Chapter 20

Novel Models to Manage Biofilms on Microtextured Dental Implant Surfaces

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Additional information is available at the end of the chapter

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Abstract

Dental implants are used extensively to replace missing teeth. To enhance their integration with the bones of the jaws, the surfaces of titanium dental implants are modified to make them hydrophilic, high energy, and microtextured. These same features make biofilm development occur readily upon exposure to the saliva. The presence of mature biofilms on dental implant surfaces drives local inflammatory responses in the adjacent soft and hard tissues (peri-implantitis), which leads to pathological loss of bone and the formation of a saucer shaped bone defects. This chapter examines the unique challenges posed by biofilms formed on highly complex dental implant surfaces, which are difficult to access for cleaning, and easily damaged by conventional cleaning approaches. We explore how biofilms can be removed from implant surfaces using a variety of novel methods, without causing surface damage or other undesirable modifications, and show how different laboratory and clinical models can be used to assess the performance of both conventional and novel methods of biofilm removal.

Keywords: biofilms, dental implants, surface characterization, debridement, lasers, particle beams

1. Introduction

Dental implants have been used for over 40 years to replace missing teeth, with good clinical success when placed in most sites in the jaws [1]. They are inserted into specially prepared channels in the bone, and once fully integrated into the bone, can be restored with a ceramic crown to replace a single missing tooth (**Figure 1**). Typically, dental implants are fabricated from commercially pure titanium (Ti), or titanium alloys which include small amounts of vanadium and aluminum. Dental implants made from ceramic materials such as sapphire and

zirconium oxide exist but are not in common use. The use of Ti is favored over other materials because it is biocompatible when inserted into direct contact with bone, resistant to corrosion, lightweight, and durable.

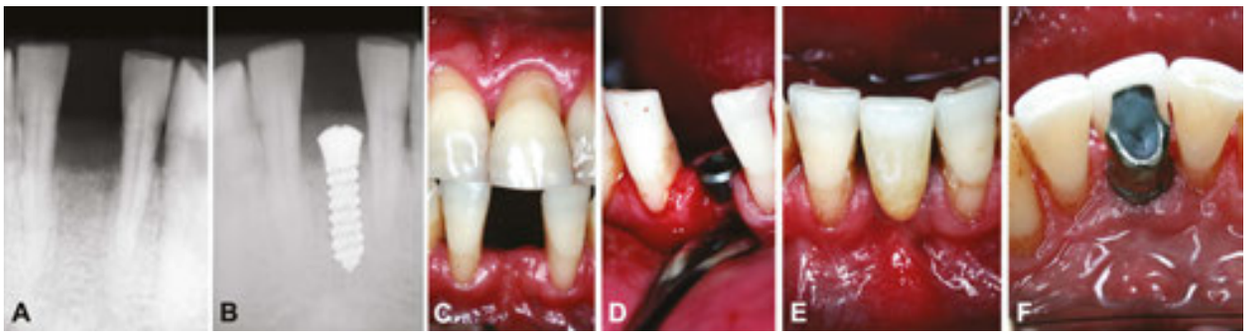

Figure 1. An overview of dental implant placement. (A) Radiograph of the site of a missing tooth showing adequate bone levels. (B) Post-operative radiograph of the same area after placement of a titanium dental implant. (C) Pre-operative clinical view of the site of the missing lower incisor tooth. (D) Immediately after placing the implant into a site prepared in the bone. The collar of the implant can be seen. The soft tissues are being displaced from the bone by the metal instrument. (E) Implant supported crown in place. (F) Mirror view showing the attachment to the implant which supports the crown.

2. The complexities of implant surfaces

While early dental implants had simple threaded forms and plain surfaces which were unmodified after milling, almost all modern dental implants have surface features which increase the surface area and surface energy, enhancing the adhesion of blood, matrix proteins, and human cells. Altering the surface of a Ti implant to increase its roughness does not compromise its biocompatibility but enhances the total area available for integration with bone [2, 3]. The surfaces of most modern dental implants are microtextured, to support and enhance osseointegration [4].

A range of methods have been used to achieve modification of the milled surface. Treatments such as titanium plasma-spraying, grit-blasting, acid-etching, and anodization create a favorable roughened, high-energy surface, which aid in the process of osseointegration [4]. The roughness of most current implant surfaces created using these methods ranges from 0.5 to 2 μm [3]. Newer surface modifications involving treatment using sulfuric acid and hydrogen peroxide can create nanoscale roughness, while technologies such as micro-arc oxidation can create nanostructured bioactive titanium oxide layers to enhance cell attachment and adhesion onto the dental implant surface. Examples of typical implant surfaces are shown in **Figure 2**. These patterns are superimposed onto a variety of different types of thread patterns (**Figure 2 A , B**). Regions which have only been milled, such as the uppermost collar region, show typical lathe marks on the surface (**Figure 2 C , J**), whereas the thread regions have microtextured surfaces.

