

Early Biofilm Formation on Rough and Smooth Titanium Specimens: a Systematic Review of Clinical Studies

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ABSTRACT

Objectives: There is a concern whether the enhancement on implant surface roughness is responsible for higher biofilm formation, which acts as an aetiological factor for peri-implant diseases. The aim of the present systematic review was to answer the following question: “Are rough surfaces more susceptible to early biofilm formation when compared to smoother surfaces on titanium specimens?”

Material and Methods: The research was performed on PubMed, Web of Science and Scopus, up to August 2021. Eligibility criteria included studies that analysed human biofilm formation on titanium specimens with distinct surface roughness (smooth vs minimally, moderate, or rough) over the experimental times of 1 or 3 days. Roughness average (Ra) and biofilm analysis parameters were extracted from selected articles. Risk of bias was evaluated using the Checklist for Quasi-Experimental Studies.

Results: Of 5286 papers, 5 were included and analysed. Smooth titanium surfaces included machined and anodized titanium/Ti-6Al-4V; machined and acid etched TiZr. Minimally, moderately, or rough surfaces comprised titanium and titanium alloys (TiZr, Ti-6Al-4V), that received surface treatments (anodization, acid-etching, blasting, hydroxyapatite-coating). No statistically significant difference on biofilm formation on rough and smooth titanium surfaces was reported by 3 studies, while more contamination on rough titanium surfaces was stated by 2 investigations. An isolated smooth surface has also been associated to higher contamination. Moderate to high quality methodological assessment of studies were identified.

Conclusions: It is not possible to assume that rough surfaces are more susceptible to early biofilm formation than smooth titanium surfaces. Additional studies are required to study this multifarious interaction.

Keywords: bacteria; biofilms; dental implants; dental implant-abutment design; peri-implantitis.

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INTRODUCTION

Peri-implant diseases are a growing concern that affects a significant number of dental implants. A systematic review, that included forty-seven clinical studies, with three to sixteen years of follow-up analysis, demonstrated that peri-implantitis was present in 9.25% and 19.83% of patients and implants respectively, while mucositis affected 29.48% and 46.83% correspondingly [1]. Although there are patient-related factors that contribute to peri-implant diseases establishment and progression [2], biofilm remains the primary etiological factor and that one can be controlled through the development of biomaterials with optimized properties [3].

Since the 1960’s, commercially pure titanium and some of its alloys are the most employed and studied materials. Being widely used as dental implants and as implant abutments due to its biocompatibility, mechanical strength and corrosion resistance properties [4]. The first implants were manufactured with a smooth or minimally rough surface. Its roughness average (Ra) values were commonly lower than 0.5 µm. Over the years, implant surfaces have been modified in order to improve osseointegration, creating irregularities that enhance cellular activity (i.e. activation of blood proteins, platelets, and osteoblasts) and, consequently, increase bone-implant contact [5]. Various treatment methods have been applied to modify surface topography and properties, creating minimally (Ra = 0.5 to 1 µm), moderately (Ra = 1 to 2 µm) and rough (Ra > 2 µm) surfaces [6].

The initial bacterial adhesion to non-shedding surfaces have been extensively studied on the last decades [3]: this process is conventionally analysed through a biochemical or a topographical point of view. Among biochemical aspects, it may be cited the interaction between ligant-receptor components, and the cell-to-cell interaction. The topographical scenario consists on the analysis of thermodynamical models, acid-base reactions and electrostatic interactions, being surface roughness

an important parameter to quantify biofilm formation on titanium [3].

Concerning a better understanding of the role of titanium surface roughness on this complex interaction, studies that had investigated human early biofilm formation on titanium specimens were researched. Authors believe that this type of methodology enables evaluation of surface roughness as a major determinant, excluding factors present on implants under occlusal load over the years, like type of prosthesis, parafunctional habits, general health, mucosa thickness, and oral hygiene. Therefore, the aim of this systematic review is to compile data from studies that evaluated human early biofilm formation on titanium specimens with minimally, moderate and rough surfaces.

MATERIAL AND METHODS

Protocol and registration

The current systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist (<http://prisma-statement.org>) and it is registered at PROSPERO (registration number: CRD42020177809).

