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Oral Microbiology Research Group

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Communication at the host-pathogen interface

Introduction/Background

The oral cavity represents a finely balanced ecosystem in which a plethora of bacterial species lives in complex communities known as biofilms. To cohabitate efficiently, bacteria within these biofilms have evolved intricate systems of communication, such as quorum sensing, cooperation or competition, involving signaling, sharing of resources and/or metabolic activities. Dysbiosis can develop and lead to periodontitis, the most common human chronic inflammatory disease.

Briefly, Streptococci, recognised as early colonisers, are followed by *Fusobacterium nucleatum*, known as a 'bridging organism'. The latter interacts with multiple bacterial species, facilitating their adherence, leading to formation of a disease-associated biofilm.

The role of *F. nucleatum* is of particular interest as it interacts with multiple species in the oral biofilm and modulates progression from health to disease. Its role in controlling oral health could be pivotal.

Five subspecies of *F. nucleatum* have been described to date, it is however unknown whether they have different roles - for example in health and disease.

AIM:
Here we are investigating the impact of subspecies of *F. nucleatum* in establishing the disease-associated biofilm by analysing mixed biofilm formation.

Methodologies

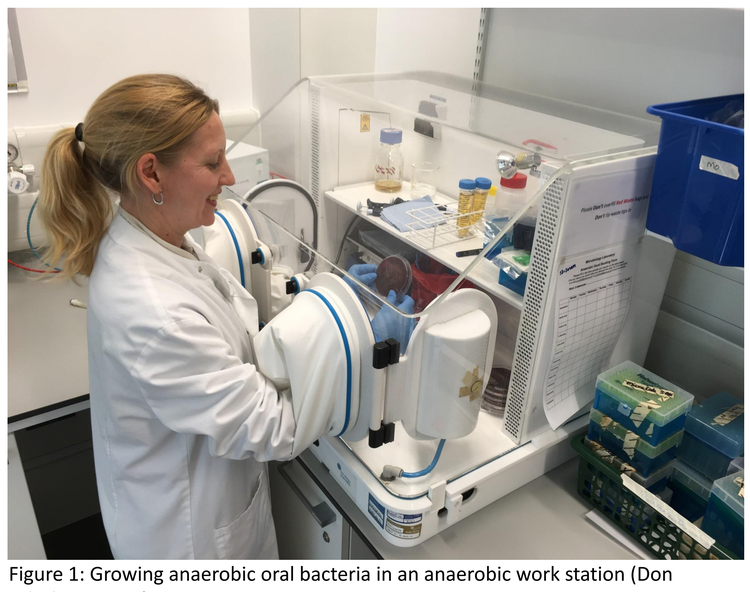


Figure 1: Growing anaerobic oral bacteria in an anaerobic work station (Don Whitley DG250).

Biofilm formation
Thermanox™ cover slips were placed into 24 well plates and inoculated with an overnight (o/n) culture of *Streptococcus mitis,* ATCC 49456 (grown aerobically, shaking at 37C in tryptic soy broth (TSB) with yeast and glucose).
After 24 h (incubated at 37C) the plate was transferred into the anaerobic cabinet (Don Whitley, see figure 1).

After 24 h (incubated at 37C) the plate was transferred into the anaerobic cabinet (Don Whitley, see picture). The supernatant was replaced with an overnight culture of the respective *F. nucleatum* subspecies (*fusiforme, nucleatum, polymorphum*), grown in Schaedler's anaerobe broth (SAB). After o/n incubation (anaerobically) the supernatant was replaced with an o/n culture of *Porphyromonas gingivalis*, W83 (grown in SAB). The biofilms were grown for 4 days, before being assayed.

Biofilm quantification and visualization
Subspecies of *F. nucleatum* were visualised after Gram staining under a standard light microscope.
Biofilms were quantified using a crystal violet staining protocol as previously described [1].
Biofilms were stained using Filmtracer™ LIVE/DEAD® Biofilm Viability Kit (Thermofisher) according to manufacturer's instructions and visualised using confocal microscopy.

Characterisation of subspecies and quantification of biofilms

Gram staining and light microscopy of *F. nucleatum* subspecies *fusiforme, nucleatum, polymorphum* has been performed (figure 2).

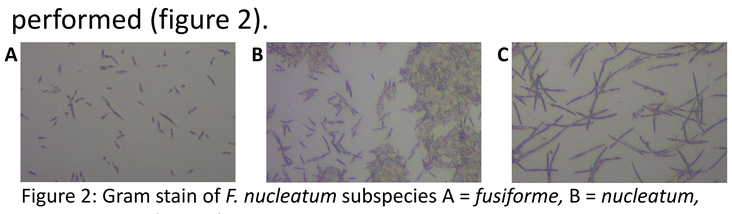
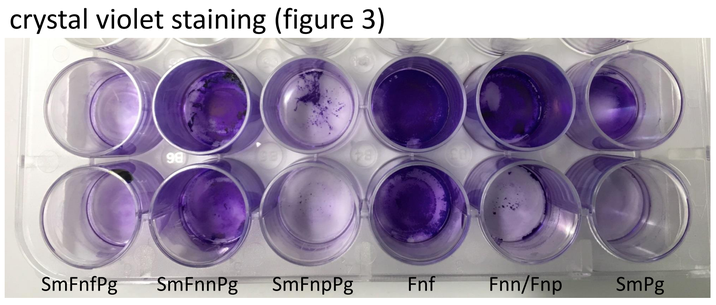


Figure 2: Gram stain of *F. nucleatum* subspecies A = *fusiforme,* B = *nucleatum,* C = *polymorphum*

