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In vivo analysis of early biofilm development and cell viability on implant-mimicking abutments at 24 h, 48 h, and 7 days

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Abstract

Introduction The microbiota associated with peri-implant diseases has been described, though information about biofilm formation and development on dental implants remains scarce.

Objectives To analyze and compare biofilm formation and distribution at 24 h, 48 h and 7 days on experimental abutments simulating dental implants in peri-implant healthy patients.

Material and methods Experimental abutments with micro-threads and a modified rough surface were placed in healthy dental implants of 10 patients. Instructions were given not to clean the abutments for the duration of the study. Exclusion criteria included the use of antiseptics or antibiotics 30 days prior to recruitment or during the study period. After 24 h, 48 h and 7 days, the abutments were removed and stained using LIVE/DEAD stain, and two sides (buccal and palatal/lingual) and two areas (supragingival and subgingival) were assessed, with measurement of the mean biofilm covering area.

Results Twenty-nine experimental abutments placed in 10 patients were assessed. The total mean biomass coverage areas were 9.3%, 16.2% and 16.8% at 24 h, 48 h and 7 days, respectively, with significant differences being observed between 24 h and the subsequent timepoints ($p < 0.05$). Significantly greater supragingival biofilm coverage was observed at 7 days in comparison with the subgingival zone (21.85% versus 11.7%; $p < 0.05$).

Conclusions Biofilm coverage on healthy dental implants increases progressively during the first 48 h and then stabilizes. The biofilm is mainly composed of live cells in the supragingival and subgingival areas. After 7 days, the supragingival areas show significantly greater biofilm coverage.

Keywords Peri-implantitis, Biofilm, Dental implant, Implant-abutment

Introduction

Dental implants are one of the most widely used treatments for restoring dental aesthetics and function. Despite the high success and survival rates [1], biological complications are a major concern in contemporary dentistry [2]. The current evidence estimates that around 43% of all patients develop peri-implant mucositis (range: 19–65%) and 22% peri-implantitis (range: 1–47%) [3–7]. A recent study [6] has reported similar prevalences of peri-implant diseases after 10 (67.9% mucositis and

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10.6% peri-implantitis) and 20 years of follow-up (47.6% mucositis and 33.3% peri-implantitis). Most authors consider that these inflammatory conditions result from a bacterial challenge and host response [8–10]. Oral biofilms seem to play a very important role in the development of these disorders [11–13], and other factors such as a history of periodontal disease, smoking, or systemic diseases may influence the progression of such biological complications [14–16].

In some cases, the rough surface of dental implants may become exposed to the oral environment due to inadequate placement, soft tissue recessions or a bone remodeling process. This in turn leads to the formation of film layer, also known as an acquired pellicle [17, 18], that allows the adhesion of early colonizing bacteria. If this layer is left undisturbed, a thick biofilm with periodontopathogenic microorganisms and an anaerobic environment in its deeper layers becomes established on the dental implants [19].

Dental implants can be colonized by a wide variety of bacterial species [20] in a short period of time [21]. The development of these biofilms can be favored by many factors such as the exposure of implant threads [22], and the roughness and chemical composition of the dental implants [23]. Since most authors report that the formation of complex biofilms with pathogenic bacteria is the key factor for the initiation and progression of peri-implant diseases, it is paramount to understand the initial stages of biofilm formation on exposed dental implant surfaces.

Previous studies on bacterial colonization and biofilm formation on dental implants are based on the analysis of experimental disks made of titanium (Ti) or zirconia (ZrO2). In vivo models have been designed to study biofilm samples collected with these disks placed in splints [24–26]. Such models have limitations, however, since they base their conclusions on the composition and structure of supramucosal biofilm, neglecting the subgingival area. Other authors have proposed the use of removed failed implants to study the biofilm structure [27, 28]. However, this approach does not allow analysis of the initial phases of bacterial colonization. To overcome these limitations, some reports have used healing abutments with different roughness profiles [17, 28–30], but such devices did not reproduce the geometry (i.e., threads) or the microscopic topography of commercially available dental implants. Indeed, according to Bermejo et al. [22, 23], increased surface roughness (microstructure) and the presence of implant threads (macrostructure) enhance biofilm formation and hamper dental hygiene [26]. Cortés-Acha et al. [21] developed a removable biofilm collector abutment with the same macro- and microstructure as dental implants in order to recover undisturbed biofilm. Their study showed that, in the absence of oral hygiene measures, extensive plaque growth can be observed on these abutments after 14 days. However, the authors did not provide data on the initial stages of biofilm formation. Thus, the aim of the present study was to analyze and compare the features of early biofilm (24 h, 48 h and 7 days) formed on healthy dental implants without oral hygiene measures.