The protocol can be assessed at:

https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020177809

Focus question

The focus question of the present study/investigation systematic review is - “Are rough surfaces more susceptible to early biofilm formation when compared to smoother surfaces on titanium specimens?” followed the PICOS (Population, Intervention, Comparison, Outcomes, and Study design) (Table 1) [7]. Population was considered as titanium specimens (P), intervention as rough surfaces (I), comparison as smooth surfaces (C), outcomes as biofilm formation (O) and study design as non-randomized prospective clinical studies (S).

Table 1. PICOS guidelines.

Patient and population (P)	Titanium specimens.
Intervention (I)	Rough surfaces.
Comparator or control group (C)	Smooth surfaces.
Outcomes (O)	Biofilm formation.
Study design (S)	Non-randomized prospective clinical studies.
Focused question	Are rough surfaces more susceptible to early biofilm formation when compared to smoother surfaces on titanium specimens?

Information sources

Electronic database researches on PubMed, Web of Science and Scopus were performed up to August 14, 2021. Additionally, hand search was performed at included papers on the reference list. When necessary, authors were contacted to obtain supplementary information. More details are available in [Appendix 1](#).

Search

The full search strategy can be found in [Appendix 1](#). Briefly, search strategy included the terms: “dental implant”, “Implant material”, “Dental Implant-Abutment Design”, “Implant Surface”, “dental devices”, “dental abutments”, “titanium”, “Biofilms”, “Dental Deposits”, “Dental plaque”, “Plaque”, “bacterial adhesion”, “bacterial colonization”, “oral bacteria”, “bacteria”, “bacterial count”, “Bacterial Load”, “bacterial attachment”, “Microbiology”, “colony count, microbial”, “microbial”, “microorganisms”, “subgingival colonization”, “initial colonization”, “in vivo”, “humans”, “patient” and, “volunteers”.

Types of publications

The types of investigations included in the present systematic review were original articles published in scientific journals.

Types of studies

The forms of studies involved in the present systematic review were prospective clinical studies that used devices to install titanium specimens at human oral cavities in order to evaluate the association among surface roughness and early biofilm formation.

Types of participants/population

Healthy adult volunteers were included in the studies selected for this systematic review.

Inclusion and exclusion criteria

Inclusion criteria

Investigations were considered eligible when they met the following criteria:

- Studies that include healthy human volunteers, approved by ethics commissions.
- Studies that analysed a smooth machined titanium

(Ti) surface ($Ra < 0.5 \mu\text{m}$) and compared it with other type of titanium surfaces with minimal (0.5 to $1 \mu\text{m}$), moderate (1 to $2 \mu\text{m}$) or rough ($> 2 \mu\text{m}$) surfaces [6].

- Studies that evaluate titanium biofilm formation in the oral cavities over the experimental times of one to three days, by employing removable oral appliance systems.

Exclusion criteria

The following exclusion criteria were observed:

- Studies that have not analysed titanium surfaces.
- Studies that employed experimental times of biofilm evaluation other than one or three days.
- Studies that have not compared a smooth machined titanium surface with a rough titanium surface (minimally, moderate, or rough surface).
- Studies that analysed strategies to destroy previously installed biofilm.
- Studies that clearly included patients with periodontal disease and nicotine consumption.
- Case reports and literature reviews.

Sequential search strategy

Two independent and calibrated reviewers (R.S.B. and K.A.B.) analysed titles and abstracts of the articles and selected articles according to the above-mentioned inclusion and exclusion criteria. Doubts about studies were discussed with a third author (C.A.M.B.). Duplicated articles were removed using reference manager software (Mendeley®, Elsevier; London, UK). No data or language restrictions were applied. Reviewers were calibrated and Kappa coefficient was calculated.

Data extraction

Two authors (R.S.B. and K.A.B.) independently performed the data collection. Any mistyping was checked for accuracy by a third reviewer (L.G.L.). The following information was collected: source information (authors, year of publication, country where the study was performed), characteristics of titanium smooth and rough groups (Ra values and type of surface treatment), roughness measurements details, biofilm analysis (experimental time, type of assay and microorganisms evaluated), as well as the main outcomes of the investigations on the relationship of titanium surface roughness and biofilm formation. If the required data was missing in the main text, efforts to contact the corresponding authors of primary studies were done by electronic mail.

