4 In Vitro and In Vivo Models to Understand Biofilm Implant Infections

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4.1 Introduction

Improvements in oral health rely on the development and implementation of new ideas and approaches including behavioural interventions, drugs or medical devices such as dental implants. The translational pathway from idea to implementation is long and complex. For new drugs and medical devices, it is essential to demonstrate safety and efficacy in preclinical models before moving to clinical trials. Models can also be used to screen compound libraries and identify promising drug candidates as well as to define the mechanism of action underpinning new therapies. Highly simplified models have advantages such as ease of use, low cost, high throughput and high measurement accuracy. However, simple models do not replicate the highly complex biology of the human body. Therefore, multiple models will be needed to fully characterise a new agent. It is essential to select the most appropriate model for the research question. This chapter describes some of the key models that have been used to investigate biofilm infections on and around dental implants.

4.1.1 Key Characteristics of Peri-Implant Biofilms

A good model will replicate the biological system as closely as possible while allowing opportunities to assess the impact of interventions on different aspects of the system and providing sufficient simplification to enable detailed measurements of system parameters. Before developing a model, it is essential to consider the key characteristics of the system to be modelled. Biofilm formation on and around dental implants has been described earlier in this book. The system is highly complex in terms of both the microbiology and the surrounding environment of host tissues. The microbiome varies according to health/disease status (Table 4.1) and in all cases, there is a complex microbial biofilm present with multiple species of bacteria [1]. The biofilm is embedded in a matrix of polymers that includes extracellular DNA and polysaccharides [2, 3]. During implant healing and in peri-implant disease, the biofilm is bathed in gingival crevicular fluid and contains host inflammatory cells and cytokines [4, 5]. Environmental factors such as smoking affect the composition of the peri-implant biofilm [6, 7]. When developing a model, it is essential to consider which aspects of the

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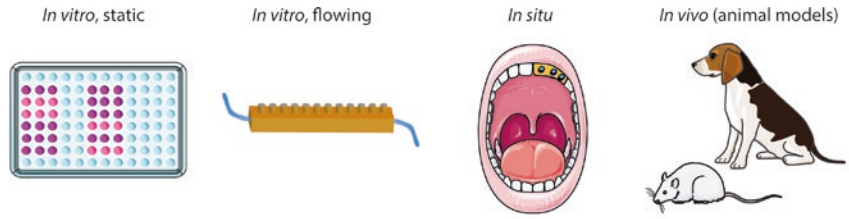
Table 4.1 Key bacterial taxa enriched in peri-implant health or disease[a]

Health	Peri-mucositis	Peri-implantitis
Neisseria spp.	*Porphyromonas* spp.	*Porphyromonas gingivalis*
Streptococcus sanguinis	*Tannerella forsythia*	*Porphyromonas endodontalis*
Streptococcus intermedius	*Treponema denticola*	*Tannerella forsythia*
Corynebacterium matruchotii	*Prevotella* spp.	*Fusobacterium nucleatum*
Rothia spp.	*Fusobacterium* spp.	*Fretibacterium fastidiosum*
Capnocytophaga spp.	*Streptococcus* spp.	*Prevotella intermedia*
Veillonella spp.	*Leptotrichia* spp.	*Treponema* spp.
Lautropia mirabilis	*Peptostreptococcaceae* XIG-5	*Filifactor alocis*
Granulicatella spp.	*Selenomonas* spp.	*Desulfobulbus* sp.
Actinomyces spp.	*Ottowia* sp.	
Lactobacillus spp.	*Lachnospiraceae* [G-3]	
	Clostridiales [F-2][G-1]	

[a]Data were extracted from the following references: [4, 8–14]



Cost	Low	Low/medium	Medium	High
Throughput	High	Medium	Low	Low
Complexity/relevance	Low	Low/medium	High	High
Interventions	Possible	Possible	Difficult*	Possible
Ethical issues	None#	None#	Discomfort/health	Animal welfare
Uses	Screening	Advanced screening	Natural biofilm analysis	Advanced testing

Fig. 4.1 Overview of models for biofilm analysis. Models need to be selected according to the intended use. Initial screening of biofilm growth or antibiofilm compounds may use high-throughput in vitro static models such as the microtitre plate system. More robust biofilms can be grown in flowing systems such as the Modified Robbins device. Biofilms grown in situ, for example, on a piece of enamel held within an intraoral stent, will most closely mimic natural dental plaque. Animal models may be required to assess toxicity or efficacy of materials including implants. *Interventions such as the application of drugs are usually not possible within in situ models, but may be applied after the biofilm is removed from the mouth. #Ethical issues for in vitro models may arise if the model incorporates body fluids such as saliva or serum. This figure includes artwork from Servier Medical Art (https://smart.servier.com/)

microbiome, host cells and tissues and environmental factors need to be incorporated.

