	0.5%	18.0%	18.7%		
		3.7%	3.7%	95.1%	93.9%	7.3%	47.6%	73.2%	7.3%	2.4%	95.1%	98.8%		
				B	**B**						**B**			

Response	**ChiSquare**	**Prob > ChiSq**
Upper posterior occlusal	9.4603	0.0238
Upper posterior buccal	15.5036	0.0014
Lower posterior occlusal	10.0161	0.0184

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

teeth surfaces and predominantly on the lower anterior labial surface ($p < 0.001$). Serious dental caries (DMFT: 6–10) was more strongly associated with biofilm on the upper posterior palatal ($p < 0.001$), lower posterior buccal ($p < 0.0010$), and lingual ($p = 0.001$, 100%) surfaces. Biofilm detected on the tongue has similar percentages between DMFT rankings (1–5 and 6–10). Even in the absence of caries disease, increased levels of biofilm on the dorsal surface of the tongue (91.5%) were found, which coincide with the simultaneous increased presence of biofilm on the lower anterior labial surface ($p < 0.001$, 88.4%).

Upper anterior labial ($p = 0.012$) and lingual surface ($p = 0.007$) staining was rarely affiliated with orthodontic disorders (2.6% each, Table 6), which was also true for periodontal disease (0%, Table 7). For the latter, biofilm on the upper posterior palatal ($p = 0.001$) and lower posterior buccal ($p = 0.001$) surfaces was more often found in the absence of than in the presence of periodontal disease.

In addition, 44.0% of the subjects had no caries, 44.8% had no periodontitis, and 69.4% had no OD.

4. Discussion

In the present study, biofilm detection points were mapped. This paper also confirms the research hypothesis about the influence of age, socioeconomic status, body mass index, and oral health on biofilm detection points. Regarding the research hypothesis that sex affects biofilm detection points, the results of this study suggest no sex-related differences.

Our study did not find any differences in biofilm detection points between boys and girls. However, despite the lack of relevant studies, sex-related differences in oral health status (caries experience, tooth loss, and periodontal disease) have been widely documented across cultures and over time; these studies also found the female population as being more vulnerable [23]. Bibliographical reports claim that such differences begin during childhood and extends into adolescence and the reproductive years [23]. Genetic variation and synergistic changes associated with female hormones, pregnancy, and the history of women's reproductive lives are some factors that can partially explain the so-called gender gap in oral health [23].

Age groups, which in this study were classified according to educational level, show significant variations in biofilm detection points. At preschool age, an increased presence of biofilm is found on the upper anterior lingual and lower posterior buccal surfaces. This is probably because at this age, there is insufficient preventive dentistry training and skills in using oral hygiene tools at the inaccessible biofilm detection points. These reasons also affect differences between primary school children and secondary school children as well as the superiority of detecting biofilm on the upper anterior lingual surface of the preschool and primary school children, as they develop teeth-brushing skills over time [7, 24]. Nevertheless, there is no relevant literature with which to compare the findings of our study.

Important differences in biofilm detection points between foreigners (generally of low socioeconomic status) and Greek schoolchildren were investigated. The specific locations of biofilm in foreign schoolchildren (upper anterior labial, upper anterior lingual, upper posterior palatal, and lower posterior buccal surfaces) and in Greek schoolchildren (lower posterior buccal surface) cannot be cross-checked with similar studies, as there are none available. However, possible differences in eating habits [25] and oral hygiene should be considered [26, 27, 28].

Another interesting finding in our study is related to the location of dental plaque according to BMI. In the group of overweight and obese schoolchildren, dental plaque seemed to be concentrated on the upper posterior occlusal and upper posterior buccal surfaces, as well as on the lower posterior occlusal surface. These findings are unique, as no relevant studies have examined these correlations. However, it could be assumed that biofilm localization on these surfaces, where one can expect biofilm to be removed by "natural cleansing," may be because overweight and obese children tend to swallow rather than chew their food,

Table 5
Cross-tabulated numerical and percentage frequencies of oral disorders according to DMFT index.

	Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
DMFT	0	A	3	3	168	143	3	229	92	3	3	166	237	1050	259
			0.3%	0.3%	16.0%	13.6%	0.3%	21.8%	8.8%	0.3%	0.3%	15.8%	22.6%		
			1.2%	1.2%	64.9%	55.2%	1.2%	**88.4%**	35.5%	1.2%	1.2%	64.1%	91.5%		
								B							
DMFT	1–5	B	22	22	279	265	23	37	266	23	3	279	290	1509	291
			1.5%	1.5%	18.5%	17.6%	1.5%	2.5%	17.6%	1.5%	0.2%	18.5%	19.2%		
			7.6%	7.6%	**95.9%**	91.1%	7.9%	12.7%	91.4%	7.9%	1.0%	**95.9%**	99.7%		
			A	**A**	**A**	**A**	**A**		**A**	**A**		**A**			
DMFT	6–10	C	1	2	36	34	38	0	38	38	5	36	38	266	38
			0.4%	0.8%	13.5%	12.8%	14.3%	0.0%	14.3%	14.3%	1.9%	13.5%	14.3%		
			2.6%	5.3%	94.7%	89.5%	**100.0%**	0.0%	**100.0%**	**100.0%**	13.2%	94.7%	100.0%		
					A	**A**	**A.B**		**A**	**A.B**	**A.B**	**A**			

Response	ChiSquare	Prob > ChiSq
Upper anterior labial	14.5240	0.0007
Upper anterior lingual	14.2207	0.0008
Upper posterior occlusal	17.1888	0.0002
Upper posterior buccal	25.2616	<.0001
Upper posterior palatal	140.395	<.0001
Lower anterior labial	212.998	<.0001
Lower anterior lingual	74.8525	<.0001
Lower posterior buccal	140.395	<.0001
Lower posterior lingual	13.0553	0.0015
Lower posterior occlusal	18.1490	0.0001
Tongue	1.0158	0,6018

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

Table 6
Cross-tabulated numerical and percentage frequencies of oral disorders according to orthodontic disorder.

	Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Orthodontic	No	A	17 1.4% 7.1%	18 1.5% 7.5%	200 16.8% 83.0%	178 14.9% 73.9%	33 2.8% 13.7%	116 9.7% 48.1%	163 13.7% 67.6%	33 2.8% 13.7%	2 0.2% 0.8%	199 16.7% 82.6%	234 19.6% 97.1%	1193	241
			B	**B**											
Orthodontic	Yes	B	9 0.6% 2.6%	9 0.6% 2.6%	283 17.3% 81.6%	264 16.2% 76.1%	31 1.9% 8.9%	150 9.2% 43.2%	233 14.3% 67.1%	31 1.9% 8.9%	9 0.6% 2.6%	282 17.3% 81.3%	331 20.3% 95.4%	1632	347

Response	ChiSquare	Prob > ChiSq
Upper anterior labial	6.2771	0.0122
Upper anterior lingual	7.2310	0.0072

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

Table 7
Cross-tabulated numerical and percentage frequencies of oral disorders according to periodontal disorder.

	Freq Share Rate Comparisons		Upper anterior labial	Upper anterior lingual	Upper posterior occlusal	Upper posterior buccal	Upper posterior palatal	Lower anterior labial	Lower anterior lingual	Lower posterior buccal	Lower posterior lingual	Lower posterior occlusal	Tongue	Total Responses	Total Cases
Periodontal	No	A	26 2.9% 14.3%	27 3.0% 14.8%	139 15.5% 76.4%	119 13.3% 65.4%	32 3.6% 17.6%	93 10.4% 51.1%	114 12.7% 62.6%	32 3.6% 17.6%	3 0.3% 1.6%	138 15.4% 75.8%	174 19.4% 95.6%	897	182
							B			**B**					
Periodontal	Yes	B	0 0.0% 0.0%	0 0.0% 0.0%	344 17.8% 84.7%	323 16.8% 79.6%	32 1.7% 7.9%	173 9.0% 42.6%	282 14.6% 69.5%	32 1.7% 7.9%	8 0.4% 2.0%	343 17.8% 84.5%	391 20.3% 96.3%	1928	406

Response	ChiSquare	Prob > ChiSq
Upper anterior labial	60.9815	<0.0001
Upper anterior lingual	63.3269	<0.0001
Upper posterior palatal	10.0352	0.0015
Lower posterior buccal	10.0352	0.0015

Share denotes percentage response per category. Rate (per case) denotes percentage of responses in each category based on the total number of cases. Letters indicate significant differences between particular cells.

thus limiting their mouths' natural self-cleaning process. Nevertheless, association studies between BMI, oral hygiene, and gingivitis in schoolchildren show conflicting results [29, 30].

