Evaluation of Anti-Inflammatory and Antimicrobial Resorcinarene-Peptides for Biomaterial Modification

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INTRODUCTION: There is an increase in the use of implantable medical devices for the repair of soft and hard tissue. Many such devices can initiate acute inflammation, or become infected when implanted, resulting in device failure. α-Melanocyte-stimulating hormone (MSH) is a potent anti-inflammatory hormone[1], which also possesses antimicrobial properties[2], produced in the body with very short peptide sequences that make it amenable for easy laboratory synthesis. The aim of this work is to immobilise short MSH peptides onto medical device surfaces using molecules called resorcinarenes, which are known to attach to a wide variety of material surfaces. This is being approached by synthesizing MSH-resorcinarene molecules with the aim of being able to 'dip and dry' treat medical devices with an anti-inflammatory and antimicrobial 'coating'.

METHODS: Surfaces were coated with two compounds, resorcinarene-PEG-OMe, without the peptide moiety, and resorcinarene-PEG-GKP(D)V in varying molar ratios (0% to 100%), and characterised using XPS.

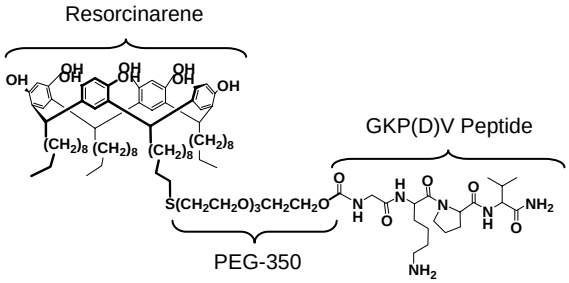

Figure 1: Schematic diagram of the GKP(D)V peptide attached to the resorcinarene compound via a PEG-350 tether, which is then coated onto glass.

The ability of the immobilized peptide to inhibit inflammatory signaling was determined by culturing RN22 Schwann cells upon the treated surfaces and measuring NF-κB/p65 inflammatory transcription factor activation.

RESULTS: *Surface Characterisation*; XPS indicated that the GKP(D)V peptide was immobilized onto the glass surface.

NF-κB Activation; Unstimulated cells exhibited predominately cytoplasmic labelling while stimulation of the cells with LPS (100 $ngml^{-1}$) caused rapid translocation, and therefore activation, of NF-κB to the nucleus. Culturing cells on resorcinarene-monoPEG-OMe coated surfaces had no effect on NF-κB activity. In contrast culturing cells on resorcinarene-monoPEG-GKP(D)V coated surfaces inhibited LPS stimulated NF-κB activation by up to 28.2±4.0% ($p<0.001$). Levels of inhibition were comparable to those observed when cells were co-stimulated with GKP(D)V at 10^{-9} M and LPS (38.9±3.3%; $p<0.001$) (Fig 2).

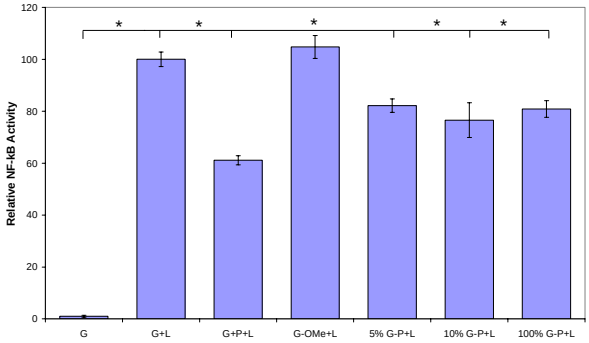

Figure 2: Immobilised GKP(D)V inhibits NF-κB activity. G, glass; L, LPS; P, GKP(D)V peptide; G-OMe, resorcinarene-monoPEG-OMe; G-P, resorcinarene-monoPEG-GKP(D)V; n=3, *$p \leq 0.05$

CONCLUSIONS: Results indicate the GKP(D)V peptide has been immobilised onto glass using resorcinarene chemistry and retains anti-inflammatory properties. Future work involves the investigation of the antimicrobial properties of KP(D)V peptide, and related melanocortin peptides, in solution and when immobilised.

REFERENCES: [1]R. P. Hill, S. MacNeil, and J.W. Haycock, (2006) *Peptides* **27**; 421-430. [2]M. Cutuli, S. Cristiani, J. M. Lipton, A. Catania, (2000) *J. Leukoc Biol* **67**; 233-239.

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