[Title page]

Implant surface factors and bacterial adhesion: a review of the literature

In-Sung Yeo[1], DDS, MSD, PhD. Assistant Professor

Ha-Young Kim[1], DDS, MSD. Graduate Student

Kyung Sub Lim[1], DDS, MSD. Graduate Student

Jung-Suk Han, DDS, MS, PhD. Professor

Department of Prosthodontics, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Korea

[1] These authors contributed equally to this work.

Correspondence to:

Jung-Suk Han, DDS, MS, PhD

Department of Prosthodontics

School of Dentistry

Seoul National University

Daehakro 101, Jongno-gu

Seoul, 110-749, Korea

Tel: +82-2-2072-2661

Fax: +82-2-2072-3860

E-mail: proshan@snu.ac.kr

Running title: Implant surface and bacterial adhesion

Key workds: Biofilm, bacterial adhesion, surface roughness, surface free energy, surface material

ABSTRACT

The microbiota that forms on implant surfaces placed in the human body can be highly resistant to antimicrobial agents and in some cases cause life-threatening infections. Consequently, to limit bacterial attachment to these surfaces and thereby minimize the risk of implant infection, the process of biofilm formation and bacterial attachment must be well-understood. The oral environment is considered to be an excellent model for research into biofilm formation and implant infection, accounting for many studies carried out in the field of dental medicine. Those studies show that the roughness, free energy, and material characteristics of the implant surface largely determine initial bacterial adhesion. This article reviews the relevant literature on these aspects of biofilm formation.

INTRODUCTION

Under favorable conditions, bacteria are able to attach to the surfaces of medical devices implanted in the human body, such as prosthetic heart valves and coronary stents. Bacterial adhesion on an implant surface is often the initial step in implant infection and can lead to biofilm formation (1). The biofilm provides a protective environment for the bacteria, making them much more resistant to antimicrobial agents (2-5). Therefore, infections that derive from biofilm formation on implant surfaces are often life-threatening (6) and their prevention requires a detailed understanding of the processes involved.

Infections in the oral cavity are also caused by biofilms that form on some oral tissues; for example, periodontitis is initiated by supra- and subgingival dental plaques that adhere to the surfaces of the teeth, and peri-implantitis is triggered by dental plaques that have become established on the surfaces of a dental implant (7, 8). The oral cavity is, however, an open growth system, in contrast to most of other structures within the human body (9). In fact, more than 500 microbial species constantly inhabit the oral cavity, in addition to those specifically bound to salivary proteins (9-12). Many of the organisms infecting the periodontium are able to survive in the oral cavity only when they can adhere to non-shedding surfaces, which is one of the characteristics of dental hard substances (7, 9). Microbial adhesion capacity in the oro-pharyngeal system forms a dynamic balance with various removal forces, such as swallowing, frictional removal by oral hygiene, masticatory friction between foods and oral structures, and salivary rinsing (10). Dentistry makes use of a wide variety of materials, such as metals, ceramics, and polymers, which are applied to restore the hard and soft oral tissues. For all these reasons, the oral cavity is

considered to be an excellent model for investigating biofilm formation and implant infection (7).

Biofilm formation on implant surfaces is similar to that on tooth surfaces in the oral cavity, although a previous study reported that the colonization pattern differs (13, 14). The biofilm microflora that colonize titanium dental implants include the same species that are found on tooth surfaces in both healthy and inflamed gingivae (13, 15-17). The first step in biofilm development on the dental implant surface is the formation of an acquired pellicle, which is bacteria-free and contains various salivary proteins, such as α-amylase, albumin, and proline-rich proteins (7, 18-24). The pellicle provides the interface between the implant surface and early colonizers (25) such as *Streptococci* and *Actinomyces* species, which reach the pellicle and the titanium surface by Brownian motion, liquid flow, and chemotaxis (1, 9). Bacterial adhesion is initiated by van der Waals forces, electrostatic forces, hydrogen bonding, and ionic bonding (9), and is further mediated by proteins in the pellicle (9, 26). The early attachment of *Streptococci* and *Actinomyces* species facilitates the late colonization by *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*, which are periodontal pathogens and the causative agents of peri-implantitis and other periodontal infections (27-29). This sequence of events points out the need to develop implant surfaces that discourage initial attachment of the early colonizers, thereby weakening both late colonization and infectious biofilm formation (1).

Surface material composition, roughness, and free energy are the three major factors known to determine initial bacterial adhesion on implant surfaces (1, 9, 28, 30, 31). In the following, we review the pertinent literature on their roles in the initial

phase of bacterial adhesion and therefore on biofilm formation. Our aim is to provide the reader with insights into the surface characteristics of implants that result in implant infections and, perhaps, into novel means that can prevent their development.

PERI-IMPLANTITIS

Peri-implantitis is defined as an inflammatory process affecting the tissues surrounding an osseo-integrated implant, resulting in the loss of supporting bone. According to the definition of the 6th European Workshop, peri-implant mucositis is limited to the soft tissues, while peri-implantitis includes the supporting bone (32). Peri-implantitis occurs in 28–56% of patients who receive an implant, and peri-mucositis occurs in about 80%. The causal relationship between biofilm formation and periodontal inflammation is well established (33-35). As mentioned above, the proportion of periodontal pathogens, e.g., *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* species as well as *Aggregatibacter actinomycetemcomitans*, motile organisms, and spirochetes (27, 28), increases during biofilm formation, gradually replacing streptococci and other early colonizers. The neighboring tissues respond to the infection such that inflammatory infiltrates develop in proximity to the biofilm. The inflammation is initially constrained to the soft tissues (peri-implant mucositis) but then extends to involve the hard tissues (peri-implantitis), with the resulting bone damage being proportional to the burden of periodontal pathogens (28).