Material and methods

A non-randomized experimental study was conducted in accordance with the declaration of Helsinki [31], and the protocol was approved by the local ethics committee (Dental Hospital of the University of Barcelona [Spain]; Ref.: 19/2015). All patients signed a written informed consent before enrollment. The CONSORT statement guidelines [32] were used as a reference to report this study.

The main inclusion/exclusion criteria and the description of the biofilm collector abutments have been published elsewhere [20]. Briefly, all patients included were systematically healthy, with controlled periodontal status, that had at least three healthy dental implants placed at the Dental Hospital of the University of Barcelona. Participants that required the use of any antibiotic or antiseptic during the study period or in the previous 30 days were excluded. Periodontal patients were only included if the disease was considered under control, with a pocket probing depth (PPD) of ≤ 4 mm and no bleeding on probing (BOP) at over 30% of the sites. The selected patients were instructed not to use the toothbrush over the abutment area, although they could brush or floss the rest of the implants or teeth, without using toothpaste. All the recruited dental implants had at least 2 mm of submucosal area and 2 mm of peri-implant keratinized mucosa.

The sample size was calculated using G*Power v.3.1.3 (Heinrich-Heine Universität, Dusseldorf, Germany), based on the assumption that a difference of 10% in subgingival biofilm coverage area after between 24 h and 7 days would be clinically significant. Considering a common standard deviation (SD) of 10%, a risk of 0.05, and a statistical power of 80%, a total of 9 patients would be required. To compensate for possible dropouts, the sample size was increased to 10 patients.

Once the biofilm collector abutment was placed, the buccal area was marked using a diamond bur, and the number of exposed threads was recorded for later analysis.

The abutments were collected at three different timepoints; after 24 h, 48 h and 7 days.

Once removed, the abutments were screwed to an implant analogue inside individual snap tubes to prevent the abutments from touching any surface. Then, the

samples were sent in sterile snap tubes with saliva at 4ºC to the microbiology laboratory of the Dentaid Research Center (Dentaid SL, Cerdanyola del Vallés, Spain).

In order to study biofilm coverage and vitality (i.e., the proportion of live and dead cells), the abutments were stained using the LIVE/DEAD Baclight Bacterial Viability Kit, L7012 (Molecular Probes, Eugene, OR, USA), and two sides (buccal and palatal/lingual) and two areas (supragingival and subgingival) were assessed.

The average biofilm covering area was measured using MetaMorph® v1.5 (Molecular Devices, LLC, Sunnyvale, CA, USA) Five regions of interest (ROIs) with the same size, and always selected in the same position, were quantified using the maximum projections obtained from each field. The 2D and 3D reconstructions were performed using Imaris Viewer® v.10.2.0 (Bitplane AG, Badenerstrasse, Zurich, Switzerland).

Statistical analysis

Data analysis was performed using the SPSS version 28.0 statistical package (IBM Corporation, Armonk, NY, USA).

The main outcome variable was the total area of the ROI covered with biofilm. Data for descriptive statistics were expressed as means and standard deviations (SD). Data normality was assessed with the Shapiro–Wilk test.

The Wilcoxon signed rank test for paired data was used to compare the abutment surface covered with biofilm (supragingival versus subgingival, buccal versus lingual, and live versus dead cells) and to compare the different timepoints (24 h versus 48 h, 24 h versus 7 days, 48 h versus 7 days). Mann–Whitney U-tests were used for comparisons between groups individually. Statistical significance was considered for $p < 0.05$.