The host tissue environment surrounding implants can only be replicated with in vivo animal models. However, there is a drive to reduce the use of animal models for ethical reasons and because findings in animals often do not replicate those in humans [15]. Appropriate in vitro models have advantages over in vivo models including reduced cost and increased reproducibility (Fig. 4.1). In situ models such as enamel chips held within stents in the mouths of volunteers can be used to replicate the growth of biofilms in the mouth. Although it is not possible to challenge these biofilms in situ with products that have not yet received regulatory approval, some models allow removal of the biofilms and assessment of responses in vitro. In general, simple in vitro models with high throughput and low cost are excellent for early-stage research such as the screening of compound libraries or biofilm formation capacity of microbes. Further characterisation requires more complex in vitro models. Due to the complexity of the implant environment, animal models are still required for a more detailed understanding of the interactions between biofilm and host.

4.1.2 In Vitro Model Systems

4.1.2.1 Static Models

The simplest model systems involve a defined inoculum such as a single species of bacteria, an inert surface and a rich medium to ensure the strong growth of the biofilm. Microtitre plates with 96 wells provide a simple, economical system for evaluating biofilm growth. Following incubation of microorganisms in the growth medium, biofilms are formed on the surface of the wells or in a ring around the air-liquid interface and can be stained with crystal violet to quantify the level of biomass formed [16]. However, it is well-recognised that stochastic variation from handling and processing samples can affect the conclusions drawn from the crystal violet biofilm assay and is therefore recommended that this is not used for detailed characterisation of biofilm formation [17]. One concern with the microtitre plate system is that biofilm formation may be affected by the settling of microbes due to gravity. To ameliorate this issue, models have been developed to grow biofilms on vertical surfaces such as pegs attached to the lid of the plate (the Calgary biofilm device) or discs of different materials suspended in clamps fitted to a custom-made lid of a 24-well plate (the active attachment biofilm model) [18, 19].

Criteria for defining medically relevant biofilms include both structural characteristics and increased recalcitrance to antimicrobial agents [20]. Staining with crystal violet provides little information about either of these. Instead, indicators of metabolic activity can be employed to understand the vitality of the biofilm. For example, tetrazolium salts such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) or 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) provide a colour change when they are reduced by metabolically active cells [21, 22]. In addition, live:dead staining can highlight specific locations where bacteria in a biofilm are inactivated by antimicrobial treatments [23]. The direct visualisation of biofilm in microwell plates requires either a relatively large well with a dipping lens or an optically clear glass bottom placed on an inverted microscope [24]. Alternatively, biofilms may be cultured on removable inserts within the microplate to facilitate microscopy. Confocal laser scanning microscopy (CLSM) has become the method of choice for biofilm visualisation since it can provide three-dimensional structural information in combination with fluorescent dyes and without any requirement for dehydrating the samples (Fig. 4.2a). Structural parameters of biofilms can then be quantified with software packages such as COMSTAT2, daime, BiofilmQ or BAIT [25–28]. However, for high-resolution images of biofilms, tools such as scanning electron microscopy (SEM) are required. In our experience, field emission SEM provides excellent structural detail of bacterial cells and matrix material (Fig. 4.2b), although it is important to note that the matrix is inevitably collapsed due to dehydration during sample processing [29]. Three-dimensional SEM approaches involving sequential imaging of slices produced by a microtome embedded in the microscope (Fig. 4.2c) or ablation of the surface by a focused ion beam can provide high-resolution structural information on microbial communities [30, 31].

4.1.2.2 Models with Fluid Flow

The major nutrient sources for oral biofilms, saliva or GCF, continuously flow into and out of the mouth. This creates additional sheer forces over the biofilms and, perhaps more importantly, leads to a continual replenishment of nutrients and removal of waste products. The incorporation of flow is a key feature of many in vitro biofilm model systems. There are three main approaches to deliver fluid flow: (i) drip feed into the system and over the biofilm samples, (ii) culture biofilms in stirred vessels, with nutrients added and removed at a constant rate and (iii) direct flow over the biofilms. Examples of each approach are shown in Fig. 4.3.