Data items

Titanium surface roughness values were evaluated in order to classify the specimens as smooth surfaces or rough surfaces, which was subdivided as minimal, moderate, and rough [6]. The following information was collected regarding plaque formation at titanium smooth and rough surfaces: biofilm thickness evaluation and bacteria density (scanning electron micrograph observation), biofilm composition and biofilm formation analysed by microbiological assays.

Risk of bias within studies

The Checklist for Quasi-Experimental Studies (non-randomized experimental studies) from The Joanna Briggs Institute Critical Appraisal tools for use in Systematic Reviews (<https://jbi.global/>) was applied. Methodological quality was categorized as low when the study reached up to 49% of affirmative answers,

moderate when the study reached 50% to 69% affirmative answers, and high when the study reached more than 70% affirmative answers.

RESULTS

Study selection

Among an initial amount of 5286 papers found in the databases, 5% were selected for Kappa calculation, based on title and abstract analysis, resulting in 95% and 100% of similarity respectively. After elimination of duplicates, 3327 papers were selected for title-based screening, 282 of which were further selected for abstract reading. Manual hand searching resulted in the inclusion of 3 more papers. Subsequently, 21 papers were selected for full text analysis, of which 17 were excluded [8-23] (Appendices 2 and 3). After full-text analysis, a total of 5 studies were maintained [24-27]. A flow chart is shown in Figure 1.