Various studies have supported the resemblance between the processes of

periodontitis and peri-implantitis (15-17, 36-40). In one of several studies comparing the microbiota of the teeth *vs.* that of implants, microbiological data from both the teeth and 127 implants in 56 subjects were evaluated by DNA-DNA checkerboard hybridization. No significant differences between the two sites were found. Other studies reached comparable conclusions (37, 40).

Due to the similar colonization of the teeth and dental implants, pre-existing periodontitis is regarded as a risk factor for peri-implantitis (41). In an *in vivo* study, plaques from 15 patients were examined by polymerase chain reaction and culture techniques. The bacterial population was shown to comprise *Porphyromonas gingivalis* (80.0%), *Prevotella intermedia* (53.3%), *Aggregatibacter actinomycetemcomitans* (46.7%), *Bacteroides forsythus* (60.0%), and *Treponema denticola* (40.0%). Pulsed-field gel electrophoresis analysis showed that the isolated *Porphyromonas gingivalis and Prevotella intermedia* strains were identical among the patients. Again, the results were confirmed in other studies (42-44).

An additional important reason for treating pre-existing periodontitis in patients receiving a dental implant was provided in another study, in which the early colonization of installed implants was investigated in 22 patients treated for advanced aggressive periodontitis and receiving supportive maintenance (45). The plaque scores of all 22 patients were below 20%. After installation of 68 non-submerged implants, the presence of five periodontal pathogens was analyzed by DNA-probes. Only five patients showed large differences in the proportion of these species compared with the baseline, while in the remaining 17, the composition and concentration of the microbiota were essentially unchanged. Moreover, 6 months later, no further changes around the implants were identified. This study

demonstrates that both the quality and the quantity of the microbiota are important in the early stage of implant placement.

Several animal studies using microswine, beagles, and monkeys have been carried out to investigate the progression of peri-implantitis (46-49). These animal models were determined to be appropriate for this purpose. In these studies, the progression of peri-implantitis was shown to be generally similar to that of periodontitis occurring around a natural tooth. Importantly, however, a previous study in cynomolgus monkeys (*Macaca fascicularis*) pointed out that an implant lacks a periodontal ligament (50). Accordingly, subsequent experiments addressed the development of peri-implantitis in the absence of the periodontal ligament system. Peri-implantitis, induced by a subgingival ligature wire resulted in more severe destruction of the marginal tissue than periodontitis. The absence of the periodontal ligament was considered to accelerate the pathogenic process. Lindhe et al. also showed that the inflammatory response around the implant was more pronounced and destructive than that around the tooth (49).

SURFACE ROUGHNESS

Extensive research shows that both the amount of plaque formation and the maturity of the plaque, with increasing numbers of motile rods, increase in proportion to the roughness of the surface (51). Several studies have investigated roughness and bacterial adhesion by altering a titanium surface. According to Pier-Francesco et al. (52), the adhesion of *Porphyromonas gingivalis*, as a cause of periodontal disease, significantly declined on a "very smooth" titanium surface, i.e., much smoother than

the one commonly used as an implant abutment (R_a = 34.57 *vs.* 350 nm, respectively). A similar decline in bacterial adhesion was not observed on smooth, rough, or very rough surfaces.

The dependence of bacterial adhesion on titanium-surface roughness was confirmed in a recent *in vitro* study (53). Among three titanium disc surfaces, an acid-etched and blasted surface showed significantly higher roughness and proportionately higher adhesion by *Streptococcus mutans, Porphyromonas gingivalis, and Fusobacterium nucleatum* than either a machined or acid-etched surface. A comparison of the purely machined titanium surface with the sand-blasted and acid-etched titanium surface, in which the surface energy was lower but the roughness was higher, showed greater bacterial adhesion on the latter, both *in vivo and in vitro*. The authors concluded that roughness (R_a) rather than surface energy is more important in promoting adhesion (54). A retrospective scanning electron microscopy study investigated 45 failed implants removed from 40 patients (55). Both the surface roughness of the implant components and the microgap between them were considered to have contributed to biofilm formation and thus to subsequent implant infection.

In an *in vitro* study, Annunziata et al. examined whether the biological response to a titanium plasma sprayed (TPS) surface could be altered by a titanium nitride coating (56). Indeed, the coating significantly decreased the roughness of the original TPS surface and reduced the adhesion and proliferation of the investigated streptococcal strains (*Streptococcus pyogenes* and *Streptococcus sanguinis*). Another *in vitro* study evaluated the attachment of *Streptococcus sanguis* to titanium surfaces of varying roughness (57). Attachment of the investigated bacteria to the titanium

surface exponentially increased with increasing surface roughness.

An animal experiment using canine mandible was performed in a study of peri-implantitis (58). Titanium implants of different surface roughness were installed at the sites of the pre-molars, which had been previously extracted. Peri-implantitis was then intentionally induced by placing cotton ligatures in a subgingival position around the neck of the inserted implants, all made of the same grade of titanium. An examination of plaque accumulation identified a larger number of plaques, greater marginal bone loss, and more severe peri-implant inflammation on the rougher implants.

A recent study using an *in vivo* model evaluated the effects of titanium surface roughness on initial bacterial adhesion by *Streptococcus sanguinis*, *Actinomyces naeslundii*, and *Lactobacillus salivarius* (59). The rougher blasted surface, with a S_a of about 1.5 μm, showed greater bacterial adhesion than the turned surface, with an S_a of 0.18 μm. An anodically oxidized surface (S_a = 0.4 μm) also promoted greater microbial attachment than the turned surface. The augmented resistance of the rougher surfaces to shear forces was suggested to cause the increased bacterial adhesion.