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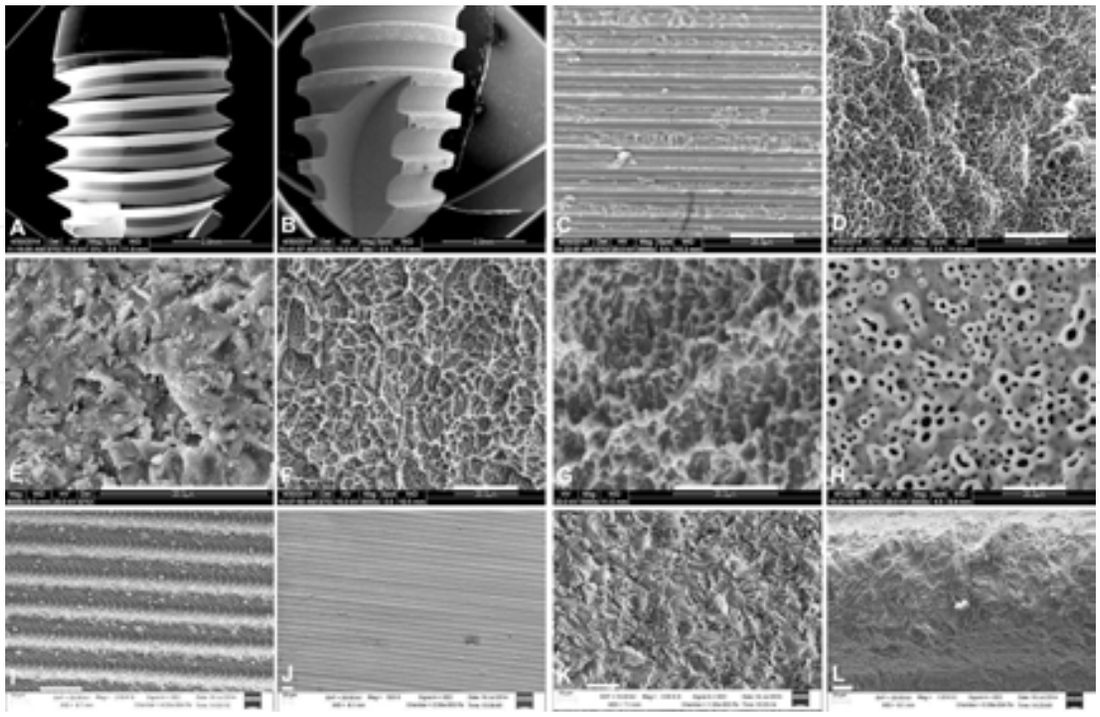

Figure 2. Scanning electron microscope images of dental implants. (A) The collar (upper) and thread regions of a 3i Biomet implant. (B) The lower end of an Ankylos implant. Note the difference in thread patterns compared to (A). (C – L) Implant surfaces of various implant brands. White scale bars represent 20 microns. (C) Non-textured surface showing lathe cutting marks (3i Biomet). (D) MIS, (E) Neoss, (F) Ankylos, (G) MID, (H) Nobel Biocare, (I) Biohorizons, (J) Southern (non-textured surface), (K) Southern ITC, (L) Southern (collar region).

3. Biofilm development on implant surfaces

The roughened implant surface which assists in achieving integration of the implant with the bones of the jaws provides an exceptionally favorable microenvironment for biofilm formation, when the surface comes into contact with saliva [2]. In vivo studies have shown that the extent of bacterial colonization of roughened Ti surfaces is greater than that of smooth surfaces [5]. Moreover, the extent of bacterial adhesion has been shown to correlate directly with the extent of surface roughness [5]. Several authors have shown that methods which increase surface roughness resulted in enhanced attachment of bacteria [6, 7]. Biofilms then develop quickly and mature rapidly, nourished by nutrients from the host, both through saliva and through gingival crevicular fluid and blood. The latter is found as a consequence of the development of inflammatory reactions in the adjacent host tissues and contributes to the growth of Gram-negative species, which utilize iron and porphyrins in their normal metabolic pathways.

Bacteria from the oral cavity readily adhere to the surfaces of dental implants, and mature biofilms develop over several days. Such biofilms can be seen on the surfaces of implants removed because of clinical failure from peri-implant inflammation, and are identical to those

which can be developed under laboratory conditions using human saliva as the sole inoculum (**Figure 3**).

Once a biofilm has become established on an implant surface, conventional methods of debridement are not effective for its removal [8]. An implant surface which is positioned below the position of the oral soft tissues cannot be reached with the bristles of a conventional toothbrush, as these only penetrate 0.5 mm into crevices around teeth and dental implants. Likewise, products used in the mouth such as mouthwashes only penetrate to a similar extent.

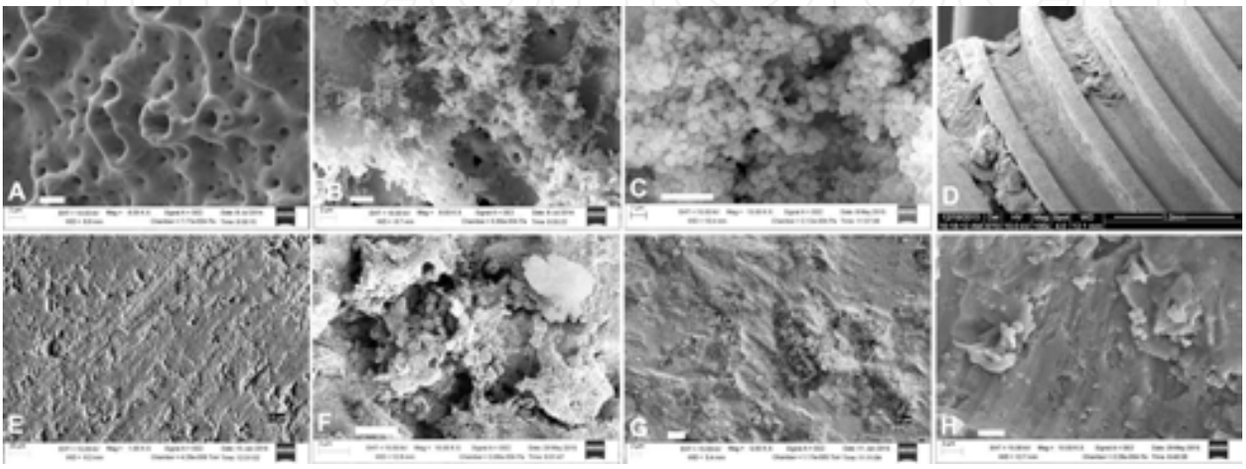

Figure 3. Rapid development of bacterial biofilm on dental implant surfaces. Scale bars in parts (A–C) and (E –G) represent 2 microns. (A) Pristine surface of a new Nobel Biocare implant. (B) The same surface after 4 days incubation in brain heart infusion broth inoculated with human saliva. (C) Four days biofilm on the surface of a Southern implant from a human saliva inoculum, which shows similar characteristics to image (B). (D) Biofilm between the threads of an implant which was removed from the mouth because of peri-implantitis, which had led to bone loss and eventual failure of the implant. (E) Damage to an abraded titanium surface (Southern implant) caused by an ultrasonic scaler with a metal tip used for 60 s to remove biofilm. Compare to **Figure 2K** which is the undamaged surface at the same magnification. (F–H) High power views showing bacteria still present and surface damage (flattening of irregularities) on different types of micro-roughened titanium surface after using an ultrasonic scaler.

