Complex periodontal biofilms comprising *Fusobacterium polymorphum, Porphyromonas gingivalis, Prevotella intermedia* and *Tannerella forsythia*, and their disruption by the Fusobacterium bacteriophage FNU1.

Background

- Periodontitis is a severe gum disease that arises from dysbiosis of the bacterial population in the oral microbiome, contributing to the formation of plaque between the gums and teeth.
- Non-surgical debridement is the gold standard treatment, but this treatment fails in up to 40% of patients.
- Treatment failure is due to an increased presence of ***Fusobacterium polymorphum*** which is a keystone bacteria in periodontal biofilms, acting as scaffolding for other pathogens, which help form a complex subgingival biofilm.

Aims of study

- To investigate the efficacy of *F. polymorphum* bacteriophage (FNU1) to disrupt
  1. Dual-species biofilms (*F. polymorphum* & *P. gingivalis, F. polymorphum* & *P. intermedia, F. polymorphum* & *T. forsythia*)
  2. Four-species complex biofilm (*F. polymorphum* & *P. gingivalis* & *P. intermedia* & *T. forsythia)*

Materials and methods

- Bacteria (1×10^8 CFU/mL) were pre-labelled with CellTrace cell proliferation assays. Total number of cells added was similar for mono-species, dual-species and four species complex biofilms.
- The biofilms were grown anaerobically at 37 °C for one week in tryptic soy broth with 0.5% (w/v) L-cysteine, 0.5% (v/v) hemin, 1µg mL^{-1} menadione and 0.5% glucose.
- On day 7, FNU1 bacteriophage was added to the biofilms.
- On day 8, visualisation of biofilms using confocal microscopy and quantitative analyses of biofilms using 0.1% crystal violet staining.
- SYBR gold and propidium iodide were used to determine viability of cells in biofilms.

Results

Periodontal pathobionts form synergistic multi-species biofilms

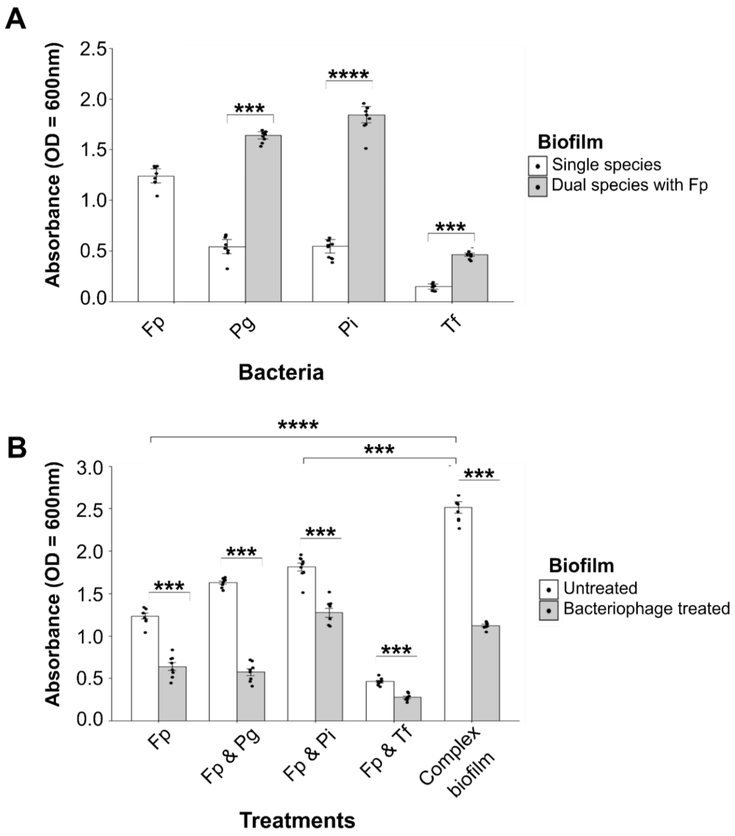

Figure 1 Biofilm quantification and synergy in dual-species and four-species complex biofilm showing: **(A)** synergy of dual-species biofilms in comparison to single-species biofilms and **(B)** capacity of bacteriophage FNU1 to disrupt single-species, dual-species and four-species complex biofilms. (Fp: *F. polymorphum*, Pg: *P. gingivalis*, Pi: *P. intermedia*, Tf: *T. forsythia*) (Wilcoxon test, *p* value < 0.01).