While bacterial adhesion declines as surface roughness decreases, there is a lower limit to this relationship, at a roughness called the “threshold R_a.” Bollen et al. reported that neither in the short- nor in the long-term was there an effect on supra- and subgingival microorganism composition when the R_a was < 0.2 μm (60). In that study, the authors connected the titanium abutment (R_a = 0.2 μm) to the fixture and sufficiently grounded the ceramic abutment (R_a = 0.06 μm) also to the fixture. After

intraoral exposure of these set-ups for 3 and 12 months, the clinical periodontal index and plaque samples were compared. Both the number and the composition of the pathogenic bacteria were found to depend on the roughness of the abutments, with an increase in probing depth and greater bleeding in response to probing determined on rougher *vs.* the smoothest abutments. This result was in concordance with those of the *in vivo* study by Quirynen et al. (61), who monitored the clinical and microbiological findings obtained with four grounded titanium abutments, with R_a values ≤ 0.2 μm, for 3 months. While spirochetes were observed only around the roughest abutment, there were no other differences in subgingival bacterial composition, providing further evidence for a threshold level below which reduced bacterial adhesion no longer confers a clinical benefit. Also, the results of this study showed that although some attachment gain (0.2 mm) was achieved in the roughest abutment, the other abutments had at least 0.8 mm of attachment loss, indicating that a certain degree of roughness may be needed for resistance against probing.

Although rough surfaces support biofilm formation in the oral cavity, surface roughness seems to have no effect on the affinities of the microbial species that cause the oral infections (late colonizers). An excellent *in vivo* study evaluated biofilm formation on titanium and zirconia surfaces of various surface morphologies, roughness, and composition (62). The investigation concluded that the roughness and composition of the surface material had little influence on biofilm formation as the biofilm matures.

At an international congress, the surface roughness of a dental implant was reported to be the primary factor influencing bacterial biofilm formation (63). However, this conclusion was contradicted in an *in vitro* study showing that roughness is less

important in bacterial adhesion than the physicochemical properties of the blasted particles modifying titanium surfaces and affecting the surface energy (64). In an *in vivo* human study using healing screws, anatase (a form of titanium dioxide)-coated surfaces were shown to be more resistant to bacterial adhesion than commercially available pure titanium surfaces, despite the fact that the former (R_a = 0.73 ± 0.05 μm) are rougher than the latter (R_a = 0.86 ± 0.06 μm) (65). A recent randomized controlled trial with split-mouth design obtained interesting results (66). Implants with a smooth turned surface and with a moderately rough anodized surface were placed in the patients' mouths. Subsequent analysis of the subgingival biofilm found no significant differences in the subgingival microbiota of the two surfaces, although the samples were taken from the subgingival area under the abutments not on the implant surfaces. Additional and more carefully designed clinical studies are required to clarify the extent to which the various properties of dental implants influence biofilm formation and the process of infection. Table 1 summarizes the above mentioned studies dealing with surface roughness and bacterial adhesion.

SURFACE FREE ENERGY

The sessile drop technique is frequently used to determine the energy of solid surfaces. It involves measurement of the contact angle between a droplet of liquid with known surface energy and the solid surface of interest. Because roughness is one of several factors affecting the contact angle, roughness itself will affect the surface free energy (SFE). Busscher et al. reported that the effects of roughness on the contact angle disappeared when R_a was < 0.1 μm (67). Thus, while the SFE is independent of roughness below certain values of R_a, further experiments must be

conducted to interpret this finding.

Recent *in vitro* research has evaluated SFE and bacterial adhesion using disc samples whose surfaces consisted of polished, partially stabilized zirconia, titanium blasted with zirconia, titanium blasted with zirconia and then acid-etched, or polished titanium (68). The surfaces of polished partially-stabilized zirconia and titanium blasted with zirconia had a lower SFE and decreased bacterial adhesion (*Streptococcus mitis and Prevotella nigrescens*). The authors concluded that SFE is the most important factor determining initial bacterial adhesion.

Sardin et al. identified a relationship between SFE and streptococcal adhesion in an *in vitro* test (26). Samples of titanium, ceramic, and enamel, all of which are used as prosthetic materials, were produced whose roughness was controlled to be approximately 0.05 μm. The contact angle of each sample was measured and the SFE was calculated with the van Oss equation. The samples were then exposed to a culture of *Streptococcus mitis*, a species dominant in early plaque formation, and bacterial adhesion was measured. Bacterial adhesion was shown to correlate with the total SFE and the proportion of the nonpolar component of the material. These findings are partially in concordance with those of Pereni et al., in which an association between SFE and bacterial retention was demonstrated, albeit using other bacterial species (69).

Almaguer-Flores et al. reported that the composition of the initial biofilm may change on the basis of surface hydrophilicity, as may the effects of microstructure and SFE according to the test species (70). However, with respect to SFE, the findings of laboratory tests do not differ significantly from those of *in vivo* studies. In a randomized controlled clinical trial evaluating bacterial adhesion on implant surfaces

(71), 12 patients each received two implants in the posterior mandible. Abutments were connected to the implants 3 months after implant insertion, with the zirconia abutment connected to one implant and the titanium abutment to the other. Five weeks later, the abutments were collected and the adhesion of *Aggregatibacter actinomycetemcomitants*, *Porphyromonas gingivalis*, and universal bacteria on each surface was analyzed. While the SFE of the zirconia surface was lower than that of the titanium surface, no significant differences between the surfaces were found with respect to the adhesion of the investigated bacterial species. Another *in vivo* human study also suggested that SFE has only a minor influence on initial bacterial adhesion, compared with surface roughness (54).

Contradictory results were obtained in an *in vivo* study suggesting that SFE affects the plaque microbiology of the supragingival area (72). Supra- and subgingival plaques were examined on a titanium abutment *vs.* a fluor-ethylene-propylene (FEP)-coated abutment with similar surface roughness after 3 months of habitual oral hygiene. The FEP abutment, with a lower SFE, resulted in a supragingival plaque with a greater representation of coccoid microorganisms, while spirochetes and motile organisms were detected only around the titanium abutment. However, the difference was slight in the subgingival areas, and clear overall effects were not evident because of the large difference between subjects. In addition to SFE, other surface characteristics, such as roughness, were reported to play a less significant role in plaque formation in subgingival than in supragingival areas (72, 73).