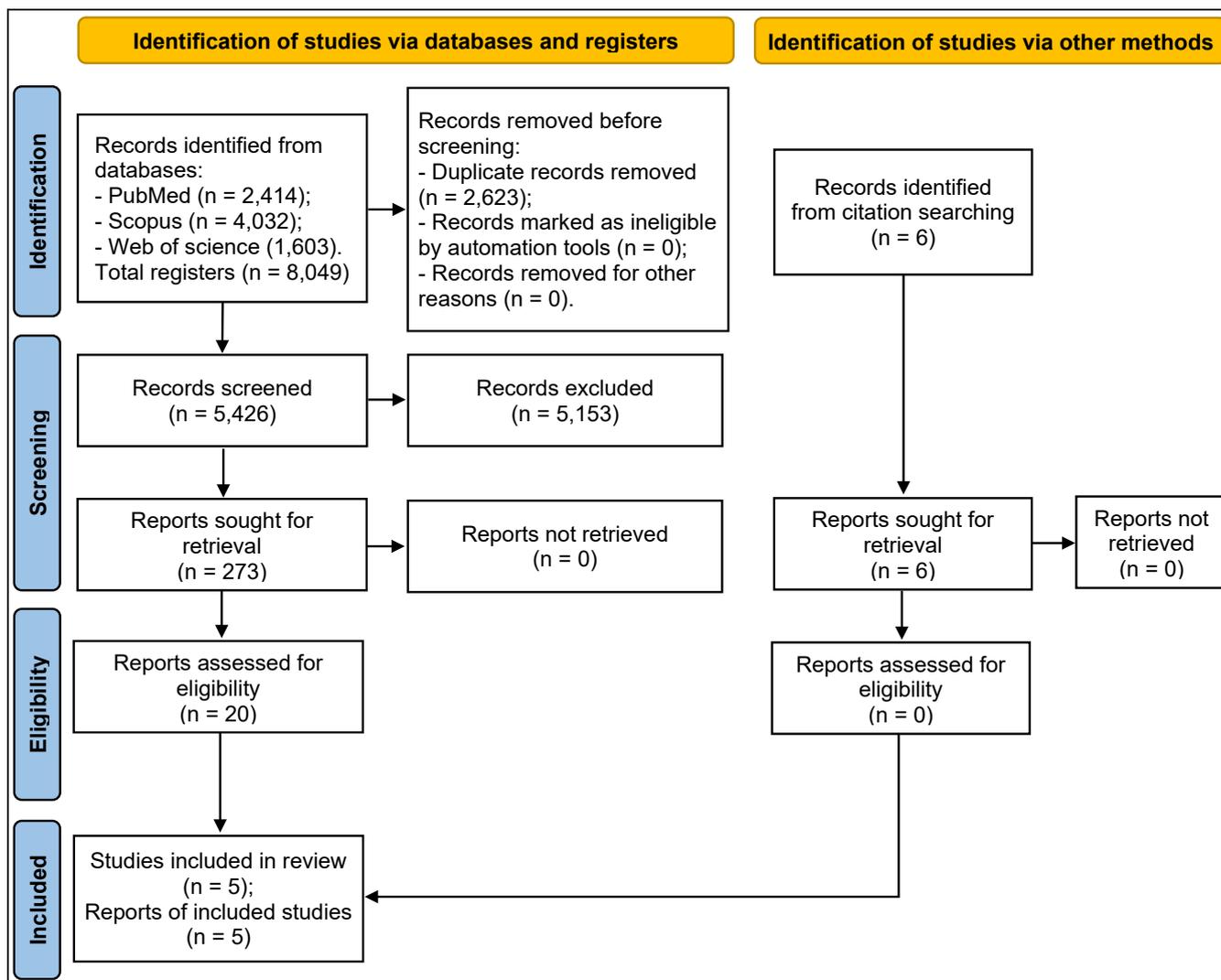


Figure 1. PRISMA flow diagram of research sources and included articles.

Study characteristics

Overall, study characteristics are exposed in Table 2. Included studies that were performed in Germany, Brazil, Italy and Switzerland, and were published between 2010 and 2020. Sample sizes varied from 6 to 16 participants, resulting in a total of 56 volunteers enrolled. All included studies employed a smooth machined titanium and some investigations have also found that other types of surfaces were considered as smooth surfaces, such as anodized titanium at 90 and 120 voltage (V) [26], Ti-6Al-4V with no treatment [28] or anodized at 100 V [26], machined and acid-etched TiZr alloy and machined Ti-6Al-4V alloy with micro-grooves [27]. Rough surfaces, were subdivided:

- Minimally rough: electrochemically anodized surface [24], blasted titanium [25], Ti-6Al-4V alloy anodized at 120 V [26], as well as machined, sandblasted and acid-etched TiZr alloy [27].
- Moderately rough: hydroxyapatite (HA)-coated titanium [25].
- Rough surfaces: sand-blasted and acid-etched titanium [28].

Three studies only included non-smokers [24,27,28], while two studies have not specified the smoking status [25,26]. All included investigations employed the criteria of recent use of antibiotics as an exclusion factor. Three of those studies included investigations reporting that participants have not used antibacterial mouth rinsing [24-26], while two investigations have not specified this criteria [27,28]. All included investigations encompassed males and females, with the exception of one, that have not specified participant's gender [24]. Additional topographical analysis included scanning electron micrograph (SEM) and contact angle analysis, which were performed by three [24,26,28] and two [24,26] studies respectively. Physicochemical analysis of investigated specimens included energy dispersive X-ray spectroscopy and X-ray diffractometry, which was realized by one study [26].

Surface roughness was analysed by mechanical [25] or three-dimensional laser profilometers [26], atomic force microscope [24], or by confocal microscopy [27]. Biofilm formation was evaluated at 1 day by two studies [26,27], at 3 days by two studies [24,28] and at 1 and 3 days by one study [25]. Methods to quantify biofilm formation or composition included fluorescence *in situ* hybridization and confocal laser scanning microscopy [24]; microbiological identification test Checkerboard DNA-DNA hybridization [25], bacteria density calculation at SEM images [26], real-time quantitative polymerase chain reaction [28], as well as by the assays safranin

staining and isothermal microcalorimetry [27].

No statistical differences on biofilm analysis among groups were reported by three studies [24,25,28]. One study reported that the minimally rough surfaces Ti-6Al-4V anodized at 120 V showed the worst contamination (compared with anodized and non-anodized Ti surfaces and Ti-6Al-4V surfaces) [26]. Another study revealed that a minimally rough (machined, sandblasted and acid-etched TiZr alloy), together with the smooth surface of machined acid-etched TiZr alloy showed the worst contamination, when compared to other evaluated groups [27].