4. Biofilm-induced peri-implant diseases

Peri-implant inflammatory diseases caused by biofilm accumulation on implant surfaces may segregated into two forms: peri-implant mucositis (PIM) and peri-implantitis (PI). These differ in the extent to which the inflammatory reaction of the host immune response extends to involve the bone surrounding the implant [9–11]. In PIM, inflammation is confined to the soft tissues surrounding the dental implant, and there is no progressive loss of supporting bone over time [10]. In peri-implantitis (PI), the biofilm-induced inflammatory process causes both changes in the soft tissues as well as progressive loss of the supporting bone [12]. Both PIM and PI are relatively common. The most recent systematic review conducted in 2015 estimated that the prevalence to be in the range of 19–65% for both PIM and PI [13]. This is consistent with recent longitudinal studies conducted in communities, where dental implant treatments are very commonly performed as the standard of care for single missing teeth [14].

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At both the microbial and immunohistological levels, there are numerous similarities between PIM and gingivitis, and likewise between PI and periodontitis. The microbial flora is dominated by Gram-negative species, with smaller numbers of streptococci and other Gram positive bacteria. The profile is similar to biofilms, which develop on implant surface sunder laboratory conditions (**Table 1**). Key pathogens which have been implicated in PI include anaerobic Gram-negative rods, motile organisms, and spirochetes which numerically are present at low levels in these biofilms [15, 16]. A point of difference between biofilm formation on implant surfaces versus teeth is that Staphylococci (particularly *Staphylococcus aureus*) and yeasts can be found in biofilms on implant surfaces, whereas these rarely occur in biofilms associated with teeth [16, 17].

Neisseria and other Gram -negative species	79%
Streptococci	7.2%
Bacilli	4.6%
Veillonella	3.5%
Gemella	1.3%
Porphyromonas	0.5%
Actinomyces	0.3%
Peptostreptococci	0.1%
Fusobacteria	0.1%

Major groupings in a typical 96 h multispecies biofilm grown on Southern dental implant surfaces from a human salivary inoculum, showing major groups according to next-gen sequencing analysis.

Table 1. Dominant organisms in the taxonomic analysis of biofilm on dental implants.

Dental implants are increasingly utilized in the restoration of partially dentate or fully edentulous patients. This raises issues of pathogens being transferred from sites with periodontitis to the surfaces of implants as a vector for infection of implant surfaces [18]. Demand for dental implants for single-tooth replacement has been driven by their lower biological cost than conventional dental bridges, and better long-term outcomes, since implants, unlike teeth, are not affected by dental caries and its complications. When failures with implant treatment occur, these may be classified as being either early or late, reflecting surgical or mechanical factors in the former, and biological factors in the latter [19]. PI accounts for most of the late failures, since the biofilm-induced inflammatory reaction causes extensive cratering of the bone, making the implant unsuitable for supporting a crown or other prosthesis [20].

The seminal work of Lang et al. [21] documented how inflammation in the implant-mucosal unit (i.e., PIM) can, in susceptible patients when biofilms are allowed to accumulate for prolonged periods of time, progress from PIM to PI, with accompanying loss of circumferential bone. This conversion is not merely an expansion in volume of the host immune response to the biofilm but represents fundamental changes in the composition of the biofilm

(such as emergence of different pathogens in a cyclical pattern) and accompanying shifts in the composition of immune cells present in the tissues and their behavior, particularly their production of inflammatory cytokines and mediators which alter host tissues, such as proteases. These add to the effects of proteases of bacterial origin, especially those produced by *Porphyromonas* species. As expected, an effective treatment would address the fundamental driving factors within the host response to the biofilm which accumulates on the surfaces of implants by removing the microorganisms and their products [22]. Reducing the biofilm volume and changing its composition should then reduce the intensity of the inflammatory response and alter its character so that the destruction of tissue no longer outweighs formation of tissue [23].

As biofilms develop on implant surfaces, the appearance of key pathogens such as Porphyromonas species is a relatively early event. As seen in **Table 1**, such organisms can be present in saliva and can reach significant levels in the biofilms which form on dental implant surfaces after a period of several days. Quirynen et al. [24] followed the colonization of newly placed implants by bacteria, took samples of the microbiota, and examined these using checkerboard DNA–DNA hybridization, cultural techniques, and by real-time polymerase chain reaction (RT–PCR). They found that bacterial species associated with periodontitis can be detected in peri-implant pockets as early as 2 weeks after implant placement.

The risk factors for peri-implant diseases are strikingly similar to the known predisposing and modifying factors for periodontal diseases. Various prospective and retrospective analyses have shown that the systemic health of the host (e.g., type II diabetes mellitus) [25], genetic traits [26], environmental factors (e.g., smoking) [27, 28], a past history of periodontitis [28], poor compliance with mechanical cleaning recommendations [27], and infrequent dental maintenance visits [29] is major risk factors for the development of peri-implantitis.

Adding to this, there are significant effects of the brand of the implant used, which reflects the different surface topography on which the biofilm will form [29–32], as illustrated in **Figure 2**. For implants with PI, on average around 30% of the bone surrounding, the implant had been lost. Several studies have reported that over periods ranging from 1 to 20 years, the prevalence of bone loss can vary from 27.8 to 47% of patients [30–32]. There is a clear message from such studies that if PI which is left untreated is a strong predictor of future implant loss.