Results

Growth of periodontal biofilms with and without FNU1 treatment

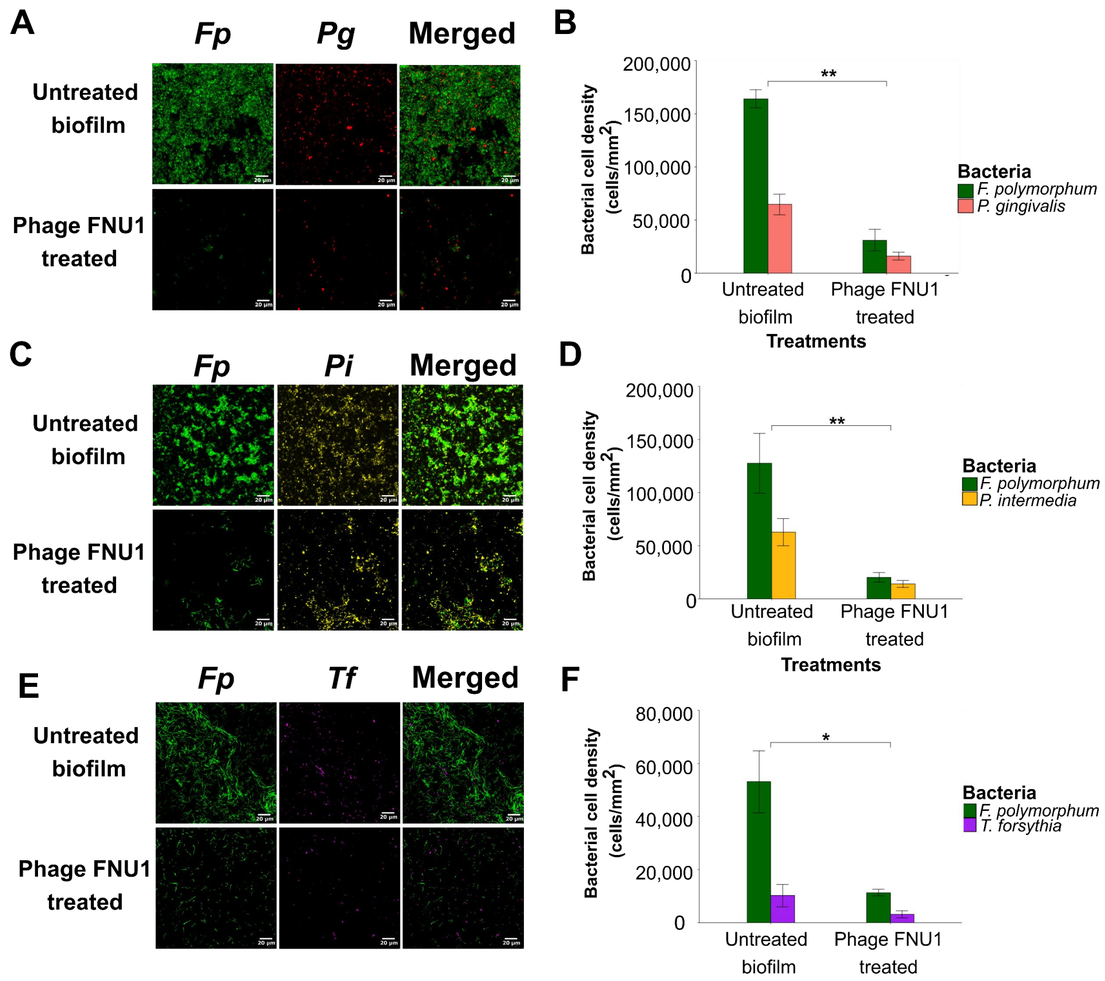

Figure 2 Confocal visualisation and quantification of bacterial proliferation of untreated and bacteriophage FNU1 treated dual-species biofilm. **(A,B)**: *F. polymorphum (Fp)* & *P. gingivalis (Pg);* **(C,D)**: *F. polymorphum (Fp)* & *P. intermedia (Pi);* **(E,F)**: *F. polymorphum (Fp)* & *T. forsythia (Tf)* (Wilcoxon rank sum test, *p* value < 0.01)