Risk of bias within studies

The non-randomized clinical studies that were analysed on this systematic review were evaluated through the Checklist for Quasi-Experimental Studies (Table 3 and 4). Three studies were considered as high methodological quality [24,25,28], while two studies were considered as moderate quality [26,27]. Some of the aspects that have drawn the author's attention as potential biases were the following:

- Absence of initial analysis other than surface roughness (i.e. SEM analysis or contact angle measurements);
- Lack of a control group (just one study employed bovine enamel slabs as control);
- Single measurements of biofilm formation (only one experimental time);
- Lack of statistical analysis to compare distinct surface roughness (it was analysed on a trustable way, but it is not possible to really assume that the surfaces were different, because statistical analysis was not performed for this parameter).

Results of individual studies

According to Al-Ahmad et al. [24], biofilm thickness means at 3 days varied between 19.78 μm and 36.73 μm . Of six evaluated groups, two groups with higher surface roughness (TiUnite[®]: Ra = 544.2 μm ; ZiUnite[™]: Ra = 488.7 μm) showed that biofilm thickness was significant correlated with higher surface roughness ($P < 0.05$). Additionally, the control group of bovine enamel slabs, showed surface roughness mean of 14.3 μm and biofilm thickness lower than 20 μm [24]. Giordano et al. [26] observed that the electrochemical treatments caused detrimental effects on biofilm grown *in vivo* at titanium grade 2 specimens. Those specimens anodized at 90 and 130 kV (Ra = 0.309 and 0.355 μm , respectively) showed more contamination than the not treated group (Ra = 0.309 μm) ($P < 0.001$).

Table 2. Summary of descriptive characteristics of included studies

Study	Year of publication	Number of patients	Country	Number of patients	Titanium groups		Roughness measurement	Experimental time/biofilm analysis/microorganisms	Main findings
					Smooth surface	Rough surfaces			
Al Ahmad et al. [24]	2010	20	Germany	20	Ti-m: 0.0544 µm	TiUnite® (electrochemical anodization): 0.544 µm (minimally rough)	Atomic force microscope in a contact mode of 50 x 50 µm²	3 days; biofilm thickness and composition analyzed through fluorescence in situ hybridization and confocal laser scanning microscopy; <i>Eubacteria</i> , <i>Veillonella spp.</i> , <i>Fusobacterium nucleatum</i> , <i>Actinomyces naeslundii</i> , <i>Streptococcus spp.</i>	Neither biofilm thickness nor composition showed statistical difference among smooth and minimally rough titanium surfaces (P > 0.05)
de Freitas et al. [25]	2011	6	Brazil	6	Ti-m: 0.47 µm	Ti-BI: 1 µm (minimally rough); Ti-HA: 1.27 µm (moderately rough)	Mechanical profilometer	1 and 3 days; biofilm composition: microbiological identification test Checkerboard DNA-DNA hybridization; 24 species (ref Sokranski)	It was not observed statistical significant differences for any species, in relation to the surfaces in the evaluated times (P > 0.05)
Giordano et al. [26]	2011	8	Italy	8	A) Ti-m: 0.306 µm; B) Ti anodized at V = 90 V: 0.309 µm; C) Ti anodized at V = 130 V: 0.355 µm; D) Ti-6Al-4V: 0.342 µm; E) Ti-6Al-4V anodized at V = 100 V: 0.436 µm	F) Ti-6Al-4V anodized at V = 120: 0.506 µm	Single measurement done on a 1.25 x 1.75 mm area attained using a three-dimensional laser profilometer	1 day; bacteria density: SEM observation of randomly selected areas (employment of scores 1 - less bacteria, 2 and 3 - more bacteria)	The minimally rough group (Ti-6Al-4V anodized at V = 120) showed the worse contamination, characterized by thicker biofilm formation, when compared to smoother surfaces (P < 0.05)
Zaugg et al. [27]	2016	16	Switzerland	16	A) Ti-m: 0.093 µm; B) ModMa: 0.287 µm; C) TAV MG: 0.128 µm	D) modSLA: 0.896 µm (minimally rough)	Images were acquired using a confocal microscope and surface roughness was determined using objective lens	1 day; biofilm formation: safranin staining assay, isothermal microcalorimetry, and SEM	The minimally rough modSLA surface, but also the smooth surface ModMA showed greater biofilm formation than other smoother surfaces (P < 0.05)
Hermann et al. [28]	2020	14	Germany	14	A) Ti-m: 0.18 µm; B) Ti-6Al-4V: 0.16 µm	A) Ti-p: 1.87 µm	Profilometric analysis, made in triplicate for each group	3 days; detection and absolute quantification of total bacteria (real-time quantitative polymerase chain reaction); biofilm composition (DNA microarray)	No statistical differences were observed on bacteria quantification between groups (P > 0.05). 16 bacteria species were identified on titanium specimens and no differences among groups was detected