5. The complexities of biofilm removal and implant debridement

While different protocols for professional care of dental implants have been suggested, it is unclear at present which is the most effective [33]. Traditional dental treatment modalities, such as the removal of biofilms using scaling instruments originally designed for debriding the roots of teeth to remove such deposits, cannot be applied in exactly the same way to threaded implant surfaces [8]. The implant surface structure has far more areas which are protected, and much of the surface is inaccessible to conventional professional instruments. Conventional dental therapies such as and scaling instruments and ultrasonic scalers been shown to have minimal effectiveness for removing biofilm and eradicating pathogens from implant surfaces through mechanical means [34].

Although there are many studies of peri-implantitis treatments, including randomized controlled clinical trials, the latest Cochrane systematic review conducted in 2012 concluded that based on current evidence, no particular treatment can be established as a gold standard approach for the treatment of peri-implantitis [33].

In dental clinical practice, chairside methods to assess the levels of key pathogens present in biofilms on the surfaces of implants or teeth do not exist, and sampling followed by genetic analysis is too expensive for routine use. Therefore, the approach taken follows a nonspecific approach, namely the removal of all biofilm from the surface, regardless of the pathogenicity of bacterial species growing within it. Since the treatment of peri-implantitis aims to also achieve re-osseointegration of the implant surface with bone, it is necessary to remove not only all viable bacteria but all traces of bacterial products such as endotoxins, in order to maximize the likelihood of success.

Treatment of peri-implantitis needs to be implemented as early as the problem is diagnosed, since the likelihood of implant failure due to PI is reduced significantly when the condition is detected early so that treatment can be instituted [35]. Such treatment involves decontamination of the implant surface, as well as surgical augmentation of the associated bony defects [36]. The desired goal of achieving re-osseointegration of the implant after decontamination, despite the use of guided bone regeneration (GBR) with or without bone grafting, is regarded as either difficult or impossible to achieve [37]. The reasons include the challenges of biofilm removal from the surface of the implant, alterations of the implant surface caused by the cleaning procedure used.

6. Methods which have attempted to clean implant surfaces

Implant surfaces are notoriously difficult to clean [38, 39]. The difficulty in cleaning the surfaces of titanium dental implants lies in the complex topography of the implant surface, as is readily apparent at high magnifications such as those used in **Figure 2**. Most implants have threads at the macro-level (e.g., **Figure 2 A , B**), which impede with the action of hand scalers and ultrasonic scalers, so that they only touch the outer parts of the threads but do not reach areas between the threads. On the microscopic level, the highly roughened surfaces mean that there is a large surface area. The microscopic roughness of the surface is a major obstacle for the removal of bacteria and their products [38], as these types of surfaces defy effective debridement by mechanical means alone [39].

The various conventional methods that have been examined for biofilm removal from implant surfaces include ultrasonic scalers fitted with various types of tips, hand periodontal curettes with steel, titanium, plastic or Teflon® tips, abrasive and polishing rubber cups and brushes, and particle beam (air powder abrasion) devices [40]. Most or all of these are found in a modern dental office. Ultrasonic scalers used with metallic tips and stainless steel hand scalers damage and scratch the surfaces of titanium implants [40–42], and for this reason, their use is contraindicated [41]. Furthermore, plastic hand scalers leave residual scaler material on implant surfaces during use [43, 44]. Examples of typical damage to surfaces from ultrasonic

scalers are shown in **Figure 3** (panels E–H), which also show residual bacteria and biofilm matrix which persist on the surface despite intense professional cleaning. No conventional cleaning method will remove all traces of biofilm from microscopically rough titanium surfaces, and the careless application of stainless steel instruments will damage the surface and encourage further biofilm growth.

Some implant designs use a smooth collar near the attachment point for the overlying crown, which is designed to be easier to clean by toothbrushing. Hand and ultrasonic scalers can readily damage this smooth surface, with the resultant scratches promoting the growth of biofilms in the supragingival areas. As this matures, it can track down the protected areas of the grooves and scratches created by dental instruments to penetrate into the subgingival environment, where it can then become established on the threads, leading to peri-implantitis. For this reason, plastic curettes and rubber polishing cups are recommended for the removal of plaque from smooth implant collars, rather than metal instruments of any type [40, 42, 45].

Within the group of conventional instruments, particle beam or air-powder abrasive methods have been shown to provide the most effective cleaning option to date [46]. The range of available particles for such devices includes aluminum oxide, calcium carbonate, sodium bicarbonate, and glycine. Several manufacturers have fabricated tip designed to apply the particle beam into subgingival implant surfaces; however, the pattern of the threads causes many regions on the surface to be protected. It must be recognized that air-powder abrasion causes undesirable microscopic alterations of titanium implant surfaces, and so are not ideal [47].

6.1. Cavitation-based approaches

Ultrasonic scalers have been used in dental practice for the removal of dental biofilms on the root surfaces of teeth [48, 49]. Modern ultrasonic scalers fall into two main categories: piezoelectric and magneto-strictive devices. A part of their cleaning action is through vibrational energy, which shatters any calcified hard deposits. Only the tip of the ultrasonic is considered active; thereby, effective debridement is limited by how much contact the tip has with the surface area of the tooth [48]. Traditional ultrasonic inserts are made from stainless steel, and these damage implant surfaces through a mechanical vibrating contact action. Typical patterns of surface damage are shown in **Figure 3**.

Ultrasonic scalers also create cavitation, with the resultant shock waves from explosions and implosions disrupting bacterial cell walls. The accompanying stream of irrigant water both cools the tip and introduces air and thereby oxygen to the area. The movement of fluid can help remove endotoxins [49]. A number of manufacturers have released the so-called "implant safe" ultrasonic tip inserts for use in both implant maintenance and for the treatment of peri-implantitis. These tips are usually made of carbon fiber, titanium, Teflon®, graphite, or plastic. A number of studies have demonstrated that ultrasonic tips designed for implant maintenance do not cause significant damage at the macroscopic level [50–52]. Paradoxically, some investigators have proposed the use of instruments that deliberately flatten the microscopically rough implant surface to reduce its roughness and thereby area

available for the attachment of bacteria [53, 54]. This is certainly a compromise since the original goal has always been to remove biofilm without causing any surface modifications.

