National Biofilms Innovation Centre

3D HOLOGRAPHY REVEALS THE IMPACT OF ANTIMICROBIALS ON BACTERIAL MOTILITY

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INTRODUCTION:

- Halitosis, caries, and periodontitis are all associated with biofilms
- Many mouthwashes contain antimicrobials to reduce the bacterial load within the oral cavity
- Antimicrobials that damage bacterial membranes may lead to disruption of motility
- Tracking cells in 3D could offer insight into the early-stage impacts of antimicrobials on bacteria via its influence on flagella-mediated motility

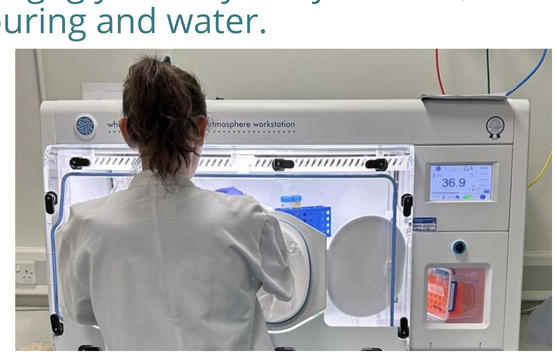
Aim: To elucidate the effects of antimicrobials on bacterial motility

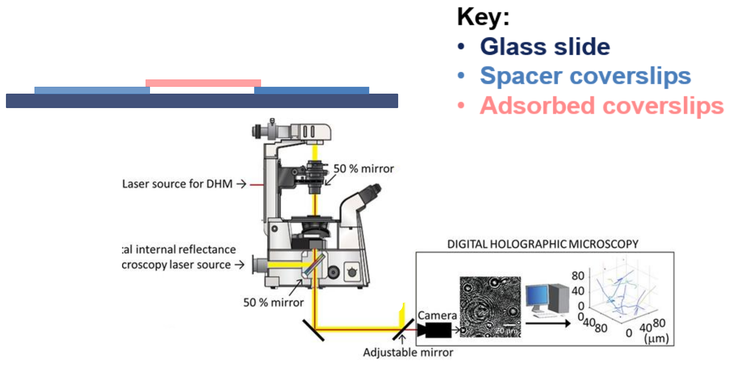
METHODS:

Antimicrobials were adsorbed onto glass coverslips for 1 min and used to create holographic imaging chambers. *Campylobacter rectus* cultured in BHI at 5% CO_2, 5% H_2, and 90% N_2 in a Don Whitley M35 Variable Atmosphere Workstation was diluted to OD_{600} of 0.01 and added to the holography chamber. Within 2 min, imaging was initiated with 18 s acquisitions (56 Hz) being taken every 10 min over 1 h using a 20x/0.75NA objective and 685 nm laser [1].

Image Analysis Methods Images were analysed using LABVIEW scripts [2] to give 3D tracks which were graphed and analysed with bespoke MATLAB scripts.

fCHX is formulated with glycerol, macrogoglycerol hydroxystearate, sorbitol, flavouring and water.