Results

Ten patients (7 women and 3 men) with a mean age of 58.2 years (SD = 10.8) were included in the study. Three study abutments were obtained for each patient, analyzed at different timepoints (24 h, 48 h and 7 days). At the 7-day follow-up, one biofilm collector abutment was excluded due to screw loosening. A total of 29 study abutments were thus analyzed: 10 at 24 h, 10 at 48 h, and 9 at 7 days. Clinical biofilm data of each patient are reported in Table 1.

The mean coverage areas were 9.3% ([95% confidence interval] 95%CI = 6.9–11.6), 16.2% (95%CI = 13.0–19.4) and 16.8% (95%CI = 13.1–20.4) at 24 h, 48 h and 7 days, respectively (Fig. 1). A statistically significant increase was observed between the 24 h timepoint and the following timepoints (48 h; $p < 0.001$ and 7 days; $p < 0.001$) (Fig. 2).

Supragingival versus subgingival

There were no statistically significant differences in mean biofilm coverage between the global supragingival and subgingival areas at 24 h ($p = 0.870$) and 48 h ($p = 0.551$). However, the mean coverage area at 7 days was significantly greater in the supragingival zone than at subgingival level: 22.0% (SD = 16.9) versus 11.7% (SD = 12.5)($p = 0.003$) (Table 2 and Fig. 3).

In the supragingival zone, statistically significant differences were found between the 24 h and 48 h timepoints ($p < 0.001$), and between 24 h and 7 days ($p < 0.001$), while in the subgingival zone the only significant differences were detected between 24 and 48 h ($p = 0.005$). No other significant differences were found (Table 3).

Table 1 Clinical and biofilm characteristics of each patient

Patient	Age	Gender	Smoking	Position	Adjacent to abutment	KT (mm)	Prosthesis	PPI	PSBI	PPD	% Supra 24 h	% Supra 48 h	% Supra 7d	% Sub 24 h	% Sub 48 h	% Sub 7d
1	68	F	No	24	T-X-I	5	B	0	0	5	9.0	26.1	-	14.7	40.5	-
2	47	F	No	36	T-X-I	2	B	0	0	4	57.5	34.5	51.0	10.6	27.8	17.1
3	67	F	No	37	T-X-X	4	B	0	0	3	13.4	35.4	25.3	7.3	5.9	14.3
4	73	F	No	35	T-X-I	5	B	0	0	4	51.9	97.2	34.4	8.3	63.8	44.9
5	47	F	No	26	T-X-T	7	SU	0	0	5	7.2	20.1	18.8	7.4	6.7	16.3
6	M	M	No	47	T-X-X-T	2	B	0	0	4	32.0	42.6	12.9	39.6	8.9	5.7
7	61	F	No	47	T-X-I	4	B	0	0	6	4.5	29.3	29.2	10.6	31.5	21.6
8	62	M	No	46	T-X-T	2	SU	0	0	4	10.2	29.5	24.5	26.8	63.8	45.9
9	72	M	No	47	T-X-I-T	4	B	0	0	3	20.8	23.4	36.5	24.1	12.1	5.7
10	43	F	No	15	T-X-T	3	SU	0	0	3	6.7	10.5	54.5	8.8	40.1	39.6

Gender: *F* Female, *M* Male, Adjacent to the abutment: *T* Tooth, *I* Implant, *x* abutment, *KT* Keratinized tissue; Prosthesis: *SU* Single unit, *B* Bridge, *PPI* Mombelli peri-implant plaque index, *PSBI* Mombelli peri-implant bleeding index, *PPD* Pocket probing depth, *% Supra* supragingival biofilm coverage area as a percentage of the entire supragingival surface, *% SubI* subgingival biofilm coverage area as a percentage of the entire subgingival surface