Drip feed systems are designed to culture biofilms under a thin film of liquid, replicating the conditions found on the exposed surfaces of teeth. This can be achieved simply by dripping growth medium onto the upper end of a microscope slide that has been tilted at an angle. A chamber for simultaneously culturing multiple

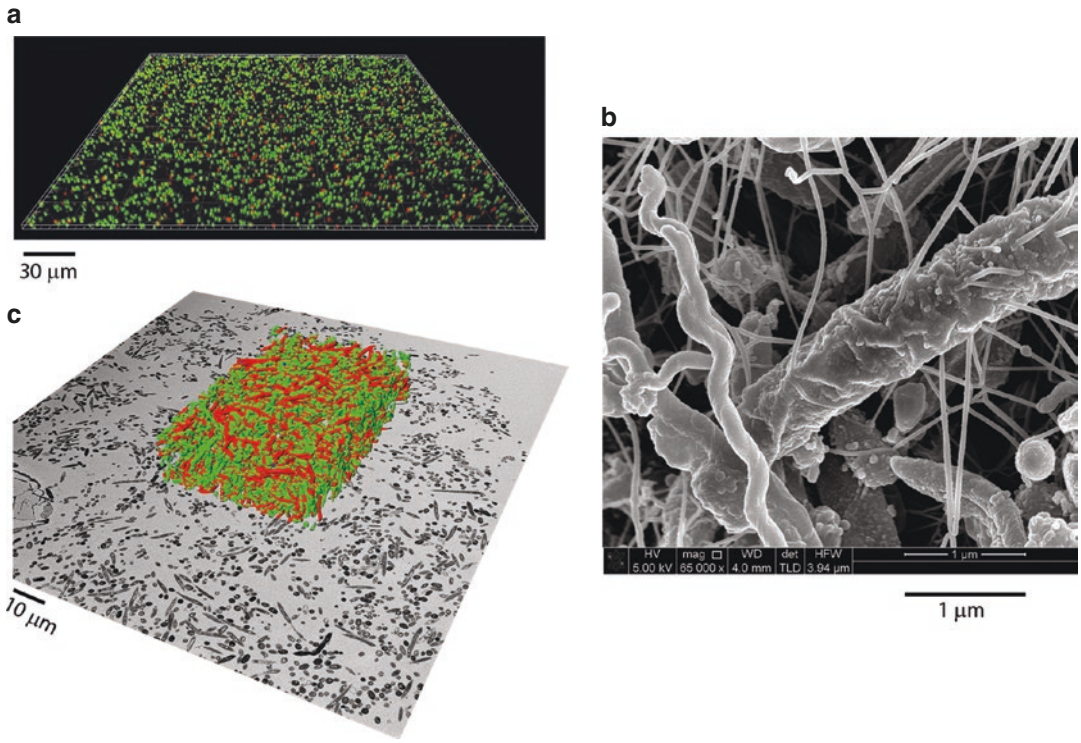


Fig. 4.2 Microscopy approaches for biofilm visualisation. (**a**) *Staphylococcus aureus* biofilm visualised by CLSM with live:dead staining. Viable cells are stained with Syto9 and appear green. Compromised cells stained with propidium iodide appear red. (**b**) Field emission SEM image of subgingival dental plaque, showing thin strings of biofilm matrix material. (**c**) Three-dimensional SEM reconstruction of a dual-species biofilm containing *Streptococcus gordonii* (artificially coloured green based on cell shape) and *Fusobacterium nucleatum* (coloured red)

biofilms has been developed and is a recognised standard test method for the quantification of *Pseudomonas aeruginosa* biofilms [32–34]. This approach has been used in oral microbiology, for example, to assess chlorhexidine tolerance in dual-species biofilms containing *Streptococcus mutans* and *Actinomyces naeslundii* [35]. The Constant Depth Film Fermenter (CDFF) also supplies nutrients by dripping them into the reactor vessel, but in this system, the biofilm samples are held horizontally (Fig. 4.3a) [36, 37]. The CDFF was originally developed by Julian Wimpenny and colleagues in the 1980s in order to culture biofilms in a steady-state and at a constant depth to provide a robust system for monitoring responses of a well-defined biofilm to perturbations [38]. Biofilm samples are held in pans that are recessed to a fixed depth (300 µm in the original design). Liquid drips into the system over paddles that scrape across the biofilm samples, which are continuously rotated by a motor underneath the system. Although long-term steady-state biofilms are difficult to achieve, the fluid flow characteristics and the ability to culture multiple biofilms within a single vessel make this system well-suited to longitudinal studies of oral biofilm formation. One disadvantage is that all samples within a CDFF vessel have the same exposure so parallel experiments require multiple vessels, which are costly and technically challenging to set up. Nevertheless, it has been shown that CDFF vessels run in parallel have good levels of reproducibility for culturing oral microcosm biofilms [39].