Also, a correlation exists between biofilm detection and dental caries. Moderate dental caries disease was associated with biofilm sites on almost all tooth surfaces, mainly on the tongue and the lower posterior buccal surface. Caries is a modern-day disease and reflects an imbalance of oral biofilm partly caused by the increased consumption of refined sugars, carbohydrates, and acidic drinks [31]. It is especially important for clinicians to prevent this disturbance of the natural microbial balance of biofilm and not just treat its consequences [32].

Our study also highlighted the extremely high level of biofilm on the tongue, which is not affected by the presence or absence of dental caries. Thus, biofilm on the dorsal surface of the tongue can be independent and is not associated with that on dental surfaces, nor is it relevant to whether or not schoolchildren apply good oral hygiene to dental surfaces. Matsui's study argues that tongue cleaning has no obvious contribution to inhibiting dental plaque formation [33]. Generally, during this research, schoolchildren were observed to be ignorant of the need to clean their lingual surfaces.

Furthermore, no statistically significant difference was found between patients with and without orthodontic disorders. Biofilm detected on the upper anterior labial and upper anterior lingual surfaces was similar in the two groups. A plausible explanation is that orthodontic appliances rather than orthodontic disorders per se might be responsible for increased biofilm accumulation; particular effort and skills are required to efficiently control microbial plaque in patients with orthodontic appliances. In our study, however, most of the children did not have any orthodontic appliances, and the disorder was not related to crowding.

This study supports the relationship between periodontal disease and biofilm localization. In particular, plaque localization on the upper posterior palatal and the lower anterior labial surfaces was found to be more frequent in the absence of periodontal disorders. Also, biofilm localization on the upper anterior labial and upper anterior lingual surfaces rarely occurred in the presence of periodontal disorders. These findings cannot be compared with previous ones, as no relevant bibliographic references have been found.

It should be noted that increased biofilm disclosure indicates incomplete or even inefficient oral hygiene and demonstrates the need for continuous and regular preventive interventions and dental sealants. Dental sealants can prevent cavities in both children and adults for years [34, 35], and using of low-viscous resin infiltrant combined with a flowable composite resin can improve the initial quality of fissure sealing compared with the exclusive use of a conventional fissure sealant, particularly in preexisting caries lesions [36].

In conclusion, effective biofilm removal in children depends on their knowledge of oral hygiene, motivation, frequency, duration and method of brushing, brush design and adhesion to tooth surfaces, their age, and the involvement of their parents in brushing. For all these reasons, children's oral hygiene programs are becoming necessary and useful, as they help reduce the prevalence of caries and gingival disease in school-aged children [37, 38, 39, 40]. Thus, the use of disclosing agents qualifies as an auxiliary tool in oral hygiene improvement programs for children [41].

Thus, this study's findings may be used (a) to guide schoolchildren, teachers, and parents or guardians in the right and efficient use of oral hygiene tools and (b) as a basis for designing new research.

4.1. Strengths and limitations of the research

This study has remarkable advantages in that (1) the study population was adequate, and (2) it is the only study that evaluates the association between biofilm location and sex, age, socioeconomic status, BMI, and oral health status (DMFT index, orthodontic disorder, and periodontal disease) in schoolchildren aged 4 to 18.

One of the limitations in this research, however, was sample selection. The sample was based on convenience (i.e., schoolchildren from the county/area where the researchers were working). Moreover, orthodontic diagnosis was based on extraoral and intraoral examination without panoramic and lateral cephalometric radiographs. Furthermore, periodontal diagnosis was based on the presence or absence of disease without classifying such diseases further.

5. Conclusions

The present study demonstrates that gender does not directly contribute to the locations of dental biofilm. The detection of biofilm in specific areas of the mouth appears to be influenced by age, socioeconomic level, body mass index, and oral health status. The presence of biofilm on the tongue is not affected by the presence or absence of dental caries. High levels of biofilm in many areas of the mouth necessitate oral hygiene programs in schools.

However, further investigation is required along with the establishment of public health programs focusing on the importance of proper oral hygiene to limit inequalities and ignorance about oral hygiene.

Declarations

Author contribution statement

Aristeidis Fasoulas, Eleni Pavlidou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dimitris Petridis, Maria Mantzorou, Kyriakos Seroglou, Constantinos Giaginis: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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