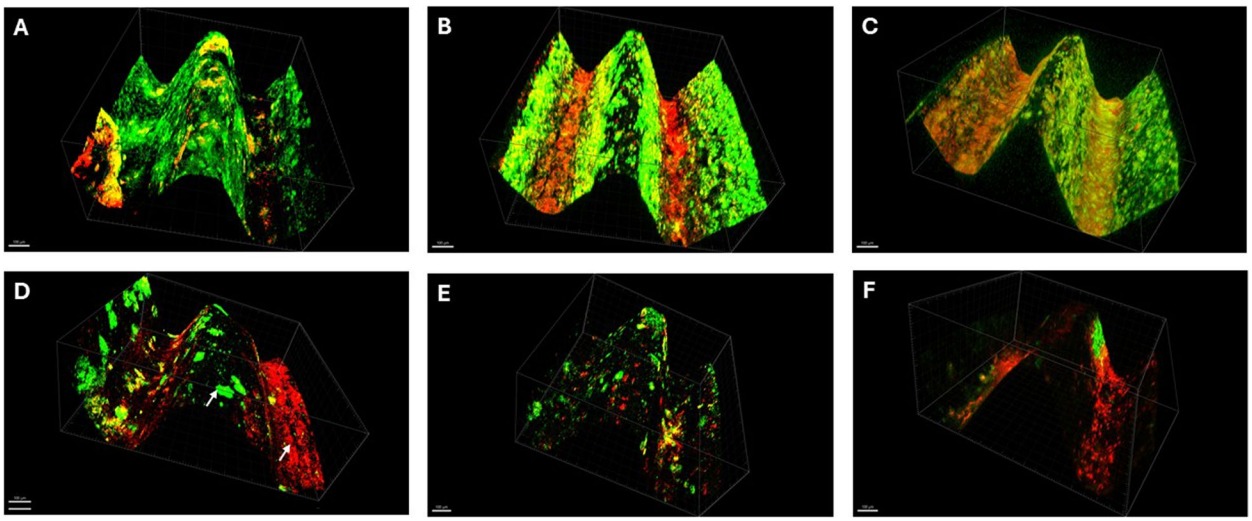

Fig. 1 Microscopic view of an abutment at 24 h, 48 h and 7 days**.** Lingual surface view of an abutment at 24 h, 48 hours and 7 days. Images obtained using Imaris Viewer® v.10.2.0 (Bitplane AG, Badenerstrasse, Zurich, Switzerland). **A** Supragingival region of the abutment at 24 h: A large amount of oral biofilm and mostly live epithelial cells can be seen. **B** Supragingival region of the abutment at 48 h: Greater biofilm coverage is observed compared to 24 h ($p < 0.001$). **C** Supragingival region of the abutment at 7 days: A large amount of live biofilm is observed on the sides of the coils and dead biofilm at the bottom of the coils. **D** Subgingival region of the abutment at 24 h: Little biofilm adhered to the surface is observed. The arrows indicate living epithelial cells (green) and epithelial cell nuclei (red) resulting from epithelial desquamation. **E** Subgingival region of the attachment at 48 h: Less biofilm coverage is observed compared to 24 h ($p = 0.005$). **F** Subgingival region of the attachment at 7 days: Stained epithelial cell nuclei (red) are observed in the valley of the whorl and little adhered microbial biofilm, mainly composed of dead bacteria

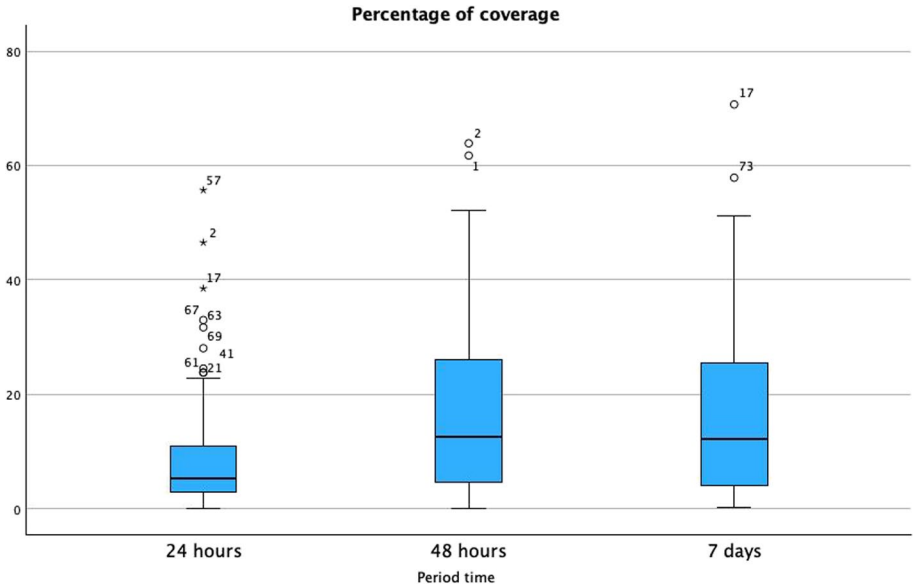

Fig. 2 Total biofilm coverage areas of the different regions of interest (ROIs) at the three timepoints (24 h, 48 h and 7 days). A statistically significant increase was observed between 24 h and the subsequent timepoints (48 h, $p < 0.001$; 7 days, $p < 0.001$)