An alternative to culturing biofilms in steady state is to grow them on coupons immersed in a more traditional fermenter, in which the free-living (planktonic) cells are in steady-state growth. For example, a two-stage chemostat system has been described for the culture of a 10-membered oral

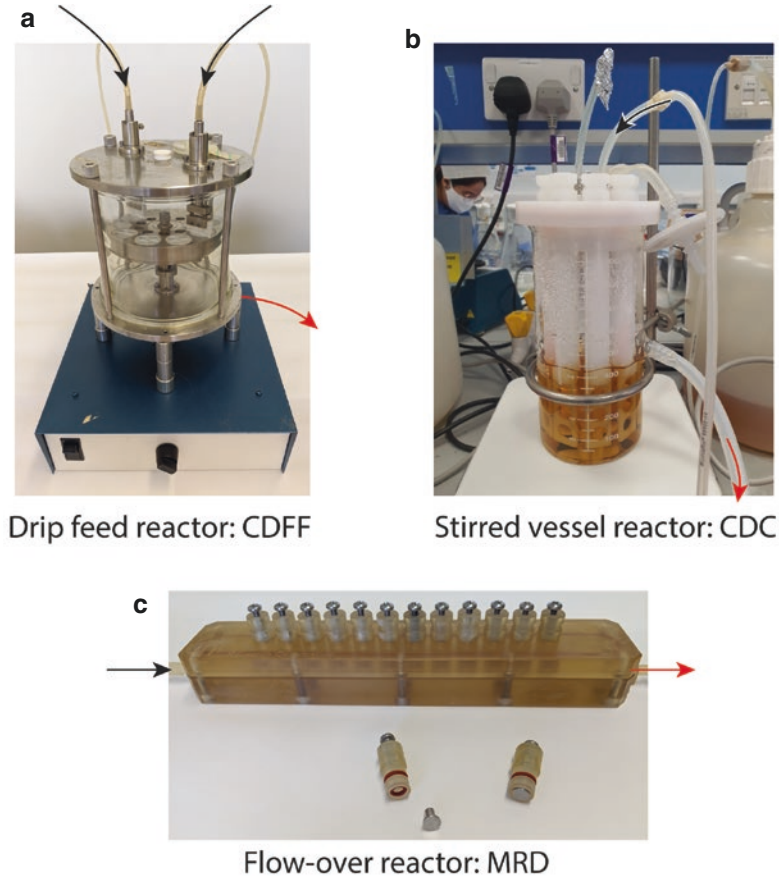


Fig. 4.3 Examples of biofilm reactors that incorporate flow. (**a**) The constant depth film fermenter (CDFF) drips medium onto paddles that scrape over the surface of the biofilm holders. Biofilms are grown on surfaces recessed at a fixed depth. The lower part of the system incorporates a motor that rotates the biofilm sample holders, ensuring they are continuously scraped by the paddle. (**b**) The CDC biofilm reactors are stirred vessels that contain biofilm samples in specialised holders. (**c**) The Modified Robbins Device (MRD) contains samples set flush against the walls of a tube. Medium is directly flowed over the samples. The direction of flow into the vessels is shown as black arrows and flow out of the systems is indicated by red arrows. In each case, fluid flow is driven by pumps (not shown)

biofilm community [40]. In this model, the first chemostat was used to obtain steady-state planktonic growth before the second-stage chemostat, containing suspended biofilm coupons, was attached to the outflow. A simpler single-stage chemostat specifically designed to hold biofilm coupons, known as the CDC Biofilm Reactor®, is commercially available from BioSurface Technologies, Bozeman, MT, USA (Fig. 4.3b). This is a relatively controllable system that is well-suited to assessing biofilm growth on dental materials or in dental unit waterlines [41, 42].