The SFE of the substratum is also related to the SFE of bacterial clusters. On low-SFE surfaces, bacterial clusters with lower SFEs were shown to predominate (74). In addition to the SFE of the substrate and of the bacteria, that of the suspending

medium is also important. In addition, the pellicle coating was shown to have homogenizing effects on the SFE, indicating the complexity of SFE effects even under defined conditions. Table 2 summarizes the above mentioned studies dealing with SFE and bacterial adhesion.

MATERIALS

Titanium is commonly used as the abutment material because of its superior biocompatibility. Recently, however, zirconia has been increasingly preferred for esthetic reasons; thus, many studies have compared zirconia and titanium. The results of Scarano et al. supported the use of zirconia (75). In that *in vivo* human study, an intraoral device adhered with either zirconia or titanium disc samples was exposed for 24 h, after which the surface was analyzed with SEM to measure the rate of bacterial covering. Significantly less adhesion was observed with zirconia (12.1%) than with titanium (19.3%), indicating the appropriateness of the former as an abutment material.

Nonetheless, many researchers reported no differences between the two materials (21, 31, 76, 77). Rasperini et al. conducted a microbiological analysis of samples collected from titanium and zirconia abutments at 6 h, 24 h, 7 days, and 14 days (76). Maximum colonization occurred after 24 h of intraoral exposure and was maintained consistently until the 14th day, with no differences between the two materials. A similar study by Brakel et al. prolonged the observation period. Bacterial composition and soft-tissue health at the 2nd post-operative week and 3rd post-operative month were not significantly different in the zirconia *vs.* the titanium group (77). In an *in vitro*

study comparing pellicle composition and bacterial binding properties, zirconia and titanium yielded similar results that were significantly different from those obtained with hydroxyapatite (21).

A recent *in vivo* study compared dental ceramics with respect to biofilm formation (78). Glass ceramic, lithium disilicate glass ceramic, yttrium-stabilized zirconia (Y-TZP), pressed Y-TZP ceramic, and a pressed mixed ceramic with Y-TZP and 25% alumina, all with similar surface roughness (mean R_a = 0.04 μm), were tested. Plaque accumulation was lowest in the pressed Y-TZP ceramic and highest in the lithium disilicate glass-ceramic, suggesting that the material itself also has an effect on biofilm formation, although this is partly related to its surface energy. The use of a gold alloy as an abutment material analogous to the use of zirconia and titanium has been examined in several animal and clinical studies aimed at estimating its biological reliability by measuring the periodontal index and assessing soft-tissue stability (79, 80).

The surface quality of a nitride coating was evaluated *in vitro* with respect to bacterial attachment (24). Commercially available, pure titanium discs were modified by four different surface treatments: laser radiation, thermal oxidation, and physical vapor deposition with titanium nitride (TiN) or zirconium nitride (ZrN). The modified surfaces were exposed to *Streptococcus mutans* and *Streptococcus sanguis* and then analyzed by fluorescence microscopy. Bacterial adhesion on the TiN and ZrN hard coatings was significantly reduced. Similar results were also shown in other *in vivo* studies (81, 82).

Bacterial adhesion on zirconia and titanium was also examined in controlled studies, as the composition of surface materials influences their corrosion behaviors,

porosities, and microstructures following exposure to the oral environment (83, 84). The observed effects on bacterial adhesion can be explained with or without reference to changes in surface roughness or SFE (78, 83, 84). Therefore, further studies, in which these variables are meticulously controlled, are needed. Table 3 summarizes the above mentioned studies dealing with the material composition and bacterial adhesion.

CONCLUSIONS

Biofilms formed on implant surfaces induce inflammation and dental-implant infection. Decreasing initial bacterial adhesion to the surface may help to restrict their formation. Surface roughness, SFE, and surface material composition are considered to be the three most important factors determining bacterial attachment to the implant surface. Overall, the rougher the surface, the greater the amount of plaque accumulation. Surface roughness is generally considered to play a larger role in biofilm formation than SFE, but some studies have reached the opposite conclusion. Surface characteristics and chemistry, such as porosity, corrosion behavior, and the composition of the surface materials, also influence bacterial adhesion to the implant surface. However, these material factors are likely to be ultimately related to surface roughness or SFE. While there have been many *in vitro* studies examining bacterial adhesion to implant surfaces, additional, clinical investigations are still required.

ACKNOWLEDGMENT

This review article was supported by the Basic Science Research Program, through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (no. 860-20110053).

REFERENCES

1. Subramani K, Jung RE, Molenberg A, Hammerle CH. Biofilm on dental implants: a review of the literature. Int J Oral Maxillofac Implants. 2009;24(4):616-626.
2. Gristina AG, Hobgood CD, Webb LX, Myrvik QN. Adhesive colonization of biomaterials and antibiotic resistance. Biomaterials. 1987;8(6):423-426.
3. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9(1):34-39.
4. Nickel JC, Ruseska I, Wright JB, Costerton JW. Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother. 1985;27(4):619-624.
5. Prosser BL, Taylor D, Dix BA, Cleeland R. Method of evaluating effects of antibiotics on bacterial biofilm. Antimicrob Agents Chemother. 1987;31(10):1502-1506.
6. Kaplan JB. Antibiotic-induced biofilm formation. Int J Artif Organs. 2011;34(9):737-751. doi: 10.5301/ijao.5000027.
7. Hannig C, Hannig M. The oral cavity--a key system to understand substratum-dependent bioadhesion on solid surfaces in man. Clin Oral Investig. 2009;13(2):123-139. doi: 10.1007/s00784-008-0243-3.
8. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol. 1995;15(3):169-175.
9. Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. J Clin Periodontol. 1995;22(1):1-14.

10. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res. 2006;17 Suppl 2:68-81. doi: 10.1111/j.1600-0501.2006.01353.x.
11. Gibbons RJ, Hay DI, Cisar JO, Clark WB. Adsorbed salivary proline-rich protein 1 and statherin: receptors for type 1 fimbriae of Actinomyces viscosus T14V-J1 on apatitic surfaces. Infect Immun. 1988;56(11):2990-2993.
12. Gibbons RJ, Hay DI. Human salivary acidic proline-rich proteins and statherin promote the attachment of Actinomyces viscosus LY7 to apatitic surfaces. Infect Immun. 1988;56(2):439-445.
13. Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007;18(4):501-508. doi: 10.1111/j.1600-0501.2007.01381.x.
14. Tanner A, Maiden MF, Lee K, Shulman LB, Weber HP. Dental implant infections. Clin Infect Dis. 1997;25 Suppl 2:S213-217.
15. Rams TE, Roberts TW, Feik D, Molzan AK, Slots J. Clinical and microbiological findings on newly inserted hydroxyapatite-coated and pure titanium human dental implants. Clin Oral Implants Res. 1991;2(3):121-127.
16. Mombelli A, van Oosten MA, Schurch E, Jr., Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol. 1987;2(4):145-151.
17. Leonhardt A, Grondahl K, Bergstrom C, Lekholm U. Long-term follow-up of osseointegrated titanium implants using clinical, radiographic and microbiological parameters. Clin Oral Implants Res. 2002;13(2):127-132.
18. Steinberg D, Sela MN, Klinger A, Kohavi D. Adhesion of periodontal bacteria to titanium, and titanium alloy powders. Clin Oral Implants Res. 1998;9(2):67-

72.

19. Sela MN, Badihi L, Rosen G, Steinberg D, Kohavi D. Adsorption of human plasma proteins to modified titanium surfaces. Clin Oral Implants Res. 2007;18(5):630-638. doi: 10.1111/j.1600-0501.2007.01373.x.

20. Steinberg D, Klinger A, Kohavi D, Sela MN. Adsorption of human salivary proteins to titanium powder. I. Adsorption of human salivary albumin. Biomaterials. 1995;16(17):1339-1343.

21. Lima EM, Koo H, Vacca Smith AM, Rosalen PL, Del Bel Cury AA. Adsorption of salivary and serum proteins, and bacterial adherence on titanium and zirconia ceramic surfaces. Clin Oral Implants Res. 2008;19(8):780-785. doi: 10.1111/j.1600-0501.2008.01524.x.

22. Kohavi D, Klinger A, Steinberg D, Mann E, Sela NM. alpha-Amylase and salivary albumin adsorption onto titanium, enamel and dentin: an in vivo study. Biomaterials. 1997;18(13):903-906.

23. Edgerton M, Lo SE, Scannapieco FA. Experimental salivary pellicles formed on titanium surfaces mediate adhesion of streptococci. Int J Oral Maxillofac Implants. 1996;11(4):443-449.

24. Grossner-Schreiber B, Griepentrog M, Haustein I, et al. Plaque formation on surface modified dental implants. An in vitro study. Clin Oral Implants Res. 2001;12(6):543-551.

25. Heuer W, Elter C, Demling A, et al. Analysis of early biofilm formation on oral implants in man. J Oral Rehabil. 2007;34(5):377-382. doi: 10.1111/j.1365-2842.2007.01725.x.

26. Sardin S, Morrier JJ, Benay G, Barsotti O. In vitro streptococcal adherence on prosthetic and implant materials. Interactions with physicochemical surface

properties. J Oral Rehabil. 2004;31(2):140-148.

27. Narhi TO, Leminen H, Haukioja A, Soderling E. Adhesion of Aggregatibacter actinomycetemcomitans and Fusobacterium nucleatum on bioactive TiO(2) surfaces. Clin Oral Implants Res. 2012. doi: 10.1111/j.1600-0501.2011.02399.x.

28. Quirynen M, De Soete M, van Steenberghe D. Infectious risks for oral implants: a review of the literature. Clin Oral Implants Res. 2002;13(1):1-19.

29. Mombelli A, Lang NP. Microbial aspects of implant dentistry. Periodontol 2000. 1994;4:74-80.

30. Linkevicius T, Apse P. Influence of abutment material on stability of peri-implant tissues: a systematic review. Int J Oral Maxillofac Implants. 2008;23(3):449-456.

31. Lee BC, Jung GY, Kim DJ, Han JS. Initial bacterial adhesion on resin, titanium and zirconia in vitro. J Adv Prosthodont. 2011;3(2):81-84. doi: 10.4047/jap.2011.3.2.81.

32. Lindhe J, Meyle J. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontol. 2008;35(8 Suppl):282-285. doi: 10.1111/j.1600-051X.2008.01283.x.

33. Listgarten MA, Hellden L. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J Clin Periodontol. 1978;5(2):115-132.

34. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. J Periodontol. 1965;36:177-187.

35. Slots J. The predominant cultivable microflora of advanced periodontitis. Scand J Dent Res. 1977;85(2):114-121.

36. Agerbaek MR, Lang NP, Persson GR. Comparisons of bacterial patterns present at implant and tooth sites in subjects on supportive periodontal therapy. I. Impact of clinical variables, gender and smoking. Clin Oral Implants Res. 2006;17(1):18-24. doi: 10.1111/j.1600-0501.2005.01190.x.

37. Leonhardt A, Adolfsson B, Lekholm U, Wikstrom M, Dahlen G. A longitudinal microbiological study on osseointegrated titanium implants in partially edentulous patients. Clin Oral Implants Res. 1993;4(3):113-120.