Vitality

A significantly larger number of live cells were detected (supragingival area plus subgingival area) (24 h, $p = 0.005$; 48 h, $p < 0.001$; 7 days, $p < 0.001$) (Table 2).

Live cells experienced a significant increase at 48 h ($p < 0.001$) and 7 days ($p = 0.001$) compared to the first 24 h. Dead cells also increased in number between the first and second day ($p = 0.005$) (Table 3).

Table 2 Percentage total coverage and according to area, cells and surface (percentage and standard deviation)

		24h	*p*-value	48h	*p*-value	7d	*p*-value
Area	Supra	7.9 (7.7)	0.870	17.4 (15.5)	0.551	22.0 (16.9)	**0.003**
	Sub	10.6 (13.0)		15.0 (12.9)		11.7 (12.5)	
Cells	Live	12.0 (12.2)	**0.005**	21.1 (13.1)	**< 0.001**	24.6 (16.7)	**< 0.001**
	Dead	6.4 (8.3)		11.4 (13.7)		9.0 (9.4)	
Surface	Buccal	7.2 (9.9)	**0.007**	16.1 (14.8)	0.870	16.7 (17.5)	0.528
	Lingual	11.3 (11.24)		16.3 (13.8)		16.7 (13.6)	
Total		9.3 (10.7)		16.2 (14.2)		16.8 (15.6)	

Supra Supragingival, *Sub* Subgingival. 24 h: 24 h; 48 h: 48 h; 7d: 7 days

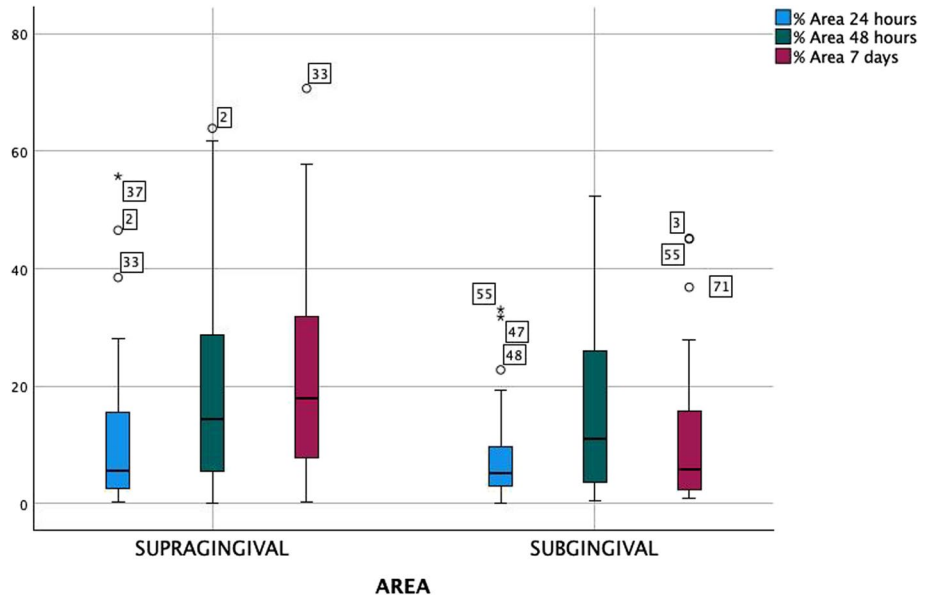

Fig. 3 Distribution between the supragingival and subgingival areas at the three timepoints (24 h, 48 h and 7 days). Mean biofilm coverage area at 7 days was significantly greater in the supragingival zone than at subgingival level (22.0% [SD = 16.9] versus 11.7% [SD = 12.5]; *p*= 0.003)