Many biofilm models simply flow growth medium directly over the substratum to culture biofilms within the channel of the device. For example, the Modified Robbins Device (MRD) is designed to hold removable sample discs of different materials flush against the wall of the vessel (Fig. 4.3c) [43]. This system provides a relatively large channel and can be used for culturing mixed-species oral biofilms that produce extensive polysaccharides which would block smaller systems [44]. Parallel MRD chambers enable comparisons of biofilm formation under different conditions and the presence of multiple sampling ports allows repeated biofilm sampling and analysis during longitudinal studies. However, it is important to note that a gradient of adhesion may be present along the device and sampling strategies should take this into account

[45]. In addition, the MRD does not permit real-time visualisation of biofilm growth or removal. This requires systems such as the flow-cell that culture biofilms on transparent cover glass [46]. Like the MRD, flow cells contain a central channel through which the growth medium is pumped. Comparisons between different treatments require multiple channels or flow cells run in parallel. The relatively small dimensions of the flow-cell make it realistic to use diluted human saliva as a growth medium for biofilms, replicating the conditions present in the mouth [46]. However, to run flow cells overnight usually requires more than 100 mL of human saliva. To reduce the need for saliva collection from volunteers, miniaturised systems such as the BioFlux microfluidics model have been employed to culture oral biofilms [47].

4.1.2.3 Importance of the Inoculum

Oral biofilms typically contain tens or hundreds of different species of bacteria, with viruses, Archaea and single-celled eukaryotes such as fungi and/or protozoa. Models often aim for simplification to increase reproducibility and facilitate analytical approaches. Some bacteria will form monospecies biofilms in vitro that allow the dissection of molecular pathways involved in surface attachment and colonisation. *P. aeruginosa* has become the model of choice for many biofilm studies due to its clinical relevance in cystic fibrosis, burn and wound infections, its genetic tractability and its ability to form structured biofilms [48]. However, *P. aeruginosa* is not a major constituent of dental plaque, except perhaps in certain populations [49, 50]. *Streptococcus mutans* is more commonly used as a target for assessing biofilm control agents due to its strong association with dental caries [51]. Biofilm formation by *S. mutans* is highly dependent on the presence of sucrose, which is utilised by extracellular glucosyltransferase and fructosyltransferase enzymes for the production of exopolysaccharides [52]. By contrast, *Enterococcus faecalis*, a persistent coloniser in root canal infections, produces biofilm matrix enriched in extracellular DNA [53]. Although extracellular matrix is readily observed in *E. faecalis* monospecies biofilms, it is difficult to replicate the dense cell–cell interactions observed in more complex systems (Fig. 4.4).

Many studies have employed defined communities of bacteria to model some of the interspecies interactions that occur in oral biofilms. When members of the community form clearly distinct cell shapes, it may be possible to distinguish them by SEM or other high-resolution microscopy (Fig. 4.4b). Selective culture can also be employed where the appropriate selective agents are known [54]. A quantitative PCR approach has been used to enumerate different species in a 14-member community [55]. This study employed a DNA cross-linking dye to bind extracellular DNA and DNA within non-viable cells so that only viable bacteria were quantified. In theory, similar approaches can be combined with deep sequencing to quantify the viable microbiome of any microbial system. However, caution is warranted with this method since complex microbial communities do not respond consistently to cross-linking agents such as propidium monoazide [56]. Nevertheless, more conventional microbiome analysis provides a powerful tool to assess the relative numbers of different taxa and enables a detailed analysis of biofilms containing the natural microbes present in the oral cavity. Consequently, there has been significant interest recently in finding systems that will allow the stable culturing of microcosm biofilm communities isolated from oral health or disease. For example, recent work has shown that saliva supplemented with 5% human serum provides an excellent growth medium for culturing the subgingival microbiota [57].

4.1.2.4 Incorporation of Host Cells and Environmental Factors

The biofilm models described above are designed to model the growth of bacteria on hard surfaces such as human enamel. However, peri-implant biofilms are also in contact with soft tissues. Although it is difficult to achieve stable long-term co-culture of bacteria with soft tissues, models have been developed to challenge cells and tissues with biofilms and biofilm products. For example, studies of invasive infections such as candidiasis have employed organotypic mod-