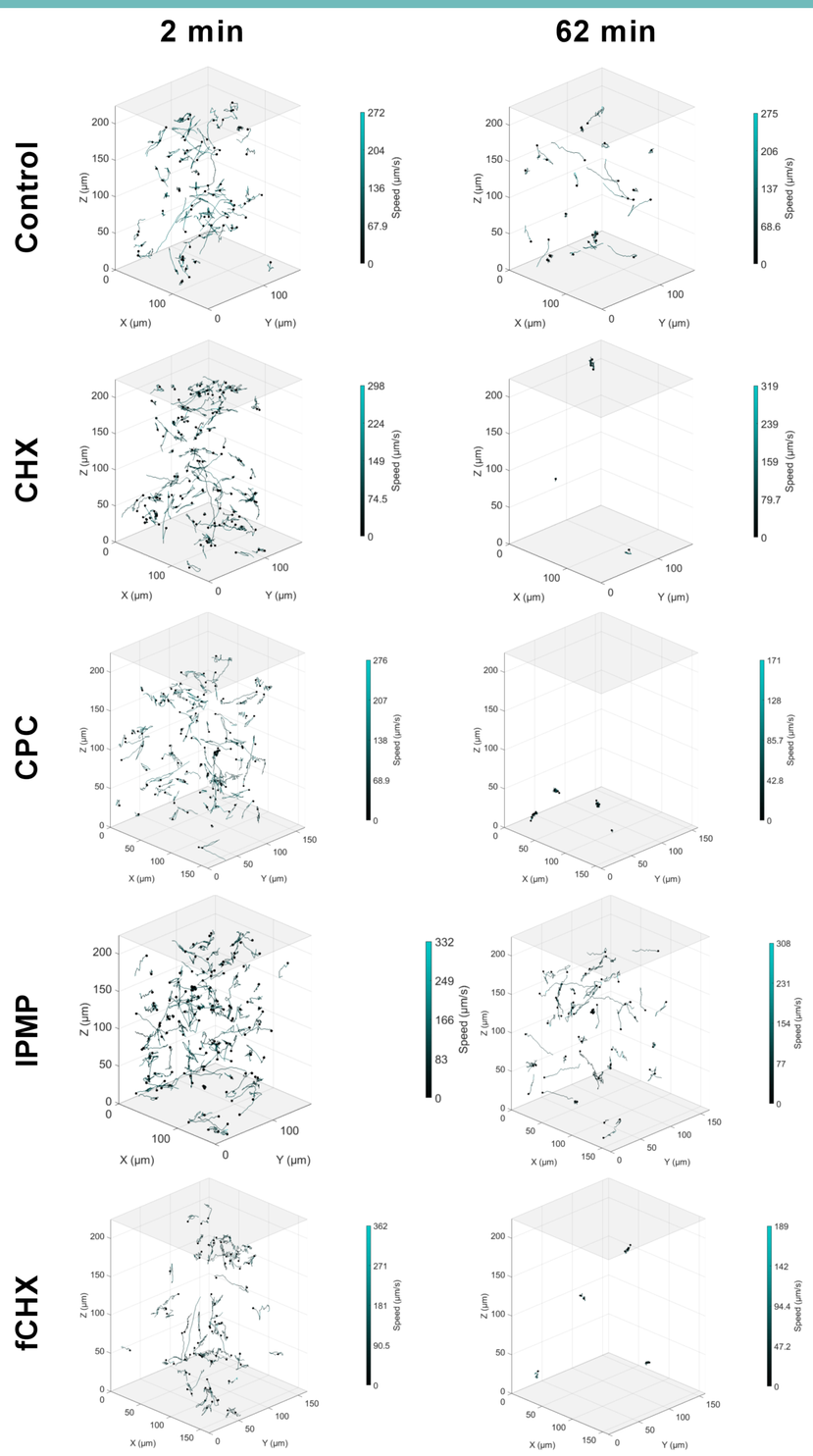
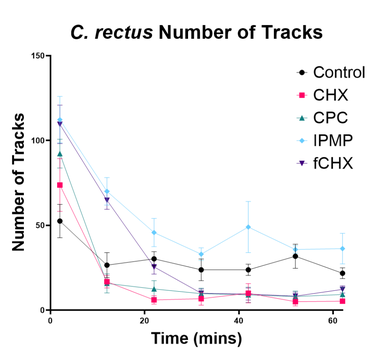
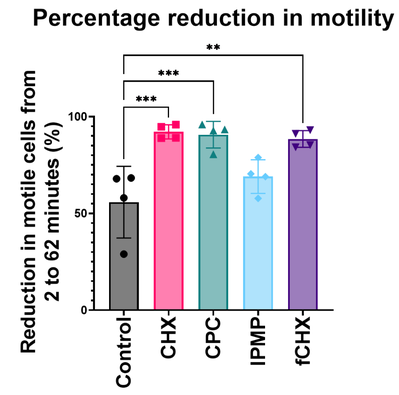


Figure 1: Reconstruction of C. rectus tracks over 18 s of imaging at (left) 2 minutes, and (right) 62 minutes). C. rectus was exposed to untreated surface (control), Chlorhexidine adsorbed surface (CHX), cetylpyridinium chloride adsorbed surface (CPC), isopropyl methylphenol adsorbed surface (IPMP), or formulated chlorhexidine adsorbed surface (fCHX).





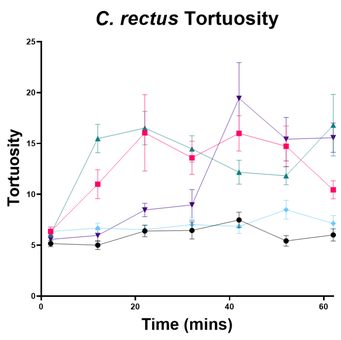


*Figure 2: (Top left) Number of tracks for each image series. (Top right) The percentage reduction in motility of the population was calculated and plotted. One-way ANOVA was conducted with comparison to the control where **= p<0.01, and ***= p<0.001. (Bottom left) tortuosity of tracks was plotted. Data presented as (mean +/- standard error of mean for number of tracks and tortuosity, and as mean +/- standard deviation for percentage reduction in motility).*

RESULTS:

- CHX, CPC, and fCHX adsorbed surfaces cause changes in the motility of *C. rectus* which can be visualised (Figure 1) and quantified (Figure 2)
- Presence of CHX, CPC, and fCHX reduced the number of motile bacteria over time and increased the tortuosity of the bacteria trajectories
- A statistically significant reduction in the number of motile cells was found for *C. rectus* exposed to CHX, CPC, and fCHX

Conclusions: CHX, CPC, and fCHX effects on flagellar-mediated motility can be qualitatively and quantitatively analysed with digital holographic microscopy

Future Work:
Grow biofilms on adsorbed surfaces to determine the correlation between the motility of *C. rectus* and its biofilm formation

REFERENCES

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2. Giuliano, C. B., Zhang, R. & Wilson, L. G. Digital inline holographic microscopy (DIHM) of weakly-scattering subjects. J. Vis. Exp. 84, 50488 (2014).

Acknowledgements: Haleon provided funding to the project described. Don Whitley Scientific Travel fund provided funding for conference attendance.

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