Table 3 Comparative results of biofilm coverage at 24 h, 48 hours and 7 days

	Area		Cells		Surface		Total
	Supra	Sub	Live	Dead	Buccal	Lingual	
24 h-48 h	**< 0.001**	**0.005**	**< 0.001**	**0.005**	**< 0.001**	**0.010**	**< 0.001**
24 h-7d	**< 0.001**	0.130	**0.001**	0.098	**0.002**	**0.043**	**< 0.001**
48 h-7d	0.106	0.071	0.414	0.489	0.975	0.888	0.978

Supra Supragingival, *Sub* Subgingival. 24 h: 24 h; 48 h: 48 h; 7d: 7 days

At the supragingival level, live cells covered a greater percentage of the area compared dead cells at 24 h ($p = 0.002$) and 48 h ($p < 0.001$) (Table 4). Statistically significant differences were observed between live cells at 48 h ($p = 0.005$) and 7 days ($p = 0.006$) compared to the first 24 h. The same differences were found between dead cells at 48 h ($p = 0.010$) and 7 days ($p = 0.028$) compared to the first 24 h (Table 5).

In the subgingival zone, significant differences between live and dead cells were observed at 7 days

Table 4 Percentage supragingival and subgingival coverage according to cells and surfaces (percentage and standard deviation)

		24 h		48 h		7d	
Supragingival			*p*-value		*p*-value		*p*-value
Cells	Live	14.8 (14.1)	**0.002**	24.1 (12.2)	**< 0.001**	31.8 (16.2)	0.106
	Dead	6.4 (10.6)		10.6 (15.1)		11.9 (10.7)	
Surfaces	Buccal	9.8 (12.9)	0.561	17.2 (17.9)	0.482	237 (19.6)	0.752
	Lingual	11.4 (13.4)		17.6 (13.0)		20.0 (14.0)	
Total		10.6 (13.0)		17.4 (15.5)		21.9 (16.9)	
Subgingival			*p*-value		*p*-value		*p*-value
Cells	Live	9.3 (9.4)	0.465	17.9 (12.9)	0.058	17.3 (14.2)	**0.003**
	Dead	6.5 (5.4)		12.1 (12.6)		6.0 (7.0)	
Surfaces	Buccal	4.6 (4.6)	**< 0.001**	15.0 (11.1)	0.725	9.8 (12.0)	0.327
	Lingual	11.2 (8.9)		15.0 (14.8)		13.6 (13.0)	
Total		7.9 (7.7)		15.0 (12.9)		11.7 (12.5)	

24 h: 24 h; 48 h: 48 h; 7d: 7 days

Table 5 Comparative results of supragingival and subgingival biofilm coating at 24 h, 48 hours and 7 days according to cells and surfaces

	Supragingival				Subgingival			
	Live	**Dead**	**Buccal**	**Lingual**	**Live**	**Dead**	**Buccal**	**Lingual**
24 h-48 h	**0.005**	**0.010**	**0.004**	**0.011**	**0.021**	0.117	**0.006**	0.247
24 h-7d	**0.006**	**0.028**	**0.003**	**0.048**	0.085	0.877	0.255	0.372
48 h-7d	0.157	0.286	0.064	0.616	0.647	**0.035**	**0.018**	0.811

24 h: 24 h; 48 h: 48 h; 7d: 7 days

Table 6 Comparative bacterial vitality (ratio of live/dead cells) between the supragingival and subgingival surfaces

	Supragingival	Subgingival	*p*-value	Total
24 h	11.6 (30.7)	1.5 (1.1)	**0.002**	6.6 (22.1)
48 h	22.0 (54.0)	3.2 (3.6)	**0.037**	12.6 (39.0)
7d	19.0 (59.9(	5.0 (5.3)	0.800	12.0 (42.5)

24 h: 24 h; 48 h: 48 h; 7d: 7 days

($p = 0.003$) (Table 4). Live cells experienced a significant increase at 48 h compared to the first 24 h ($p = 0.021$), while dead cells showed a significant difference between 48 h and 7 days ($p = 0.035$) (Table 5).