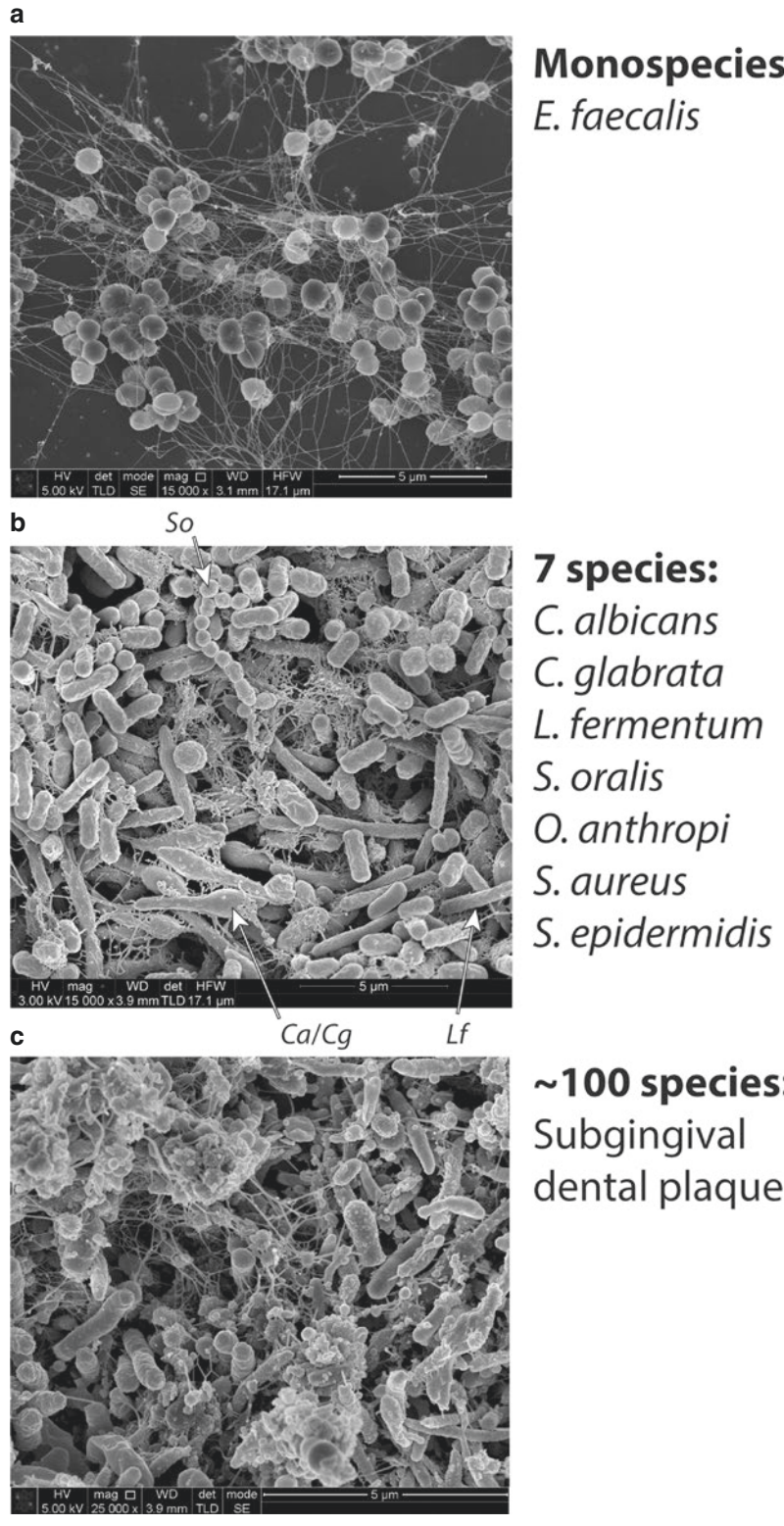


Fig. 4.4 Levels of microbial complexity in biofilm models visualised by FE-SEM. (**a**) Monospecies biofilms of *E. faecalis* contain extracellular material that appears as strings between the microbial cells. However, cells do not adopt the densely packed arrangements of more complex biofilms. (**b**) A 7 species biofilm including two fungi (*Candida*) and five bacteria isolated from tracheoesophageal speech valves. The bacteria are *Lactobacillus fermentum*, *Streptococcus oralis*, *Ochrobactrum anthropi*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Different species can be distinguished by their cell shape and arrangement such as *S. oralis* (*So*, strings of cocci), *L. fermentum* (*Lf*, relatively thin rods) or *Candida* (*Ca*/*Cg*, large yeast, pseudohyphae or hyphae). Matrix material is also visible. (**c**) Subgingival dental plaque on a recently-extracted tooth contains many different cells that cannot easily be identified without staining. Extracellular matrix material is abundant

els as substrates for the development of biofilms [58]. Similarly, *Aggregatibacter actinomycetemcomitans* has been shown to reduce the expression of keratin by gingival epithelial cells in an organotypic model of gingival tissue [59]. An alternative approach to investigate biofilm-host interactions is to culture biofilms in a transwell system and apply them to tissue culture cells on the surface of plastic dishes [60]. The use of these and other systems to investigate the immune response to oral biofilms and these have been reviewed in detail recently by Brown et al. [61].