38. Mombelli A, Marxer M, Gaberthuel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. J Clin Periodontol. 1995;22(2):124-130.

39. Nakazato G, Tsuchiya H, Sato M, Yamauchi M. In vivo plaque formation on implant materials. Int J Oral Maxillofac Implants. 1989;4(4):321-326.

40. Quirynen M, Listgarten MA. Distribution of bacterial morphotypes around natural teeth and titanium implants ad modum Branemark. Clin Oral Implants Res. 1990;1(1):8-12.

41. Sumida S, Ishihara K, Kishi M, Okuda K. Transmission of periodontal disease-associated bacteria from teeth to osseointegrated implant regions. Int J Oral Maxillofac Implants. 2002;17(5):696-702.

42. Papaioannou W, Quirynen M, Van Steenberghe D. The influence of periodontitis on the subgingival flora around implants in partially edentulous patients. Clin Oral Implants Res. 1996;7(4):405-409.

43. Quirynen M, Papaioannou W, van Steenberghe D. Intraoral transmission and the colonization of oral hard surfaces. J Periodontol. 1996;67(10):986-993.

44. Takanashi K, Kishi M, Okuda K, Ishihara K. Colonization by Porphyromonas gingivalis and Prevotella intermedia from teeth to osseointegrated implant

regions. Bull Tokyo Dent Coll. 2004;45(2):77-85.

45. De Boever AL, De Boever JA. Early colonization of non-submerged dental implants in patients with a history of advanced aggressive periodontitis. Clin Oral Implants Res. 2006;17(1):8-17. doi: 10.1111/j.1600-0501.2005.01175.x.

46. Eke PI, Braswell LD, Fritz ME. Microbiota associated with experimental peri-implantitis and periodontitis in adult Macaca mulatta monkeys. J Periodontol. 1998;69(2):190-194.

47. Hanisch O, Cortella CA, Boskovic MM, James RA, Slots J, Wikesjo UM. Experimental peri-implant tissue breakdown around hydroxyapatite-coated implants. J Periodontol. 1997;68(1):59-66.

48. Hickey JS, O'Neal RB, Scheidt MJ, Strong SL, Turgeon D, Van Dyke TE. Microbiologic characterization of ligature-induced peri-implantitis in the microswine model. J Periodontol. 1991;62(9):548-553.

49. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res. 1992;3(1):9-16.

50. Schou S, Holmstrup P, Reibel J, Juhl M, Hjorting-Hansen E, Kornman KS. Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth: stereologic and histologic observations in cynomolgus monkeys (Macaca fascicularis). J Periodontol. 1993;64(6):529-537.

51. Quirynen M, van der Mei HC, Bollen CM, et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. J Dent Res. 1993;72(9):1304-1309.

52. Amoroso PF, Adams RJ, Waters MG, Williams DW. Titanium surface modification and its effect on the adherence of Porphyromonas gingivalis: an

in vitro study. Clin Oral Implants Res. 2006;17(6):633-637. doi: 10.1111/j.1600-0501.2006.01274.x.

53. Badihi Hauslich L, Sela MN, Steinberg D, Rosen G, Kohavi D. The adhesion of oral bacteria to modified titanium surfaces: role of plasma proteins and electrostatic forces. Clin Oral Implants Res. 2011. doi: 10.1111/j.1600-0501.2011.02364.x.

54. Burgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res. 2010;21(2):156-164. doi: 10.1111/j.1600-0501.2009.01815.x.

55. O'Mahony A, MacNeill SR, Cobb CM. Design features that may influence bacterial plaque retention: a retrospective analysis of failed implants. Quintessence Int. 2000;31(4):249-256.

56. Annunziata M, Oliva A, Basile MA, et al. The effects of titanium nitride-coating on the topographic and biological features of TPS implant surfaces. J Dent. 2011;39(11):720-728. doi: 10.1016/j.jdent.2011.08.003.

57. Pereira da Silva CH, Vidigal GM, Jr., de Uzeda M, de Almeida Soares G. Influence of titanium surface roughness on attachment of Streptococcus sanguis: an in vitro study. Implant Dent. 2005;14(1):88-93.

58. Albouy JP, Abrahamsson I, Berglundh T. Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics: an experimental study in dogs. J Clin Periodontol. 2012;39(2):182-187. doi: 10.1111/j.1600-051X.2011.01820.x.

59. Frojd V, Chavez de Paz L, Andersson M, Wennerberg A, Davies JR, Svensater G. In situ analysis of multispecies biofilm formation on customized titanium surfaces. Mol Oral Microbiol. 2011;26(4):241-252. doi: 10.1111/j.2041-

1014.2011.00610.x.

60. Bollen CM, Papaioanno W, Van Eldere J, Schepers E, Quirynen M, van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. Clin Oral Implants Res. 1996;7(3):201-211.

61. Quirynen M, Bollen CM, Papaioannou W, Van Eldere J, van Steenberghe D. The influence of titanium abutment surface roughness on plaque accumulation and gingivitis: short-term observations. Int J Oral Maxillofac Implants. 1996;11(2):169-178.

62. Al-Ahmad A, Wiedmann-Al-Ahmad M, Faust J, et al. Biofilm formation and composition on different implant materials in vivo. J Biomed Mater Res B Appl Biomater. 2010;95(1):101-109. doi: 10.1002/jbm.b.31688.

63. Lang NP, Berglundh T. Periimplant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011;38 Suppl 11:178-181. doi: 10.1111/j.1600-051X.2010.01674.x.

64. Rodriguez-Hernandez AG, Juarez A, Engel E, Gil FJ. Streptococcus sanguinis adhesion on titanium rough surfaces: effect of shot-blasting particles. J Mater Sci Mater Med. 2011;22(8):1913-1922. doi: 10.1007/s10856-011-4366-8.