In terms of clinical outcomes, Karring et al. treated 11 patients diagnosed with PI and cleaned the implants either with an ultrasonic scaler or plastic hand scalers. They found no clinically relevant difference in the outcomes obtained [55]. No instrumentation applied by the dentist can resolve peri-implantitis (or periodontitis) if the oral hygiene of the patient remains poor, and bleeding scores remain high, which indicates persisting biofilms and persistent inflammation. Despite the advent of new ultrasonic scaler inserts made of titanium, plastic, or graphite, the general consensus in the literature is that ultrasonic scalers have the same fundamental limitations as hand instruments in that cannot access the undercuts of the implant found between the threads [56]. Their zealous use causes surface alternations [52, 57]. Moreover, the treated surface is not yet biologically compatible, since biofilm and endotoxins remain [58].

6.2. Particle beam systems for the removal of biofilm

Particle beam (air polishing /air abrasion) units have been marketed for the treatment of the roots of teeth affected by periodontal disease, because of their ability to disrupt biofilms [59], while causing little damage to the roots of teeth or the adjacent oral soft tissues [60]. They are well suited for repeated use at the same site, in contrast to hand instruments which when used repeatedly on the same tooth can cause significant removal of tooth structure from the root surface [60, 61].

The principle behind these particle beam devices is that steady flow of compressed air accelerates abrasive particles, which then impact on the tooth surface and fracture or abrade away deposits, including biofilms and external stains [62]. Ideally, the powder used should not damage the target and preferably would also exert some modest antibacterial actions [60]. A number of manufacturers now produce particle beam devices and powders for different periodontal applications. The tip designs vary according to the mode of clinical application (supragingival or subgingival tip) since these require different angulations for applying the particle beam at the appropriate working distance from the surface being cleaned. The powders available vary in particle size, shape, composition, and density [62], and include sodium bicarbonate, calcium carbonate, bioactive glass, pumice, and glycine [61]. The choice of powder type and the application method used both influence the effectiveness of biofilm removal and the potential for tissue harm [60]. Logically one would want to avoid powders which are harder than grade 4 or 5 titanium, as these could damage the surface and roughen it even more, enhancing the problems caused by the attachment and growth of bacteria [63]. This is exactly the same issue as discussed earlier for stainless steel instruments such as ultrasonic scalers and hand scalers, which will damage titanium implant surfaces [64].

The first study of implant surface debridement using a particle beam approach was undertaken by Barnes et al. [65], who used four different implant systems and exposed samples on the bench to particle beams for 0.5 s up to 10 s. Using scanning electron microscopy (SEM), no major differences between the surfaces were found. Since that time, numerous studies have examined the effects of particle beams on implant surfaces. Most in vitro studies and narra-

tive reviews based on these have concluded that particle beams are a safe treatment for decontaminating a titanium implant surface without causing major modifications to the surface [46, 52, 66]. The extent of surface damage is influenced strongly by the choice of abrasive powder [46], with sodium bicarbonate and aluminum oxide (alumina) powders being more likely to damage the implant surface than glycine [46]. There has been emerging support in the most recent literature for the use of glycine powder as the particle material of choice, due to it exerting bacteriostatic actions when used at a 10% concentration [67], having a low risk of air emphysema [68], and causing less damage to implant surfaces than sodium bicarbonate.

6.3. Laser-based methods

As discussed above, the complete removal of biofilm from titanium implants has proved elusive to date. Traditional dental instruments used to debride root surfaces have proved particularly ineffective [69]. Lasers have been suggested as an alternative means of decontaminating dental implants [39, 70], with some studies using Er:YAG lasers showing nearly complete removal bacteria and debris from titanium surfaces [36, 38]. The logic behind using lasers relates to the various photothermal bactericidal effects of lasers as well as their ability to create photomechanical effects such as cavitation when used in a way that generates cavitation in water [71]. The three-dimensional effects created by the scatter of laser energy, when combined with the shear forces generated by cavitation from a static laser tip would seem to be a very promising approach. The scatter of laser energy from a microscopically rough surface would enhance the extent of photothermal disinfection achieved, so that under certain conditions laser treatment could render the implant surface not only decontaminated but also sterile. This stands in marked contrast to the effects of hand or ultrasonic scalers or particle beam devices, none of which can produce a sterile surface [72]. Likewise, decontamination and detoxification of a titanium implant surface cannot be achieved with hand curettes alone [36]. In contrast, with a laser, the ability to decontaminate the implant is limited primarily by the degree of access that the laser energy has to affected implant surfaces. The choice of system used to deliver laser light then becomes an important consideration, with aspects such as the physical size and light distribution properties of the sapphire tips, glass, and non-glass optic fibers, or hollow waveguides used to deliver laser light having an effect [73].

The biocompatibility of a laser-treated surface must also be considered. Guided bone regeneration or bone grafting may be used to treat peri-implant bone loss; however, these surgical techniques both require a meticulously clean implant surface in order to achieve a good outcome [74]. Romanos et al. [75] established that cell attachment and morphology after laser irradiation is equal to that of sterile implant surfaces. Kreisler et al. [47] examined the biocompatibility of contaminated implant surfaces after treatment with either a particle beam device or the Er:YAG laser. The lowest cell growth and proliferation was seen for contaminated Ti surfaces, while cell growth was significantly greater on sterile (new), air powder-treated, and Er:YAG laser-cleaned surfaces.