The incorporation of dietary factors into biofilm models is easily achieved by adjusting the growth medium as needed. However, exposure to cigarette smoke and e-cigarette vapour is an important factor for periodontal disease and peri-implantitis that is more difficult to reproduce in the laboratory. The simplest approach is to add tobacco smoke extract, nicotine or e-cigarette liquids to the biofilm growth medium. For example, the addition of cigarette smoke extract to planktonic *Streptococcus gordonii* or *Porphyromonas gingivalis* causes changes in gene expression [62]. More recently, systems have been developed to expose biofilms to smoke or vapour [63]. Exposure to e-cigarette vapour increases the expression of genes encoding glucosyltransferase, competence and glucan-binding proteins and enhances biofilm formation by *S. mutans* [64]. In a more complex model involving mixed-species biofilms and host cells, exposure to tobacco smoke led to enhanced immunogenicity of commensal biofilms, but a dampening down of the inflammatory capacity of pathogen-rich biofilms [65]. Overall, these data highlight the complex interplay between microbes, host and environmental factors that can only be replicated by sophisticated model systems.

4.1.3 In Situ Models

The development of a model to culture marine 'bacterial films' in situ was perhaps the first example of biofilm research [66]. In situ models are also widely used for developing biofilms within the oral cavity that can then be extracted and analysed. A wide range of models have been developed and these have been reviewed elsewhere [67]. Of course, these models are restricted to areas of the mouth that can be accessed without causing harm. Therefore, the majority of in situ models have been developed for studying early microbial colonisation of teeth or the development of cariogenic biofilms. One innovative approach is the development of a combined system that develops biofilms in situ and then utilises a 3D-printed microfluidic flow-cell device to continue culturing biofilms in vitro [68]. This system has been employed to demonstrate the effects of exposing in situ-grown biofilms to sucrose on the pH in different areas of the biofilm. The model could also be employed to challenge natural biofilms with experimental agents that are not yet approved for use in clinical studies.

4.1.4 In Vivo Models

A major limitation of in vitro and in situ models for research on peri-implant biofilms is that they do not include the interaction with alveolar bone that is a critical factor for stabilising the implant. Brånemark introduced the term 'osseointegration', which was initially described as 'a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant' [69] and later redefined as 'clinical osseointegration implies histologic osseointegration, it is necessary [there is] a contiguous contact between the alveolar bone and the implant surface' [70]. Many factors influence osseointegration such as the implant surface topography, chemical composition and surface roughness [71]. The process of osseointegration is still actively studied as it is not yet fully understood [72].

The use of animal models is the only way currently to include osseointegration in addition to biofilm formation and to translate the experimental knowledge for clinical uses related to dental implant applications. Several different parameters need to be considered such as the variety of dental implants [73] and materials used [74], in addition to the factors that cause implant failure including biofilm formation and treatment [75]. Models may address peri-implant mucositis (inflammatory lesion of the soft tissues around the implant) or peri-implantitis (affecting supporting bone)

since both are caused by microbial biofilms. It is important to note that peri-implantitis varies from periodontitis in terms of the rate of progression, the extent of lesion formation and the composition of cells in the lesion [76]. It is still not clear how closely the microbiome of peri-implantitis resembles that of periodontitis, but a review by Rakic et al. [77] suggested that there are common species found in cases of periodontal disease and peri-implant infection, but their microbiome status is not identical.

Previously, many studies modelling peri-implant infections employed large animal models such as dogs and pigs [73, 78, 79]. However, due to housing, ease of handling and commercial availability with different genetic backgrounds, small laboratory animals such as rodents are gaining some interest for implant-associated animal model research [80, 81]. These animal models are induced to form peri-implant infections by various methods such as implantation of a device colonised with a human pathogen [82], split-mouth model [83] or ligatures tied around implants to facilitate the accumulation of bacteria [81].