The comparison of bacterial vitality (ratio of live/dead cells) between the supragingival and subgingival surfaces revealed statistically significant differences at both 24 h and 48 h (11.6 [SD = 30.7] versus 1.5 [SD = 1.1], $p = 0.002$; and 22.0 [SD = 54.0] versus 3.2 [SD = 3.6], $p = 0.037$, respectively). However, at 7 days, no statistically significant differences were observed (Table 6).

Buccal versus lingual/palatal

Significant differences between the buccal and lingual/palatal sides were only observed at the 24 h timepoint ($p = 0.007$) (Table 2).

In the supragingival zone, significant differences were found on the buccal aspect between 24 h and the subsequent timepoints (48 h, $p = 0.004$; 7 days, $p = 0.003$). Similar results were found on the lingual/palatal aspect, with differences being observed between 24 and 48 h ($p = 0.011$) and 7 days ($p = 0.048$) (Table 5).

With regard to the subgingival zone, a higher coverage area was found on the lingual aspect versus the buccal aspect at 24 h ($p < 0.001$) (Table 4). In the case of the latter, differences were observed between the first day and 48 h ($p = 0.006$) and 7 days ($p = 0.018$) (Table 5).

Discussion

The aim of the present study was to describe how oral biofilms develop on exposed healthy dental implants after 24 h, 48 h and 7 days. Specific removable biofilm collector abutments mimicking dental implants were used for this purpose [20, 21]. The in vivo model employed has several advantages, since it allows a more realistic

approach to the study of biofilm formation on exposed dental implants [20, 21]. The present study showed a significant increase in biofilm coverage between the first (9.3%) and second (16.2%) day, after which the growth seemed to stabilize (16.8% coverage at 7 days). It is also important to mention that the biofilm was mainly composed of live cells in both the supragingival and the subgingival areas.

Other authors have also described early biofilm formation in in vitro and in vivo settings. Bermejo et al. [22, 23] found the highest number of total bacteria to be registered after 96 h in dental implants placed on a stent. On the other hand, Herrmann et al. [33] studied materials with different surface roughness characteristics (sand-blasted acid-etched titanium implant surface, smooth implant collar, titanium abutment [Ti6AI4V] and zirconium dioxide abutment [ZrO2]), and recorded a biofilm increase from day 3 to day 31 for all the materials—though proliferation was seen to be greater with a rough implant surface and lower in the case of a smooth implant collar. These results underline the importance of the macrostructure and microstructure of the materials when considering biofilm growth. Our study focused on analyzing biofilm coverage and vitality on recently exposed rough surface dental implants (24 h, 48 h and 7 days) and showed significant growth during the initial stages. From a clinical point of view, these results indicate that exposed rough surfaces can be easily colonized in a very short period of time. Therefore, it is essential that patients understand the need to maintain very strict and regular hygiene of these areas of exposure. On the other hand, clinicians should be aware that the presence of soft tissue dehiscences or peri-implant bone remodeling might increase the likelihood of presenting peri-implant diseases.

The methodology used in this study also allowed us to compare the differences in biofilm formation between the supragingival and subgingival zones. Our results showed a similar biofilm growth pattern in both zones during the first two days (Fig. 1). However, significant differences were found after 7 days (22% coverage in the supragingival zone versus 12% in the subgingival zone), which could be explained by the presence of the peri-implant soft tissue, since this mucosal barrier might reduce biofilm formation [19]. Other authors such as Elter et al. [29] found important differences when comparing the supragingival (17.3%; SD = 23.1%) and subgingival zones (0.8%; SD = 1.0%) and concluded that the presence of supragingival biofilm does not lead to a significant increase of bacteria in subgingival zones. The data of the present study evidenced biofilm coverage in the supragingival zone consistent with that reported by other authors using rough surface healing abutments [29, 30]. Cortés-Acha et al. [21], using the same methodology, analyzed biofilm accumulation after 14 days and observed greater biofilm coverage of the supragingival zone than in the subgingival zone (38% versus 21%), which is in line with our own findings (21.9% versus 11.7%). Therefore, the peri-implant soft tissue could be considered as a protective barrier, though early bacterial colonization will also occur in the submucosal zone. In this regard, Monje et al. [34] considered that the lack of keratinized mucosa could be a local predisposing factor for peri-implant diseases in patients with inadequate oral hygiene. Indeed, its absence could be associated with mucosal recessions, discomfort, reduced buccal depth and more plaque accumulation, all of which might contribute to increase inflammation.