Infrared lasers can exert powerful photothermal effects which can inactivate or destroy bacteria. The highly water absorbing far infrared energy from a carbon dioxide laser has potential application for the destruction of bacteria. Deppe et al. [36] found that the carbon

dioxide laser when used for disinfection gave faster initial healing than conventional methods. Nevertheless, the long-term outcomes were not significantly different, particularly when bone levels were compared after 4 years. The authors of this study also pointed out that the shape of the defect could have prevented the perpendicular delivery of laser energy and that optimal therapy with this laser when used for disinfection would require changes to the delivery system of the laser to make laterally emitting or side firing. They also noted that bleeding from the surgical site during the procedure would have reduced the amount of laser energy reaching the implant surface, and this attenuation by water absorption may have put the actual levels of energy reaching the implant surface well below those required for sterilization.

As well as the Er:YAG and carbon dioxide lasers already mentioned, other lasers have been found to be of benefit in the treatment of peri-implantitis. Bach et al. [89] found that near infrared diode laser irradiation reduced the rate of recurrence of peri-implantitis to only 7%, most likely because of the disinfecting action of this laser. Likewise, several wavelengths of laser light have been shown to impede the progression of bone resorption in peri-implantitis treatment regimens [77–80].

6.4. Guided Er: YAG and Er,Cr:YSGG lasers for implant surface decontamination

Sterilization and cleaning of implant surfaces by infrared lasers has been demonstrated in several reports [39, 70, 81], and surface decontamination has been reported for both CO_2 and Er:YAG lasers. Bone has been found to reattach to implants after infrared laser irradiation in a peri-implantitis models in dogs, suggesting that laser treatment leaves a biocompatible surface [78].

A key issue is that while laser irradiation can rapidly reduce the bacterial load on an implant surface, it may not be able to render the surface sterile in all circumstances, depending on the geometry of how the laser light interacts with the biofilm on the implant surface. The ability of laser irradiation to reduce bacterial viability is influenced by the implant surface roughness. Kreisler et al. [74] found greater bacterial killing for laser energy delivered at right angles to the surface for microbial deposits on smooth surfaces, and lower effectiveness for those on rough surfaces. They also showed that intensity (power density) strongly influences the disinfecting action.

In order to optimize the effect of laser energy, it is important to achieve a side-firing effect so that laser light applied using a fiber which is parallel to the long axis of the implant is directed onto the implant surface at an optimal angle. Simplistically, one could consider this angle 90 ° to the surface; however, the presence of micro- or nano- roughness on surfaces means that a spread of angles should be even more effective. Depending on the light wavelengths used, such the optical fibers used to deliver energy to the side of a dental implant may be plain glass, glass which has been modified with fluoride, germanium, or other dopants to enhance infrared light transmission, or rare earth element compounds such as germanium or gallium oxides. The latter are used with middle infrared lasers (Er:YAG and Er,Cr:YSGG).

Fibers with plain 90 ° ends (from a right angle cleave of the fiber) emit light with a typical divergence of 18°–20° . Cone-shaped, periscope, and other specialized applicators have been developed for the ends of optical fibers, to make them have enhanced side-firing actions [73, 76]. An alternative approach is to modify the end of the fiber itself, through various physical processes such as acid etching and particle abrasion [73]. Using such methods, it is possible to create radial-firing tips with cone-shaped ends to provide a broader pattern of light collection and emission than a right angle cleaved end. The most interesting modifications to the surfaces of glass and ceramic optical fibers involve the combination of various processes including tube etching, particle abrasion, and further etching, which creates unique surface architectures known as the "honeycomb" surface, to increase transmission and collection of visible red and infrared light [73, 76]. Various modifications of the parameters used for this technique are required for doped glass fibers (e.g., a longer primary etch stage for fluoride-doped glass), or for fibers containing germanium.

The applications of such honeycomb surfaces include broad lateral dispersion of visible red light as well as near and middle infrared light, for photodynamic and photothermal disinfection of subgingival areas and confined spaces, including biofilms present inside the root canals of teeth. This type of optical fiber technology also reduces thermal stress in adjacent hard and soft tissues [77]. It can also be used for fluorescence detection of biofilms on complex surfaces, including those which are only several cell layers thick, and of free-floating planktonic bacteria [78–81]. There is potential application for the automated detection and removal of biofilms from implant surfaces [82–84]. The value of laser fluorescence systems for detecting subgingival deposits on the roots of teeth is well established, even for those which have become calcified to become subgingival dental calculus [85]. The debriding action to remove biofilms then comes from the ability of the laser to generate cavitation in a water irrigant or water-based fluid. Various optical fiber modifications can enhance dramatically fluid agitation for cleaning complex surfaces and spaces, which are difficult to access [86, 87].

6.5. Laser-induced damage to implant surfaces

An important issue to consider with lasers is whether irradiation causes adverse changes to the implant surface [70]. One would expect that higher peak powers would cause greater alterations, and this has been shown for CO_2 lasers, which can cause undesirable implant surface alterations when used in the super-pulsed mode (when there are very high peak powers), but less damage occurs when the same laser is used in continuous wave mode [82]. Likewise, the Er:YAG laser, which normally operates in free running pulsed mode, can cause damage to titanium surfaces when used at very high peak power settings [45, 88]. Such areas have a melted volcanic appearance, which contrasts with the adjacent surface (**Figure 4**). For this reason, laser parameters such as peak power must be kept below the point where melting or surface ablation of titanium occurs, and water flow rates must be sufficient to minimize effects of plasma formation.

A further issue when using a powerful laser is the possibility of adverse thermal effects on bone. If the laser energy is absorbed strongly into titanium, not only can the surface be damaged, but the heat generated can be transferred to the adjacent bone [70, 71]. For safe

clinical use, the temperature elevation which occurs in the peri-implant bone as a result of laser irradiation should be <10° C, since bone temperatures of 47° C or above may result in bone necrosis [83]. Using fibers and tips, which emit laser energy in a side-firing manner, lowers the total irradiance of the bone, while still achieving even irradiation along the length of the exposed threads fiber. Low average powers will also preserve the morphological and chemical characteristics, which provide titanium with its excellent biocompatibility.

