Gingipain Inhibitors Penetrate And Inhibit Gingipains In *Porphyromonas gingivalis* Biofilms

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CORTEXYME

Poster #2121

Background

Porphyromonas gingivalis (Pg), a keystone pathogen in the development of periodontal disease (PiD), is an assaccharolytic bacterium that relies on proteases called gingipains for its virulence and survival. Small molecules directed at inhibiting *Pg* growth and survival require effective penetration into bacterial biofilms for maximum efficacy in PiD and other diseases associated with *Pg* including Alzheimer's disease, atherosclerosis, and diabetes. A library of highly potent, oral, selective small-molecule gingipain inhibitors with brain penetration were developed and a lead molecule atuzaginstat (COR388) is currently being assessed in the GAIN Trial, a pivotal Phase 2/3 study in mild-to-moderate Alzheimer's Disease which includes an assessment of its effect on periodontal disease in a substudy (the REPAIR study). Efficacy of atuzaginstat in PiD was demonstrated in a naturally occurring aged dog model, including a decrease in gingipain activity and bacterial burden in subgingival plaque biofilms and a reduction in periodontal pocket depths (ref 1). Here, we present the ability of gingipain inhibitors to penetrate *in vitro* surface attached biofilms.

Surface attached *Pg* biofilms were optimized on hydroxyapatite (HA) discs and analyzed for robustness of the biofilms as well as gingipain inhibitor penetration. Fluorescent viability stain and scanning electron microscopy methods confirmed robust biofilm formation. Growth kinetics showed that biofilm production was mature at 48 hours and measurable lysine-gingipain (Kgp) and arginine-gingipain (RgpB) activity was seen using protease substrate cleavage assays and Cy5 conjugated activity-based probes. Biofilms demonstrated decreased susceptibility to broad-spectrum antibiotic amoxicillin relative to planktonic growth as expected for robust biofilms. Atuzaginstat and COR588, two potent Kgp inhibitors, exhibited significant time and concentration-dependent inhibition of lysine-gingipain activity within these biofilms with other inhibitors having varying levels of potency in biofilms. These inhibitors maintained their lysine-gingipain target selectivity within the biofilms.

Method Development: Hydroxyapatite Surface Attached Biofilms

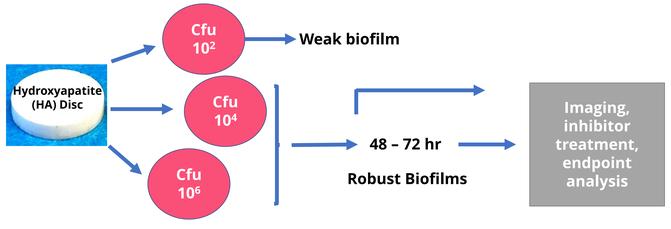


Figure 1. Development and Optimization of Hydroxyapatite surface-attached *P. gingivalis* biofilms. Various parameters were optimized including wash conditions, growth kinetics, and inoculum concentration.

Results: Biofilm Characterization and Lysine-Gingipain Inhibitor Permeability of the Biofilms

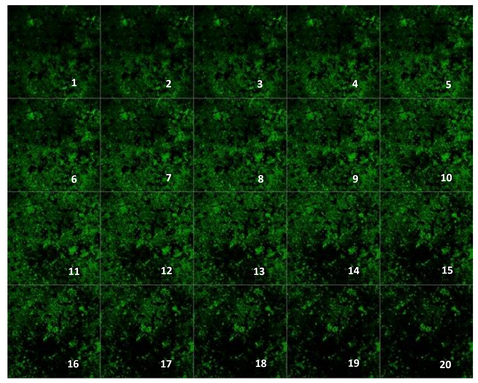


Figure 2. Confocal scanning laser microscopy (CLSM) shows robust and viable HA surface attached *P. gingivalis* biofilm formation Z-stack *P. gingivalis* biofilm at 72h stained with FilmTracer LIVE/DEAD Biofilm Viability Kit and imaged live on a Zeiss LSM880 Confocal multiphoton upright microscope using a 20x water dipping objective. The twenty slices shown had a total thickness of 20 µm, with each slice showing an area of 425x425 µm.