In our study, a consistently higher proportion of live microorganisms was found at all three timepoints. The supragingival versus subgingival live/dead ratio was significantly higher at 24 h (11.6 versus 1.5; $p = 0.002$) and 48 h (22.0 versus 3.2; $p = 0.037$), respectively. At 7 days, the live/dead cell ratio remained higher, but no significant differences were detected (19.0 versus 4.9; $p = 0.800$). These findings are in accordance with those of a previous study and seem to indicate that supragingival locations have more favorable environmental conditions for biofilm viability [21]. The supragingival zone is more exposed to oxygen, saliva flow and mechanical disruption from tongue and cheek movement, which can favour aerobic microbial growth and reduce cell death. In contrast, the subgingival environment is more protected, anaerobic and nutrient-limited, potentially promoting microbial stress and reduced oxygen availability, leading to a higher proportion of dead cells. A larger quantity of live bacteria may suggest a potential risk of infection development, showing that rough implant surfaces enhance bacterial growth. It should be emphasized that the participants in the present study were instructed not to clean the area, and this could have led to an overestimation of the results.

At 24 h, the total bacterial coverage was higher on the lingual surface, and this was also observed in the subgingival zone. Significant increases were observed on both the buccal and lingual surfaces between 24 and 48 h and between 24 h and 7 days. The increase between 48 h and 7 days was not significant. We believe this to be due to the fact that the lingual surface has poorer access for hygiene, which favors biofilm formation. This biofilm progresses rapidly in the first 48 h and maintains its levels up to 7 days.

The small sample size involved might be considered one of the main limitations of the present study. However, patient recruitment was very difficult due to the strict inclusion/exclusion criteria and the required number of appointments in a short period of time. Likewise,

the addition of intermediate timepoints (e.g., 72 or 96 h) could have provided a more detailed understanding of biofilm growth dynamics, but this was not feasible due to the clinical burden of additional placement/removal appointments. However, to our knowledge, this is the first in vivo human study to characterize the early stages of biofilm formation directly on dental implants surfaces, highlighting its translational relevance despite the absence of intermediate timepoints. Additionally, the participants were instructed to avoid oral hygiene measures in the biofilm collector abutment area. Another important drawback is that the present outcomes can only be extrapolated to patients with healthy dental implants. However, the main aim of this study was to provide information about early biofilm formation on dental implants as an initiating factor of peri-implant diseases. Finally, the impact of other factors such as implant location (i.e., maxilla versus mandible), age, sex and type of edentulism were not assessed.

Conclusions

Biofilm coverage on exposed healthy dental implants progressively increases during the first 48 h and then seems to stabilize. This biofilm is mainly composed of live cells in both the supragingival and the subgingival zones. The presence of the mucosa does not seem to affect the growth of the biomass in the initial phases (48 h), but supragingival zones have a greater biofilm coverage after 7 days.

Abbreviations

Ti	Titanium
ZrO2	Zirconia
PPD	Pocket probing depth
BOP	Bleeding on probing
SD	Standard deviation
ROIs	Regions of interest
CI	Confidence interval

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Clinical trial number

Not applicable.

Authors' contributions

KMG: Study concept/design, data collection and drafting of the manuscript. BCA: Study concept/design and data collection. MGG: Study concept/design, data analysis/interpretation and drafting and approval of the manuscript. ASO: Study concept/design and data analysis and interpretation, statistics, critical review and approval of the manuscript. VB: Study concept/design and data analysis and interpretation, statistics, critical review and approval of the manuscript. RF: Study concept/design, data interpretation, funding acquisition, critical review and approval of the manuscript. EVC: Study concept/design, data interpretation, supervision, critical review and approval of the manuscript. KMG: Study concept/design, data collection and drafting of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee (CEIM) of the University of Barcelona Dental Hospital (Ref.: 19/2015), and the study was conducted in accordance with the Declaration of Helsinki on human studies [31]. All patients signed a written informed consent before enrollment. The CONSORT statement guidelines [32] were used as a reference to report this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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