In summary, the concept of using lasers to treat implant surfaces holds considerable promise, yet certain technical issues remain to be addressed, including controlling the laser effect (for example, through fluorescence feedback), achieving the correct geometry for delivery of laser energy (such as using side-firing fiber tips), and controlling undesirable thermal effects on the titanium surface and on the adjacent supporting bone [73–75]. The laser-treated surfaces have high biocompatibility, and this is reflected in the clinical studies that have been undertaken to date and produced promising results [89–95].

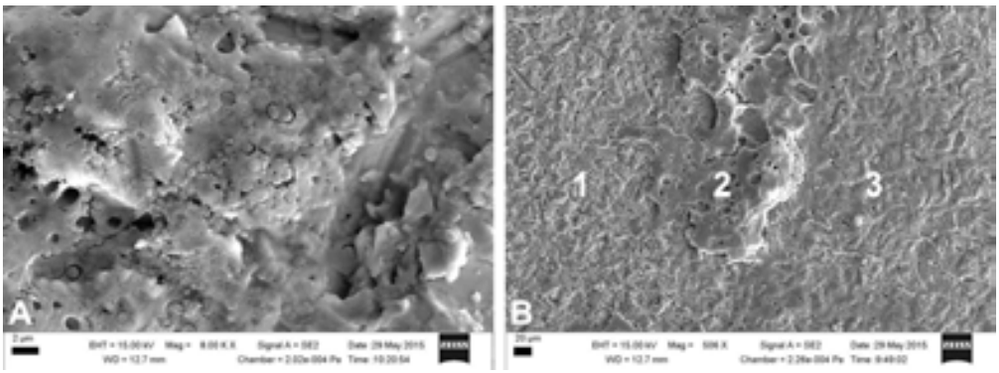

Figure 4. (A) Laser debridement. Biofilm growing on micro-rough abraded titanium surface from a saliva inoculum after 4 days, prior to laser treatment. The scale bar represents 2 microns. Individual bacteria are embedded into a dense matrix. (B) The surface after application of 120 mJ Er:YAG laser pulses with a fine mist water spray. The original abraded surface can now be seen (1), as well as a large central area where the titanium surface has been melted by laser pulses (2), and regions with remaining biofilm which have not yet been treated (3).

7. Laboratory models for assessing biofilm removal from implant surfaces

One of the most informative ways to assess how well a particular method can clean biofilm from an implant surface is to use a physical material which replicates the adhesive nature of biofilm, covers the surface at the microscopic level, and requires a similar process for its removal. The model which fulfils these three requirements involves the application of permanent marker ink of a certain type. The ink model was first described by Sahrmann et al. [96] . In our laboratory, when the same model is used, an abutment is attached to each implant so it can be handled without touching the surface, and the implants are dip-coated in a cyan blue indelible ink (Sharpie Fine Point Permanent Marker, Sanford L.P., Illinois, USA). This ink forms a uniform, visually detectable biofilm-like layer over the implant surface and penetrates well to cover fully the regions between the threads. The implants are inspected

under a light microscope to confirm an even distribution of ink over the implant surface. Each implant is subsequently mounted in an acrylic resin block (Sawbones, Pacific Research Laboratories, Washington USA) prepared with a 6- mm-deep, circumscribed saucer-shaped defect at 60° to simulate the bony defects found in sites of peri-implantitis environment. Implants are fixed into the Sawbones by screwing them in with two revolutions, to the desired position, typically so the third thread of the implant is level with the base of the intra-bony defect. After applying particular treatments, ink removal is then assessed by analysis of the area of ink remaining [96, 97]. We have developed a special system to photograph the ink distribution across the surface, which involves digitally stitching images from macro-photography so that the sharply in focus regions are combined into one image which shows the entire surface. This is suitable for quantitative analyses of the area of ink removed (**Figure 5**). At the microscopic level, the ink be identified using SEM in backscatter mode as its low atomic number signal appears dark which contrasts well with the higher atomic number signal from the underlying titanium.

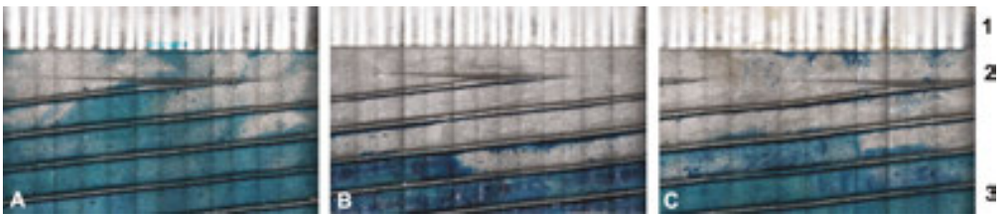

Figure 5. Ink model of biofilm removal from implant surfaces using blue ink placed onto 10- mm-long 4 mm-diameter Southern implants (ITC 410) fixed into Sawbone with peri-implant defects. Zone 1 represents the implant upper collar, and Zone 2 represents the area, where the ink has been removed and the underlying grey implant surface can be seen, and Zone 3 is the ink, which has remained in the deeper regions of the defect. Three different treatments have been applied in an attempt to clean the surface. (A) Ultrasonic scaler for 15 s. (B) Ultrasonic scaler for 120 s. (C) Particle beam device with glycine powder for 30 s. Note the persistence of dye on the areas which are shadowed by the threads, while the adjacent easily accessible areas have little dye. No treatment has reached to the base of the defect.

7.1. Mixed biofilm models

Biofilms which grow on implant surfaces contain multiple species, which are derived from the approximately 700 species of bacteria which are found in the saliva. These bacteria form a complex multispecies microorganism community in the biofilm, along with fungi such as *Candida albicans*. Using single species models in the laboratory cannot replicate the complexity or the biofilms which form in the clinical situation. Some laboratory studies have developed a mature anaerobic biofilms from multiple strains of known primary, secondary, and tertiary colonizers enriched in a high protein broth [98–100]; however, a major limitation in such studies is that the biofilms have been grown on flat hydroxyapatite (HAP) discs.