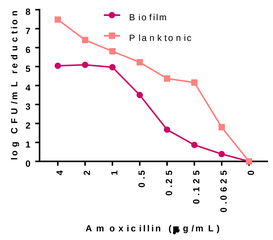


Figure 3. Resistance of *P. gingivalis* biofilms to antibiotic amoxicillin, compared to planktonic growth, confirms robust biofilm formation.
The difference in log CFU/mL between vehicle and each treatment concentration was calculated to assess the log CFU/mL kill achieved in both culture types using the formula: (Vehicle log CFU/mL) — (amoxicillin X µg/mL log CFU/mL).
The plot demonstrates a reduced sensitivity to amoxicillin in the bioiflms compared to planktonic *P. gingivalis*.

Characteristic of robust biofilm formation, *P. gingivalis* biofilms are less sensitive to broad spectrum antibiotic due to compromised biofilm penetration.

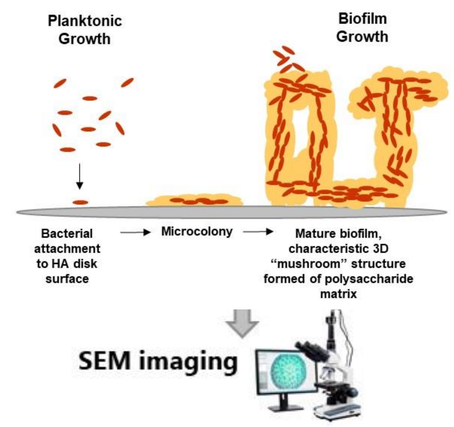


Figure adapted from: Vasudevan, J. *Biofilms: Microbial Cities of Scientific Significance.* Microbiology and Experimentation (2014): v1(3) 84-98.

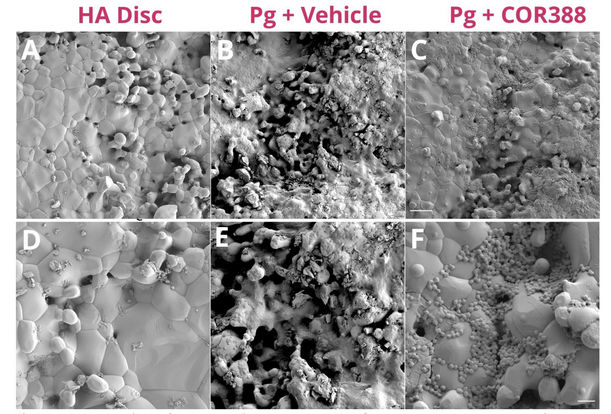
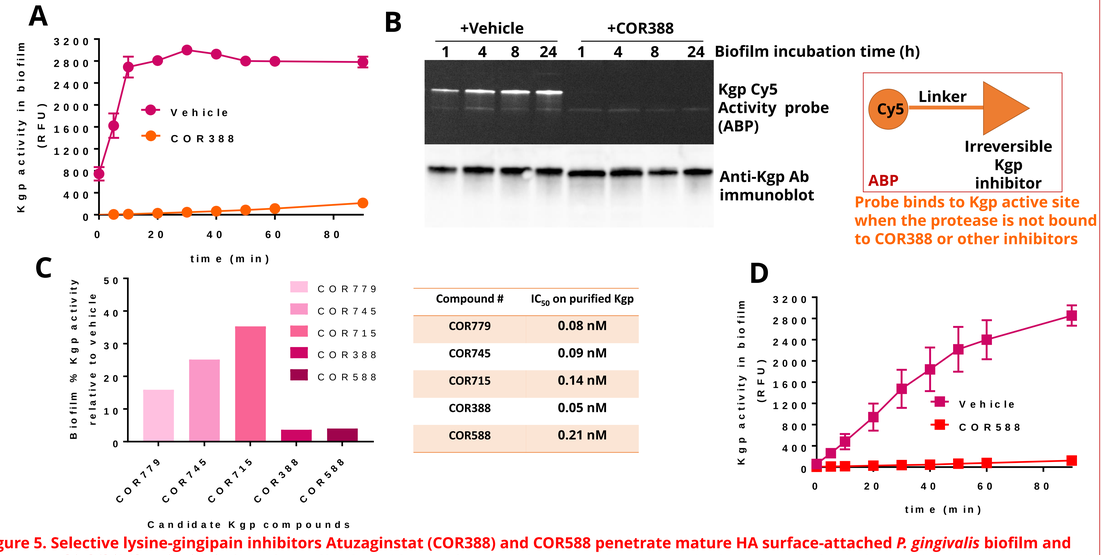


