

Evaluation of Anti-Inflammatory Calixarene-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, resulting in device failure. The co-delivery of anti-inflammatories together with the device is proposed as a therapeutic strategy to reduce excessive inflammation. α -Melanocyte-stimulating hormone (MSH) is a natural and potent anti-inflammatory hormone 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 calixarenes, which are known to attach to a wide variety of material surfaces. This is being approached by synthesizing MSH-calixarene molecules with the aim of being able to 'dip and dry' treat medical devices with an anti-inflammatory 'coating'.

METHODS: Initial studies indicated the anti-inflammatory properties of the analogue of MSH, GKP(D)V, were retained when joined to a PEG-350 tether. The PEG-GKP(D)V moiety was then attached to the calixarene molecules and coated onto glass coverslips. Surfaces were coated with two compounds, calixarene-PEG-OMe, without the peptide moiety, and calixarene-PEG-GKP(D) in varying molar ratios (0% to 100%).

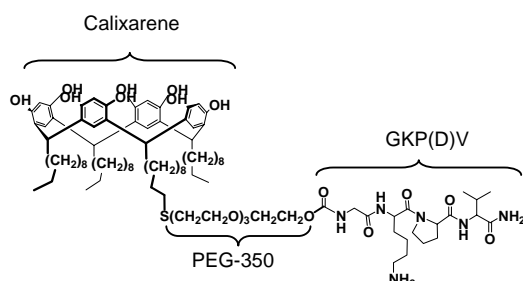


Figure 1: Schematic diagram of the GKP(D)V peptide attached to the calixarene compound via a PEG-350 tether, which is then coated onto glass.

The surface was characterised using XPS, MALDI-ToF-MS and contact angle goniometry. Human dermal fibroblast cells were then cultured onto the coated glass coverslips for 48 hours and stimulated with TNF- α (1000Uml⁻¹) for 120 minutes. Cells were then immunolabeled for the p65 subunit of NF- κ B to monitor acute inflammatory signalling. Whole cell counts were

performed, cytoplasmic labelling indicated inactive NF- κ B and nucleic labelling indicated active NF- κ B.

RESULTS:

Surface Characterisation. XPS and MALDI-ToF-MS indicated that the GKP(D)V peptide was immobilized onto the glass surface.

NF- κ B Activation. Unstimulated cells exhibited predominately cytoplasmic labelling regardless of the surface upon which they were cultured. Stimulation of the cells with TNF- α caused rapid translocation, and therefore activation, of NF- κ B to the nucleus. Culturing cells on calixarene-PEG-OMe coated surfaces had no effect on NF- κ B activity. In contrast culturing cells on calixarene-PEG-GKP(D)V coated surfaces inhibited TNF- α stimulated NF- κ B activation by up to 14.5 \pm 3.0% (n=3, p=0.001). Levels of inhibition were comparable to those observed when cells were cultured on to glass and then incubated with both GKP(D)V at 10⁻⁹ M and TNF- α (9.4 \pm 2.6; n=3, p=0.003).

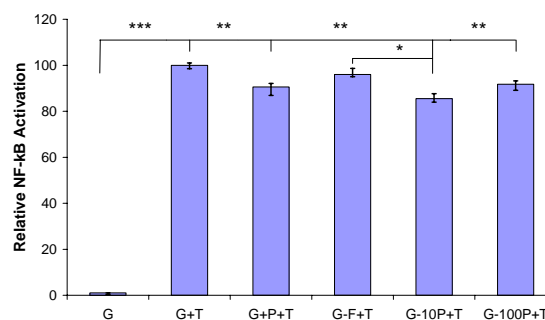


Figure 2: Immobilised GKP(D)V inhibits NF- κ B activity. TNF- α (1000Uml⁻¹); P, GKP(D)V peptide; G, glass; F, calixarene-PEG-OMe; G-P, calixarene-PEG-GKP(D)V; n=3, *p \leq 0.05, **p \leq 0.001, ***p \leq 0.005.

DISCUSSION & CONCLUSIONS: Initial results indicate the GKP(D)V peptide has been immobilised onto a glass surface using calixarene chemistry and retains anti-inflammatory properties. This strategy supports ongoing research into its application as an anti-inflammatory coating for biomaterials.

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