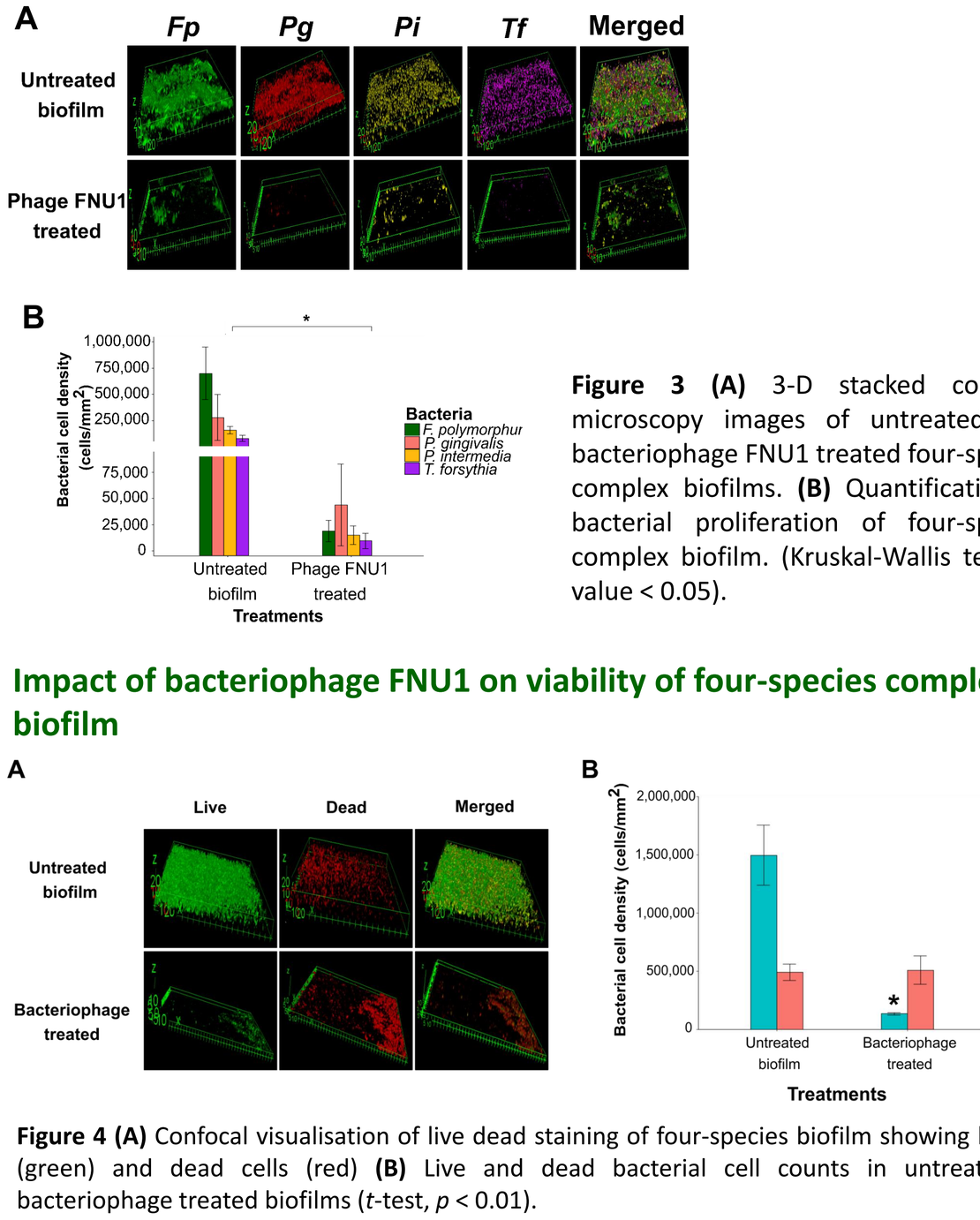

Figure 3 (A) 3-D stacked confocal microscopy images of untreated and bacteriophage FNU1 treated four-species complex biofilms. **(B)** Quantification of bacterial proliferation of four-species complex biofilm. (Kruskal-Wallis test, *p*-value < 0.05).

Impact of bacteriophage FNU1 on viability of four-species complex biofilm

Figure 4 (A) Confocal visualisation of live dead staining of four-species biofilm showing live cells (green) and dead cells (red) **(B)** Live and dead bacterial cell counts in untreated and bacteriophage treated biofilms (*t*-test, $p < 0.01$).

Conclusions

- FNU1 bacteriophage, though specific to *F. polymorphum*, significantly disrupted complex biofilms composed of all four periodontal pathogens tested here.
- Targeting a single keystone bacteria using bacteriophages can lead to collapse of complex pathogenic biofilms.

Future directions

- Build library of bacteriophages targeting other oral pathobionts e.g. *P. gingivalis*, *P. intermedia* and *T. forsythia* for which no bacteriophages have been characterised to date.
- Test combinations of bacteriophages in disrupting complex pathogenic periodontal biofilms.

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