65. Scarano A, Piattelli A, Polimeni A, Di Iorio D, Carinci F. Bacterial adhesion on commercially pure titanium and anatase-coated titanium healing screws: an in vivo human study. J Periodontol. 2010;81(10):1466-1471. doi: 10.1902/jop.2010.100061.

66. Quirynen M, Van Assche N. RCT comparing minimally with moderately rough implants. Part 2: microbial observations. Clin Oral Implants Res. 2012;23(5):625-634. doi: 10.1111/j.1600-0501.2011.02255.x.

67. Busscher HJ, van Pelt AWJ, de Boer P, de Jong HP, Arends J. The effect of surface roughening of polymers on measured contact angles of liquids. Colloids and Surfaces. 1984;9(4):319-331. doi: 10.1016/0166-6622(84)80175-4.

68. Al-Radha AS, Dymock D, Younes C, O'Sullivan D. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. J Dent. 2012;40(2):146-153. doi: 10.1016/j.jdent.2011.12.006.

69. Pereni CI, Zhao Q, Liu Y, Abel E. Surface free energy effect on bacterial retention. Colloids Surf B Biointerfaces. 2006;48(2):143-147. doi: 10.1016/j.colsurfb.2006.02.004.

70. Almaguer-Flores A, Olivares-Navarrete R, Wieland M, Ximenez-Fyvie LA, Schwartz Z, Boyan BD. Influence of topography and hydrophilicity on initial oral biofilm formation on microstructured titanium surfaces in vitro. Clin Oral Implants Res. 2012;23(3):301-307. doi: 10.1111/j.1600-0501.2011.02184.x.

71. Salihoglu U, Boynuegri D, Engin D, Duman AN, Gokalp P, Balos K. Bacterial adhesion and colonization differences between zirconium oxide and titanium alloys: an in vivo human study. Int J Oral Maxillofac Implants. 2011;26(1):101-107.

72. Quirynen M, Van der Mei HC, Bollen CM, et al. The influence of surface-free energy on supra- and subgingival plaque microbiology. An in vivo study on implants. J Periodontol. 1994;65(2):162-167.

73. Elter C, Heuer W, Demling A, et al. Supra- and subgingival biofilm formation on implant abutments with different surface characteristics. Int J Oral Maxillofac Implants. 2008;23(2):327-334.

74. Weerkamp AH, Uyen HM, Busscher HJ. Effect of zeta potential and surface

energy on bacterial adhesion to uncoated and saliva-coated human enamel and dentin. J Dent Res. 1988;67(12):1483-1487.

75. Scarano A, Piattelli M, Caputi S, Favero GA, Piattelli A. Bacterial adhesion on commercially pure titanium and zirconium oxide disks: an in vivo human study. J Periodontol. 2004;75(2):292-296. doi: 10.1902/jop.2004.75.2.292.

76. Rasperini G, Maglione M, Cocconcelli P, Simion M. In vivo early plaque formation on pure titanium and ceramic abutments: a comparative microbiological and SEM analysis. Clin Oral Implants Res. 1998;9(6):357-364.

77. van Brakel R, Cune MS, van Winkelhoff AJ, de Putter C, Verhoeven JW, van der Reijden W. Early bacterial colonization and soft tissue health around zirconia and titanium abutments: an in vivo study in man. Clin Oral Implants Res. 2011;22(6):571-577. doi: 10.1111/j.1600-0501.2010.02005.x.

78. Bremer F, Grade S, Kohorst P, Stiesch M. In vivo biofilm formation on different dental ceramics. Quintessence Int. 2011;42(7):565-574.

79. Abrahamsson I, Cardaropoli G. Peri-implant hard and soft tissue integration to dental implants made of titanium and gold. Clin Oral Implants Res. 2007;18(3):269-274. doi: 10.1111/j.1600-0501.2006.01326.x.

80. Vigolo P, Givani A, Majzoub Z, Cordioli G. A 4-year prospective study to assess peri-implant hard and soft tissues adjacent to titanium versus gold-alloy abutments in cemented single implant crowns. J Prosthodont. 2006;15(4):250-256. doi: 10.1111/j.1532-849X.2006.00114.x.

81. Grossner-Schreiber B, Teichmann J, Hannig M, Dorfer C, Wenderoth DF, Ott SJ. Modified implant surfaces show different biofilm compositions under in vivo conditions. Clin Oral Implants Res. 2009;20(8):817-826. doi: 10.1111/j.1600-0501.2009.01729.x.

82. Scarano A, Piattelli M, Vrespa G, Caputi S, Piattelli A. Bacterial adhesion on titanium nitride-coated and uncoated implants: an in vivo human study. J Oral Implantol. 2003;29(2):80-85. doi: 10.1563/1548-1336(2003)029<0080:BAOTNA>2.3.CO;2.

83. Meier R, Hauser-Gerspach I, Luthy H, Meyer J. Adhesion of oral streptococci to all-ceramics dental restorative materials in vitro. J Mater Sci Mater Med. 2008;19(10):3249-3253. doi: 10.1007/s10856-008-3457-7.

84. Milleding P, Wennerberg A, Alaeddin S, Karlsson S, Simon E. Surface corrosion of dental ceramics in vitro. Biomaterials. 1999;20(8):733-746.

Table 1. Summary of the reviewed articles evaluating the Influence of surface roughness on biofilm formation.

