Cell vs. bacterial viability in the presence of host defence peptides and RGD

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INTRODUCTION: More than 2 million people/year suffer a bone fracture in the UK[1]. Reconstruction of bone defects represents a major clinical challenge and is addressed using a number of medical devices. Although medical device compositions and applications may differ widely, all attract microorganisms and represent niches for medical device associated infections. For open fractures, the risk of infection can be 55%[2]. These infections are often resistant to many of the currently available antibiotics and represent a huge and growing financial and healthcare burden. The aim of this study was a fundamental understanding of how the presence of host defence peptides (HDPs)[3] and/or RGD can influence the outcome of cell vs. bacterial viability and proliferation.

METHODS: The antimicrobial activity of four HDPs; K5, K6, E6 and 1018, alone or in combination with RGD, was tested against three bacterial strains; *Staphylococcus (S.) aureus*, *S. epidermidis* and *Pseudomonas aeruginosa*, and their biofilms under both static and dynamic flow conditions using a commercially available microfluidic system, the BioFlux. Their performance was compared to Vancomycin, Polymyxin and Nisin, using a live/dead assay and luminescence.

The cytocompatibility of the above HDPs, alone or in combination with RGD was tested using breast cancer cell lines 231 and 468, human G-292 osteosarcoma cells, human antipose-derived stem cells (ADSCs, Lonza) and a MTT assay.

The performance of HDPs and/or RGD was also tested in *S. epidermidis* –G-292 co-culture systems using the BioFlux. *S.* epidermidis was introduced after an overnight G-292 culture.

RESULTS: The HDPs were effective against both Gram-positive and Gram-negative bacterial strains in the presence or not of RGD, while the RGD did not affect at all the bacteria viability. The E6 performed better against bacterial suspensions while the other three HDPs performed better against biofilms that were established using the BioFlux system. The antibiotics were more effective against bacterial suspensions than the HDPs at low concentrations such as 8 μg/ml; however the HDPs, in contrast to the antibiotics, were effective against both Gram-positive and negative strains and their biofilms, and performed better than Nisin.

The presence of HDPs at concentrations higher than 8 μg/ml significantly reduced the cell viability with the increase in HDPs concentration. The ADSCs were the most susceptible. The presence though of RGD at low concentrations in parallel to HDPs significantly increased the cell viability in comparison to HDPs alone.

In the case of *S. epidermidis* – G-292 co-culture systems in the BioFlux, the presence of HDPs significantly enhanced the cell viability, in comparison to the co-culture system without treatment, while the bacterial viability was reduced. The parallel presence of HDPs/RGD further supported cell viability and proliferation without compromising the antimicrobial and antibiofilm performance of the HDPs.

DISCUSSION & CONCLUSIONS: These results show promising signs for the use of RGD in combination with HDPs towards the preparation of antimicrobial materials that allow tissue integration. Orthopaedic implants would therefore be a great application for this kind of combined HDPs/RGD systems.

REFERENCES: [1] L.J. Donaldson, I.P. Reckless, S. Scholes, *et al.*, (2008). J Epidemiol Community Health **62**: 174-180.

[2] Open fractures at: http://orthotrauma.co.uk/open_fracture.htm

[3] A.L. Hilchie, K. Wuerth, R.E.W. Hancock. Nature Chemical Biology, 2013; **9**: 761-768.

ACKNOWLEDGEMENTS: Dr S. Farmer, Dr E. Haney, Dr A. Hilchie (UBC) are acknowledged for their support with the antimicrobial and cytocompatibility assessment of HDPs and Dr S. Patel (Fluxion) for his help with the BioFlux. This work and MGK secondement at UBC were funded through WELMEC under grant number WT 088908/Z/09/Z and iMBE. This template was modified with kind permission from eCM Journal.