Ti-m = machined titanium; Ti-BI = titanium blasted with aluminum oxide particles; Ti-HA = titanium coated with hydroxyapatite; SEM = scanning electron microscopy; ModMa = machined and acid-etched TiZr alloy; TAV MG = machined titanium aluminum vanadium alloy with micro-grooves; modSLA = machined, sandblasted and acid-etched TiZr alloy; Ti-p = pure sand-blasted acid-etched titanium.

Table 3. Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)

Q1	Is it clear in the study what is the ‘cause’ and what is the ‘effect’
Q2	Were the participants included in any comparisons similar?
Q3	Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
Q4	Was there a control group?
Q5	Were there multiple measurements of the outcome both pre and post the intervention/exposure?
Q6	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?
Q7	Were the outcomes of participants included in any comparisons measured in the same way?
Q8	Were outcomes measured in a reliable way?
Q9	Was appropriate statistical analysis used?

Table 4. Results of The Checklist for Quasi-Experimental Studies (non-randomized experimental studies) from The Joanna Briggs Institute Critical Appraisal

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Total (% score yes)	Methodological quality
Al-Ahmad et al. [24]	Yes	No	88	High							
de Freitas, et al. [25]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	77	High
Giordano et al. [26]	Yes	Yes	No	No	No	Yes	Yes	Yes	No	66	Moderate
Zaugg et al. [27]	Yes	Yes	No	No	No	Yes	Yes	Yes	No	55	Moderate
Herrmann et al. [28]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	77	High

Total = ΣY/applicable items (the not applicable items were excluded from the sum). Methodological quality was categorized as low when the study reaches up to 49% score “yes”, moderate when the study reached 50% to 69% score “yes”, and high when the study reached more than 70% score “yes”.

Adversely, it was observed that the electrochemical treatment at titanium Grade V showed controversial effects on bacterial contamination, being detrimental to the group anodized at higher voltage (120 kV, Ra = 0.506 μm), while the group anodized at lower voltage (100 kV, Ra = 0.436 μm) have not shown differences to the Ti Grade V not anodized group (Ra = 0.342 μm) [26]. Herrmann et al. [28] have analysed total bacteria cell counts. Statistical differences were observed among the Ti-machined group (Ra = 0.18 μm) and the ZrO2 group (Ra = 0.74 μm) - bacteria cell counts of 4.43 (SD 9.38) and 5.63 (SD 4.83) x 10⁸ respectively (P < 0.05). Biofilm mass was evaluated through safranin staining (absorbance of 530 nm) by Zaugg et al. [27] and it has varied from 0.611 for the machined TiZr group (Ra = 0.093 μm) and 1.17 for the machined sandblasted acid-etched TiZr group (Ra = 0.896 μm) (P < 0.05) [27].

Considering biofilm composition, Al-Ahmad et al. [24], have not found differences between groups (P > 0.05). de Freitas et al. [25] has just analysed biofilm composition on different titanium specimens (machined: Ra = 0.47 μm; blasted: Ra = 1 μm; HA-coated: Ra = 1.27 μm) and could not find statistical differences on this parameter’s either (P > 0.05). Biofilm composition was analysed only descriptively Herrmann et al. [28].

Synthesis of results

All included studies analysed smooth and rough titanium surfaces in the oral cavity over the experimental times of 1 or 3 days in order to evaluate biofilm formation on the surfaces. In summary, three [24,25,28] of the five included studies have not found statistical differences on biofilm formation among smooth and rough titanium specimens (P > 0.05), while two studies [26,27] found that minimally rough titanium specimens showed the worst contamination (P < 0.05). An isolated smooth surface (machined and acid-etched TiZr alloy) have also been associated to a high level of surface contamination (P < 0.05) [27].