To ensure that a biofilm is established with features more like those found in vivo, we have developed complex multispecies biofilms on titanium disks with surface micro-roughness, and on dental implants under laboratory conditions (**Figures 3 B, C,** and **4A**). For this purpose, we have used human stimulated saliva to inoculate a broth of brain heart infusion (BHI) medium enriched with 5% defibrinated sheep or horse blood and 1 mg/mL menadione, which

is then kept under anaerobic conditions (0% O_2, 20% CO_2, and 80% N_2) at 37°C. This medium is rich in protein and hemoglobin in order to encourage the growth of facultative and obligate anaerobes. The saliva is collected from healthy adult subjects who have refrained from toothbrushing and other oral hygiene practices for 12 h prior to the collection of stimulated saliva, collected whilst chewing on sterile paraffin wax for 5 min. The incubation times of 72–96 h which we have used in these studies are the same as those used by Sánchez et al. [100] in their studies of the growth of pure species using the same BHI growth medium . Their work showed that by 12 h the early colonizers had adhered, the intermediate colonizers appeared at 24 h, the late colonizers were found after 48 h, and the biofilm reached a steady state between 72 and 92 h after initiation. Therefore, this model using BHI supports the development of a biofilm that is similar in composition and structure to a subgingival biofilm in vivo.

A key aspect of the process of biofilm formation is the deposition of a glycoprotein pellicle layer by the adsorption of salivary glycoproteins onto the pristine titanium surface before it is placed into the broth. Surfaces of titanium discs are abraded with alumina particle beams and then steam sterilized before being placed into the collected saliva for 5 min, to allow a pellicle layer to form. The same process is undertaken for titanium implants. The discs or implants are then placed into the BHI broth and incubated under anaerobic conditions. The resulting biofilms on the discs and implants can then be treated with various methods, and the extent of remaining biofilm assessed using vital staining with confocal microscopy, or scanning electron microscopy. For the latter, an appropriate fixation regimen involves 24 h in 10% neutral buffered formalin solution, followed by rinsing in 0.1 M cacodylate buffer solution for 30 min, and then post-fixing in osmium tetroxide for 1 h. The fixed samples can then be dehydrated with graded ethanol solutions (50–100%), dried, and placed onto aluminum stubs using conductive carbon tabs, and sputter coated with a 10- nm-gold layer, prior to being viewed using secondary electron emission or backscatter modes under high vacuum conditions.

7.2. In situ models

We have also developed an in situ model of biofilm formation on implants, using a specialized removable oral appliance [101]. The rationale behind this work is that past studies of implant biofilms have been laboratory based and have used only single species biofilms of oral bacteria. They have little or no direct relevance to clinical patient care. It was desirable to have a reproducible in situ model with naturally formed complex biofilms of mixed species, which should form under low oxygen conditions in an environment which is partially protected from the washing action of saliva, but able to access nutrients from the saliva. There should be contact with normal host protective mechanisms such as the gingival crevicular fluid produced around the gingivae. To meet these objectives, a removable appliance was designed which uses a removable dental bleaching tray as its base. This appliance carries an implant on its side, which is located within a tube and held against the oral soft tissues beside the gingival crevice (**Figure 6**). Using this model, we have generated realistic biofilms on dental implants in 48 h and then used these to test the effectiveness of various debridement methods. Other groups have likewise developed methods for developing dental plaque on implants using in

situ appliances [102–104], and this will likely be a productive approach for future studies. A particular advantage of our own system is that it can use both a flat titanium disc and an actual commercial titanium implant, whereas other models use flat titanium discs. While flat surfaces are easier to both clean and analyze, they lack features such as threads which make them hard to clean.

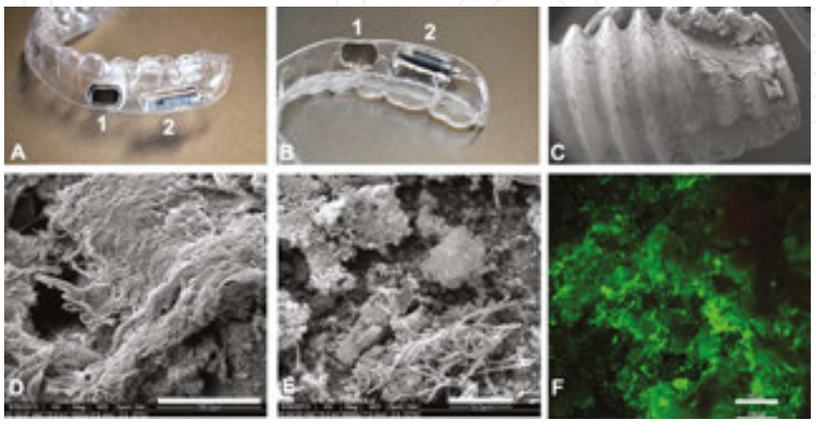

Figure 6. Removable dental appliance for the development of biofilms on dental implants. (A , B) The appliance design showing the flat titanium disc (1) and the titanium dental implant (2) mounted into a vacuum-formed removable appliance. (C–E) Low, medium, and high power SEM views of a 48 h biofilm on the implant in the in situ model. Scale bars in panels (D) and (E) are 100 and 10 microns, respectively. (F) Vital staining of biofilm grown on a flat titanium surface, using confocal microscopy.

8. Conclusions

The complex surface properties of titanium dental implants which give them excellent biocompatibility also facilitate the attachment of bacteria and the development of biofilms. The macroscale and microscale topographies of threaded implants make these difficult to clean with conventional dental instruments. Technologies such as particle beams and pulsed lasers appear promising in terms of better biofilm removal from surfaces. The development of various clinical and laboratory models for dental implant biofilms allows the systematic comparison of different approaches to biofilm removal.

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