Ligature-induced defect animal models are employed to mimic a natural peri-implantitis lesion [81, 82, 84]. This technique involves the induction of mucositis and peri-implantitis lesions by the introduction of ligatures around the implant neck in a submucosal position in areas of plaque formation [84]. This experimentally induced invasive procedure is associated with spontaneous disease progression in a majority of sites [79] and is more often employed to study osseointegration than biofilm infection per se [82]. The use of ligatures will initiate an inflammatory response and induce bone destructive processes [85]. Changes in the microbial composition of peri-implant pockets will also be influenced by ligatures [86].

4.1.4.1 Canine Models

Canine models are commonly used to investigate biofilm accumulation and spontaneous periodontitis [87] in studies of dental implant application and its association with peri-implantitis [78]. Human and canine bones possess similarities including bone weight, density and composition and dogs are able to use human-sized implants. However, there are some limitations since the rate of remodelling and apposition may vary within and/ or between dogs [72, 80].

Studies have employed the split-mouth design in a beagle dog model to evaluate peri-implant tissue clinically, radiographically, microbiologically and histologically [83, 88, 89]. In the split-mouth model, each dog hemi-mandible will be randomly assigned by matched pair design to either test implant group or control implant group. To examine the role of oral hygiene, the control implant can be brushed daily while the test implants are left untouched [88]. From this work, the test group had differences in total bacteria, *Fusobacterium* spp., *A. actinomycetemcomitans* and *Porphyromonas gingivalis* and there were significant increases in probing depth, bleeding-on-probing and clinical attachment level versus baseline that were consistent with the onset of peri-implantitis. Furthermore, the split-mouth model adapted from previous studies [83] can also be used for the detection, classification and measurement of peri-implant bone defects. Various analysis methods are available and a recent study has shown advantages of cone beam computed tomography (CBCT) compared with intra-oral (IO) radiography for the assessment of bone defects [89].

4.1.4.2 Rodent Models

Rodent models of polymicrobial peri-implantitis have been used to investigate the inflammatory response to human microbial biofilms [90, 91]. For example, a split-mouth implant model in specific pathogen-free female ex-breeder Sprague-Dawley rats was used to investigate and quantify the implant-associated biofilm. Besides the establishment of a three-step implantation method for titanium implants [92], this model can be excellent for analysing microbial growth when the biofilm formation process is left to occur naturally after the inoculation of human-derived oral bacteria *Streptococcus oralis*, *Fusobacterium nucleatum* and *P. gingivalis* orally [90]. This approach is similar to a previous report that used Wistar rats and established reproducible biofilm quantification using fluorescence staining and confocal scanning laser microscopy [91]. In addition, PCR was used for the identification of microbial taxa present in the samples. A blinded clinical inspection of the implantation site was done by a dentist and any

signs of infection were evaluated at the end of the experiment using an established mucosa index based on the gingival index described by Löe [93]. Koutouzis et al. [91] and Sun et al. [75] reported that bacterial colonisation/infection around implants caused significant IgG and IgM antibody responses. There was advanced bone resorption, and extensive inflammation with granulation tissue and PMNs observed in the peri-implantitis model.

The rat model was reported to have 79.6% successful osseous initial integration [90], similar to a report by Koutouzis et al. [91]. This high success rate may be due to the implant assembling steps practised in both studies. This technique overcame problems with the establishment of a direct bone-implant interface reported in previous studies that were caused by movements of the implant during osseous integration [92]. Furthermore, there were signs of mild to moderate peri-implant mucositis in animals with confirmed polymicrobial infection, which were absent in control animals treated with antibiotics [90].

This animal model allows an easy insertion and removal of the implant abutment when needed. This advantage is that this not only protects the abutment from wear and abrasion or unwanted colonisation by endogenous bacteria but also provides the possibility to observe and quantify three-dimensional biofilm formation of this model using confocal scanning laser microscopy of the abutment and retaining screw. Besides the established clinical situation where there is bacterial leakage from the implant-abutment connection, they highlighted that this model still needs a further modification due to the presence of a cavity under the retaining screw that may act as a good hiding place for the biofilm community to form [94, 95].

4.2 Summary

There is still much to learn about the development of microbial biofilms on dental implants and the surrounding tissues. Good model systems are essential for enhancing our understanding of these processes and how to control them. In addition to the in vitro, in situ and in vivo models described here, it is likely that we will see a rapid expansion in the use of in silico models, in part driven by the development of artificial intelligence and deep learning [96]. Ultimately, these models will lead to the development of improved dental implants that provide strength, longevity and that are able to remain free of pathogenic infections over their lifetime of use.

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