DISCUSSION

Studies evaluating surface properties that control bacteria colonization and consequently, biofilm formation, are designed essentially to avoid undesired biological response, since contamination of the surrounding peri-implant tissues could compromise implant treatment prognoses [29]. Considering that the development of materials with optimized surface properties could be helpful to prevent peri-implant diseases [30], this systematic

review aimed to clarify aspects regarding the role of surface roughness in biofilm formation on titanium specimens. A limitation of the present study was the huge heterogeneity among surface treatments and biological analyses employed on articles found on literature about this topic, which made it impossible to perform meta-analysis. Qualitative synthesis provided the observation of outcomes as follows: no differences on biofilm formation on rough and smooth titanium surfaces [24,25,28]; more contamination on rough titanium surfaces [26,27]; or on smooth surfaces [27].

Those results corroborate the high complexity of biofilm formation surfaces on the intricate system of the oral cavity [3].

Since implant surface modifications were first investigated, the role of dental implant surface roughness on biofilm formation has been extensively studied and reported in the literature [31]. For example, in an animal study, Berglundh et al. [32] compared the progression of induced peri-implantitis on sandblasted-acid etched (SLA) titanium surfaces with polished titanium surfaces during a 5-months evaluation period. For those implants, progression of bone loss was faster at the rougher SLA surface and inflammatory lesions were bigger, when compared to smoother surfaces, leading the authors to suggest that progression of peri-implantitis could be more pronounced at implants with moderately rough surfaces than at implants with smooth surfaces [32].

In a recent systematic review, human prospective and retrospective studies were analysed to evaluate if modern rough implants were more susceptible to peri-implantitis than conventional implants with polished surfaces (follow-up of included articles varied from 1 to 15 years) [33]. Clinical parameters such as bleeding on probing, suppuration, plaque accumulation and probing pocket depth were assessed in 8 papers, totaling 2992 implants. Meta-analysis could not be performed, but authors postulated that implants with rough surfaces were more susceptible to biofilm accumulation at short-term follow-up periods, while implants with machined surfaces were associated to more plaque accumulation and higher peri-implant bone loss at long-term evaluation [33]. The present systematic review employed short-time evaluations of biofilm formation on titanium specimens installed at human mouth. This type of methodology was selected to investigate the association among biofilm formation and surface roughness because there are inherent limitations of employing *in vitro* biofilm models, such as lack of molecular mechanisms that are present in human oral cavity, as well as topographical aspects

and host-pathogen interactions [34]. On the contrary, a systematic review that included 57 papers that investigated peri-implantitis prevalence on long-term follow-up periods (mean of 56.8 months) have identified lack of prophylaxis as a risk factor for disease establishment (that one consequently leads to biofilm accumulation). However, other factors have also influenced, such as smoking, diabetes mellitus and history of periodontitis (medium and medium-high level of evidence) [35].

Finally, it is important to add that since surface roughness alone often does not explain differences in biofilm formation on distinct implants, additional physicochemical and topographical analyses are required, in order to achieve optimal understanding of this complex interaction among surfaces and biofilm formation on titanium specimens. Additionally, at human oral cavity, there are other factors than biofilm that can trigger the development and the progression of peri-implant diseases.

CONCLUSIONS

Based on the included clinical studies, a distinct situations were observed, being not possible to estimate if a specific type of surface roughness is more susceptible to biofilm formation than other. Since surface roughness alone often does not explain differences in early biofilm formation at human oral cavity, future research requires additional physicochemical, and topographical analyses of specimens, as well as evaluation of patient local and general factors.

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Appendix 1. Database search strategy (August 14th, 2021)