Figure 4. Scanning electron microcopy (SEM) shows COR388 compromises robust characteristic mushroom-like structure of the *P. gingivalis* biofilm A&D) HA Discs; B&E) biofilms treated at 48 hr with vehicle, fixed at 72 hr; C&F) 48 hr biofilms treated at 48 hr with COR388 (1µM), fixed at 72 hr; A-C) SEM images taken at 1000x magnification at a 10µm scale; D-F) SEM images taken at 4000x at a 2µm scale. All biofilms seeded at 10^6 inoculum and fixed with 4% PFA.



Compound #	IC_{50} on purified Kgp
COR779	0.08 nM
COR745	0.09 nM
COR715	0.14 nM
COR388	0.05 nM
COR588	0.21 nM

Figure 5. Selective lysine-gingipain inhibitors Atuzaginstat (COR388) and COR588 penetrate mature HA surface-attached *P. gingivalis* biofilm and engage and inhibit the gingipain target A) Biofilms formed from a 10^6 CFU/mL culture over 48 hr were treated with COR388 (1µM) for 1hr, harvested, and Kgp activity measured in the lysates using a fluorescent substrate cleavage assay described previously with measurements taken over 90 min (ref 2). B) Biofilms in (A) were incubated for times indicated with COR388, harvested, and Kgp activity and active site engagement by COR388 detected in lysates using an activity-based Cy5-linked probe as described previously (ref 2). The presence of Kgp in the lysates was confirmed using an anti-Kgp antibody. C) Kgp activity was used as a screen in biofilms to characterize inhibitor efficacy in penetrating penetrate biofilms and blocking the protease. Biofilm Kgp activity after incubation with a selected panel of discovery compounds (all at1µM) is shown following 1hr biofilm treatment, lysis, and 60 min reads of Kgp enzyme assay. The table on the right highlights the IC_{50} values in purified Kgp. E) Biofilms formed from a 10^4 culture over 48 hr were treated with COR588 (1µM), demonstrating the high biofilm potency of this additional Kgp inhibitor.

Summary

- **Robust biofilms of *Pg* were used to characterize the biofilm penetration of lysine- gingipain inhibitors**
- **Atuzaginstat and COR588 quickly penetrate formed biofilms to inhibit lysine-gingipain (Kgp) specifically at concentrations relevant for therapeutic efficacy in PiD**
- **Atuzaginstat treatment compromises the structural integrity of *Pg* biofilms**
- **Biofilm penetration and gingipain target engagement in subgingival plaque has been published in a natural dog model of periodontal disease (ref 1) and is discussed in poster #1756 at IADR General Session 2021.**

References, Disclosures, and Acknowledgements

References:
1) Arastu-Kapur et al, Pharmacol Res Perspect. 2020 Feb;8(1):e00562
2) Dominy et al, Sci Adv. 2019 Jan 23;5(1):eaau3333

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