Authors	Experimental condition	Sample morphology	Sample material	Range of surface roughness (R_a)	Influence of roughness for biofilm formation
Quirynen et al. 1993	In vivo (human)	Implant abutment	Titanium	0.35 µm - 0.81 µm	Major
Amoroso et al. 2006	In vitro	10 X 10 X 1 mm Square	cp-Titanium	0.035 µm - 0.450 µm	Major
Badihi Hauslich et al. 2011	In vitro	Ø 6mm Disc	Titanium alloy (Ti6Al4V)	0.054 µm - 0.183 µm	Major
Bürgers et al. 2010	In vivo (human) and in vitro	Ø 9 X 2 mm Disc	Titanium	0.15 µm - 0.95 µm	Major
Annunziata et al. 2011	In vitro	Ø 15 X 1 mm Disc	Titanium alloy (Ti6Al4V) – TiN coating	3 µm – 6 µm (S_a)	Moderate*
Pereira da Silva et al. 2005	In vitro	10 X 10 X 1 mm Square	cp-Titanium	0.17 µm - 3.17 µm	Major
Albouy et al. 2012	In vivo (Labrador dog)	Implant fixture	Titanium	N/A	Major
Fröjd et al. 2011	In vitro	Ø 8mm Disc	cp-Titanium	0.18 µm - 2.0 µm (S_a)	Moderate*
Bollen et al. 1996	In vivo (human)	Implant abutment	cp-Titanium , ceramic	0.06 µm - 0.21 µm	Minor (below a certain "threshold R_a "-0.2 µm)
Quirynen et al. 1996	In vivo (human)	Implant abutment	cp-Titanium	0.05 µm - 0.21 µm	Minor (below a certain "threshold R_a "-0.2 µm)
Al-Ahmad et al. 2010	In vivo (human)	Ø 5 X 1 mm Disc	Titanium, zirconia	0.014 µm - 0.544 µm	Minor
Rodriguez-Hernandez et al. 2011	In vitro	Ø 5 X 2 mm Disc	cp-Titanium	0.34 µm - 8 µm	Minor
Quirynen et al. 2012	In vivo (human)	Implant fixture, implant abutment	Titanium	0.05 µm - 32 µm	Minor

cp-Titanium, commercially pure titanium.

Moderate* is used when other factors affect similar influence on biofilm formation as surface roughness exist.

Table 2. Summary of the reviewed articles evaluating the Influence of surface free energy on biofilm formation.

Authors	Experimental condition	Sample morphology	Sample material	Range of surface roughness (R_a)	Influence of surface free energy for biofilm formation
Sardin et al. 2004	In vitro	Ø 11 mm Disc	Casting alloys, Ceramic, Titanium	0.03 µm - 0.13 µm	Not significant
Al-Radha et al. 2012	In vitro	Ø 5 mm Disc Ø 6 mm Disc	Titanium, Zirconia	0.043 µm - 0.15 µm	Major
Pereni et al. 2006	In vitro	30 X30 X 1 mm Square	Stainless steel, Silicone	0.08 µm - 0.25 µm	Major
Almaguer-Flores et al. 2012	In vitro	Ø 15 X 1 mm Disc	Titanium	Pretreatment titanium – < 0.2 µm Acid etched – < 0.8 µm SLA or hydrophilic SLA – 3.2 µm	Positive correlation
Salihoglu et al. 2011	In vivo (human)	Implant abutment	Titanium, Zirconia	N/A	Not significant
Quirynen et al. 1994	In vivo (human)	Implant abutment	Titanium	0.81 µm - 0.82 µm	Major (supragingiva) Not significant (subgingiva)
Weerkamp et al. 1988	In vitro	4 X 4 mm Square	Human teeth	N/A	Moderate*

Moderate* is used when other factors affect similar influence on biofilm formation as surface free energy exist

Table 3. Summary of the reviewed articles evaluating the influence of material compositions on biofilm formation.

Authors	Experimental condition	Sample morphology	Compared sample material	Range of surface roughness (R_a)	Influence of material composition for biofilm formation
Lima et al. 2008	In vitro	Ø 10 X 2 mm Disc	Titanium, Zirconia	N/A	Not significant
Lee et al. 2011	In vitro	Ø 12 mm Disc	Resin, Titanium Zirconia	0.059 µm - 0.179 µm	More attachment on resin Similar between titanium and zirconia
Scarano et al. 2004	In vivo (human)	Disc	Titanium, Zirconia	0.73 µm - 0.76 µm	Less attachment on zirconia
Rasperini et al. 1998	In vivo (human)	4 X 3 X 1 mm rectangular form	Titanium, Novel ceramic	0.6 µm - 0.7 µm	Not significant
van Brakel et al. 2011	In vivo (human)	Implant abutment	Titanium, Zirconia	0.21 µm - 0.236 µm	Not significant
Bremer et al. 1994	In vivo (human)	3 X 3 X 1.5 mm Square	Glass ceramic, Lithium disilicate glass ceramic, Zirconia, HIP zirconia, HIP zirconia with 25% alumina	0.04 µm	Least attachment on zirconia
Größner-Schreiber et al. 2001	In vitro	Ø 10 X 2 mm Disc	Titanium nitride Zirconium nitride Oxidized titanium Titanium	0.14 µm - 0.20 µm 1.00 µm (laser-radiated titanium)	Less attachment on titanium- and zirconium-nitride coatings
Scarano et al. 2003	In vivo (human)	Ø 4 X 13 mm Implant	Titanium-nitride Titanium	0.76 µm - 0.79 µm	Less attachment on titanium-nitride coating
Größner-Schreiber et al. 2009	In vivo (human)	Ø 10 X 2 mm Disc (titanium) 0.7 to 0.9 cm^2 (area) X 1 mm (thickness) Square to rectangular (glass)	Zirconium-nitride Roughened titanium Polished titanium Glass	0.03 µm - 0.1 µm 0.19 µm (roughened titanium)	Less attachment on zirconium-nitride coating
Meier et al. 2008	In vitro	14.4 X 14.4 X 0.2 mm Square	Glass, Glass ceramic, In-ceram alumina, In-ceram zirconia, Zirconia	0.24 µm - 1.34 µm	Not significant

HIP zirconia, a hot isostatically pressed zirconia ceramic.