Database	Search
PubMed (n = 2,414)	((“dental implant” [Title/Abstract] OR “dental implants” [MeSH Terms] OR “Implant material” [Title/Abstract] OR “Dental Implant-Abutment Design” [MeSH Terms] OR “Implant material” [Title/Abstract] OR “Implant Surface” [Title/Abstract] OR “dental devices” [Title/Abstract] OR “dental abutments” [MeSH Terms] OR “titanium” [MeSH Terms]) AND (“Biofilms” [MeSH Terms] OR “Biofilm” [Title/Abstract] OR “Dental Deposits” [MeSH Terms] OR “Dental plaque” [MeSH Terms] OR “Plaque” [Title/Abstract] OR “bacterial adhesion” [MeSH Terms] OR “bacterial colonization” [Title/Abstract] OR “oral bacteria” [Title/Abstract] OR “bacteria” [MeSH Terms] OR “bacterial count” [Title/Abstract] OR “Bacterial Load” [MeSH Terms] OR “bacterial attachment” [Title/Abstract] OR “Microbiology” [MeSH Terms] OR “colony count, microbial” [MeSH Terms] OR “microbial” [Title/Abstract] OR “microorganisms” [Title/Abstract] OR “subgingival colonization” [Title/Abstract] OR “initial colonization” [Title/Abstract]) AND (“in vivo” [Title/Abstract] OR “humans” [Title/Abstract] OR “human” [Title/Abstract] OR “patient” [Title/Abstract] OR “patients” [Title/Abstract] OR “volunteers” [Title/Abstract] OR “volunteer” [Title/Abstract])
Scopus (n = 4,032)	(TITLE-ABS-KEY (“dental implant” OR “dental implants” OR “Implant material” OR “Implant material” OR “Implant Surface” OR “dental devices” OR “dental abutments” OR “titanium”) AND TITLE-ABS-KEY (“Biofilms” OR “Biofilm” OR “Dental Deposits” OR “Plaque” OR “bacterial adhesion” OR “bacterial colonization” OR “oral bacteria” OR “bacterial count” OR “Bacterial Load” OR “bacterial attachment” OR “Microbiology” OR “colony count” OR “microbial” OR “microorganisms” OR “subgingival colonization” OR “initial colonization”) AND TITLE-ABS-KEY (“in vivo” OR “humans” OR “human” OR “patient” OR “patients” OR “volunteers” OR “volunteer”) AND NOT TITLE-ABS-KEY (in AND vitro) AND NOT TITLE-ABS-KEY (review))
Web of Science (n = 1,603)	(TS=(“dental implant” OR “dental implants” OR “Implant material” OR “Implant material” OR “Implant Surface” OR “dental devices” OR “dental abutments” OR “titanium”) AND TS=(“Biofilms” OR “Biofilm” OR “Dental Deposits” OR “Plaque” OR “bacterial adhesion” OR “bacterial colonization” OR “oral bacteria” OR “bacterial count” OR “Bacterial Load” OR “bacterial attachment” OR “Microbiology” OR “colony count” OR “microbial” OR “microorganisms” OR “subgingival colonization” OR “initial colonization”) AND TS=(“in vivo” OR “humans” OR “human” OR “patient” OR “patients” OR “volunteers” OR “volunteer”) NOT ALL=(review) NOT ALL=(“in vitro”)) AND (DT=(“ARTICLE”))

Appendix 2. Papers found at electronic data bases and evaluated through full text reading

Source	Included	Reason for exclusion
Conserva et al. [8]	No	Different experimental times of biofilm evaluation
Desch et al. [9]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Gatewood et al. [10]	No	Inclusion of patients with periodontal disease and nicotine consumption
Grössner-Schreiber et al. [11]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Hannig [12]	No	Have not analysed titanium surfaces
Leonhardt et al. [13]	No	Have not analysed titanium surfaces
Macedo et al. [14]	No	Have not analysed titanium surfaces
do Nascimento et al. [15]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
do Nascimento et al. [16]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Ferreira-Ribeiro et al. [17]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Rimondini et al. [18]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Scarano et al. [19]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Scotti et al. [20]	No	Evaluation of strategies to destroy previously installed biofilm
Wang et al. [21]	No	Lack of comparison of smooth machined titanium surface with a rough titanium surface
Al-Ahmad et al. [23]	Yes	Not applicable
Al-Ahmad et al. [24]	Yes	Different experimental times of biofilm evaluation
de Freitas et al. [25]	Yes	Not applicable
Herrmann, et al. [28]	Yes	Not applicable

Appendix 3. Papers found through manual searching and evaluated through full text reading

Source	Included	Reason for exclusion
Bürgers et al. [22]	No	Different experimental times of biofilm evaluation
Giordano et al. [26]	Yes	Not applicable
Zaugg et al. [27]	Yes	Not applicable