Quantification of mixed and single species biofilms by crystal violet staining (figure 3)



Biofilm quantification

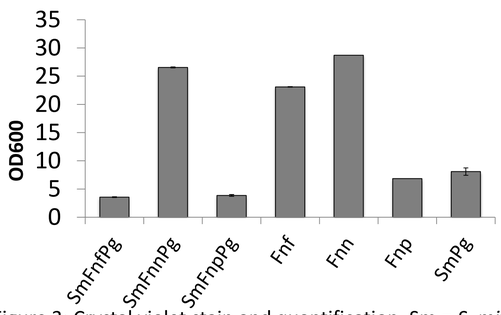
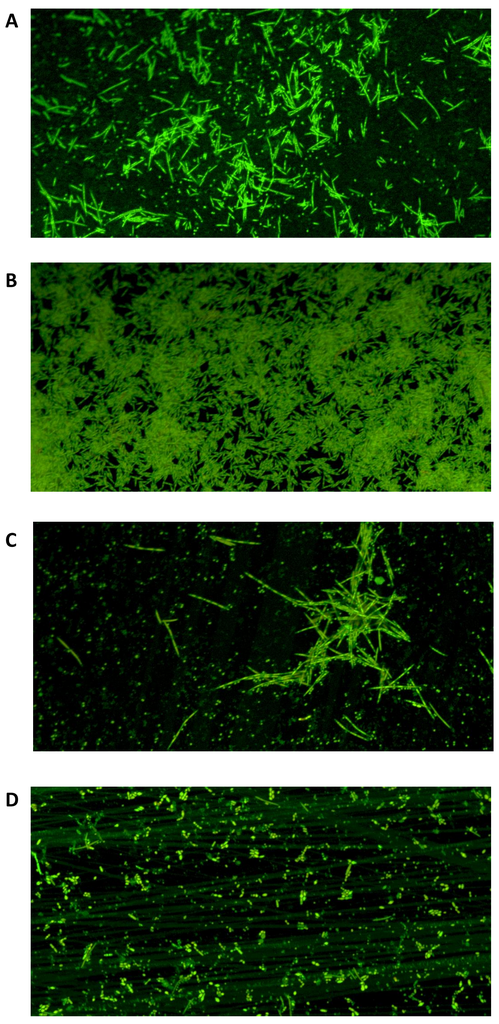


Figure 3: Crystal violet stain and quantification. Sm = *S. mitis*, Fnf – *F. nucleatum ssp. fusiforme*, Fnn – *F. nucleatum ssp. nucleatum*, Fnp – *F. nucleatum ssp. polymorphum*, Pg – *P. gingivalis*

Multispecies biofilms



Duplicates of the biofilms that were quantified (figure 3) were visualised using confocal microscopy (figure 4). Clear differences could be observed between the subspecies.

Figure 4:
A = SmFnfPg
B = SmFnnPg
C = SmFnpPg
D = SmPg

Sm = *S. mitis*,
Fnf – *F. nucleatum ssp. fusiforme*,
Fnn – *F. nucleatum ssp. nucleatum*,
Fnp – *F. nucleatum ssp. polymorphum*,
Pg – *P. gingivalis*

Future Work Plans

Aggregation of the bacteria in oral biofilms has been studied previously and is relatively well documented. Their means of communication and in particular their mechanisms of recruitment are less well understood.
We aim to further study the impact of the subspecies of *F. nucleatum* in bacterial cell-cell communication and bacteria-host interactions.

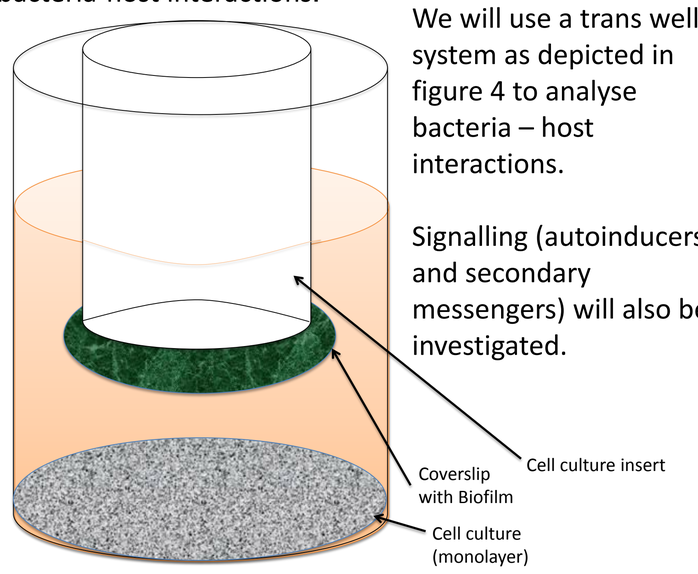


Figure 5: Trans-well biofilm model, modified from [1]
The model can be used to study biofilm (bacterial) – cell (host) interactions.

We will use a trans well system as depicted in figure 4 to analyse bacteria – host interactions.

Signalling (autoinducers and secondary messengers) will also be investigated.

Future work will compare the 5 *F. nucleatum* subspecies on a genomic, transcriptomic and metabolomic level to understand impact on health and disease further.

References & Acknowledgments

[1] Millhouse E, Jose A, Sherry L, Lappin DF, Patel N, Middleton AM, Pratten J, Culshaw S, Ramage G. Development of an *in vitro* periodontal biofilm model for assessing antimicrobial and host modulatory effects of bioactive molecules. *BMC Oral Health*. 2014 Jun 28;14:80. doi: 10.1186/1472-6831-14-80.

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