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Guest Editorial

Polymicrobial infections, biofilms, and beyond

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The dental world has been pivotal in a number of important areas and discoveries relevant to infectious diseases. In the very early days, van Leeuwenhoek, the father of microbiology, discovered animalcules in his own dental plaque (Selenomonads: 1676) and overthrew the prevalent traditional belief in spontaneous generation (Schierbeek 1959). Incidentally, Leeuwenhoek also viewed the first multispecies biofilm that is the dental plaque biofilm (Costerton et al. 1999). More recently, the study of bacterial adhesion as a determinant of successful colonization (Gibbons & Houte 1975) and the concept of a distinct type of immunity against infection at mucosal surfaces (Michalek et al. 1976) were pioneered by dental researchers. The term ''dental microbial plaque'' predates the more modern term ''biofilm'' and dental caries and periodontal disease offer classical examples of microbial biofilms that induce human disease (Costerton et al. 1999). In fact, few discussions on pathogenic biofilms omit the canonical example of dental plaque.

Since the 1970s biofilm research has mushroomed but our typical incorporation of only one species within our *in vitro* experiments has not kept pace with our multi-species biofilm knowledge. Work from our laboratories and others have limited themselves to analysing the effect of typically one species on cell lines or more recently primary culture cells, despite our appreciation that host–pathogen interactions involve the participation of multiple host cells and microbial species. Clearly, the use of recognized and widely available laboratory strains permits the comparison of experiments between laboratories but they are unrepresentative of the true challenge that host cells face from the subgingival microbial plaque biofilm. Indeed, the use of even primary cells, typically gingival epithelial cells or phagocytes, is a step in the right direction but remains a poor substitute for the real multi-layer and multi-cell type tissue challenge in the disease state.

Why do we persist in performing these limited assessments of this multi-bacterial and multi-host cell interaction? Again, the necessity to utilize reductionist approaches to understand basic biological principles, repeatability across laboratories, and reduction of variance to draw conclusions on mechanisms and outcomes of host–parasite interactions are at play here. The challenges of dealing with the enormous variability generated by ''real life models'' are viewed as insurmountable without sophisticated high-throughput approaches, and equally sophisticated statistical modeling to rationalize, interpret, and integrate the findings. Even under these conditions, however, the reductionist approaches would still remain relevant as a source of mechanistic information for better interpretation of the data from ''real life models'', which are indeed greatly needed for understanding human disease. Thus the current paper by Polak and colleagues on ''Mouse model of experimental periodontitis induced by *P. gingivalis*/*F. nucleatum* infection'' is welcomed and applauded. There are lessons for all of us in these results and for the planning of new experiments. Of course the mouse is an animal that (1) cannot faithfully reproduce all aspects of human periodontal disease initiation and progression; (2) has cells that differ in their response to their human counterparts; (3) are by and large genetically identical and thus genetic variability is unaccounted for; (4) the bacteria used are merely two of at least 150 organisms in any dental plaque biofilm; (5) these bacteria are applied in planktonic solution, and at a growth phase inconsistent with what would occur in the biofilm. Nevertheless, this paper does represent an important step forward that may move us to a new understanding of host–parasite interaction.

Although the study by Polak and colleagues has successfully used two human periodontal pathogens, as opposed to the traditional use of one species (usually *Porphyromonas gingivalis*), it should be emphasized that the implantation of a single human periodontal pathogen does not necessarily constitute a mono-infection, unless the mice are specifically germ free. In fact, the vendors' claim that their mice are pathogen free may not be quite accurate, as the mice may not be free of potential periodontal pathogens. Our laboratories have noticed development of naturally occurring murine periodontitis starting at about 9 months of age and further increasing as a function of age, as is the case with human periodontitis. Even young mice can develop periodontitis caused by their own flora, if their ability to control their indigenous bacteria is compromised by genetic defects in their phagocytes, although the presence of antibiotics prevents development of the disease (Beertsen et al. 2008). Based on these considerations, it is thought that in the single-organism oral gavage model, *P. gingivalis* may initiate experimental murine periodontitis, at least in part, by modifying the endogenous subgingival biofilm to acquire enhanced virulence (Graves et al. 2008). One such plausible mechanism, which is based on its

remarkable ability to exploit several strategies for immune evasion (reviewed in Kinane et al. 2007) is that *P. gingivalis* may undermine the host response in ways that favour microbial outgrowth and periodontal disease development. Of course, the paper by Polak and colleagues who introduced a second human periodontal pathogen is a step closer to the human disease and adds additional advantages. In that model, *P. gingivalis* may interact synergistically with *Fusobacterium nucleatum* for promoting their mutual survival interests. This interaction is well tried out as the two organisms have long co-existed and co-evolved in human mouths as opposed to the putative ''ad-hoc'' interactions of *P. gingivalis* with the murine oral flora, discussed above.

P. gingivalis appears to offer a panoply of critical virulence properties to a mixed-species biofilm (e.g., corrupting host immunity or generating peptide nutrients through specific proteolytic activity). It, therefore, becomes evident that approaches to neutralize *P. gingivalis* may also impact upon the whole biofilm. This notion is of course a testable hypothesis, the testing of which is facilitated by more complex models like the one developed by Polak and colleagues. Would neutralization of key virulence properties of *P. gingivalis* have an impact on the survival of *F. nucleatum* or other periodontal species that may additionally be used in mouse periodontitis models? It should be noted, however, that the generation of such ''human'' periodontal biofilms on the teeth of mice or other animal models should be conclusively demonstrated and compared with the ''native'' biofilms of the human tooth surfaces; simply observing enhanced virulence when adding two human periodontal pathogens, as opposed to each one alone, suggests but does not prove cooperative or synergistic interactions between the two species.

Dental research, particularly periodontal, has made enormous strides since the days of Leeuwenhoek but our knowledge and application of microbial findings remains largely untranslated, despite the funding and the intellectual talent applied. The call for more meaningful translational research is appropriate: we are dealing with disease rather than albeit fascinating biology; thus we are tasked with developing appropriate laboratory models of periodontal disease that do not stray too far from what we should expect to see clinically and from our histopathological knowledge. The paper by Polak and colleagues in this issue shows that polymicrobial infection with *P. gingivalis*/*F. nucleatum* causes more alveolar bone loss and more inflammation than either bacterium alone. The results suggest that oral infection of mice with a mixture of *P. gingivalis* and *F. nucleatum* are more meaningful than each species alone in models of experimental periodontitis. However, we are still a long way from mimicking experimental gingivitis and chronic periodontitis conditions in the laboratory. Thus, we look with anticipation to a future in which multi-cell models of periodontal tissue may be challenged and to standardized subgingival biofilm mimics, so that we can conduct reproducible and translatable pathogenicity studies that may have a bearing on the true clinical disease process.

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