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Biofunctionalized instructive polycaprolactone scaffolds for the specific recruitment of blood endothelial cells

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Introduction: Functionalisation of polycaprolactone (PCL) scaffolds to allow the recruitment of circulating endothelial progenitor cells (EPCs) requires the 'decoration' with specific molecules. This facilitates specific cell recruitment, tissue formation, and enhancement of material biocompatibility. TPS (TPSLEQRTVYAK) is a novel peptide selected for its high affinity to endothelial cells derived from blood progenitors (blood outgrowth endothelial cells; BOECs)[1]. In this work, we aimed to biofunctionalize jet sprayed PCL[2] with a cysteine-containing TPS sequence using the sulfhydryl-reactive-crosslinker maleimide.

Methods: The effect of TPS (1- 1000µM) on the proliferation of BOECs (grown from peripheral blood mononuclear cells; PBMCs) was determined by AlamarBlue® assay, and CXCL8, prostacyclin (6-ketoPGF$_{1\alpha}$) and endothelin(ET)-1 release by ELISA. Sulfhydryl-crosslinking of TPS to PCL films and scaffolds was done by surface activation using 20% ethylenediamine (EDA) for 1hr at 40^{o}C with shaking. PCL was incubated with PEG-NHS-Prop-Mal (1mg/ml) for 2hrs at room temperature with agitation, followed by overnight incubation with 1mg/ml of the peptides in sodium bicarbonate solution (50mM; pH9). The effect of modification on PCL wettability was estimated by contact angle (CA) measurement, and the efficiency was confirmed using a fluorescently tagged peptide. The ability of modified-PCL to attract BOECs was studied using cell capture assay; pre-stained BOECs were incubated with TPS-modified-PCL for 1hr, followed by counting the number of adhered cells per cm^2.

Results: Treating BOECs with increasing concentrations of TPS significantly increased their proliferation ($p<0.05$,n=6; 142.85±23.41%). CXCL8 release was significantly reduced by 1000µM TPS (3109.97±778.77 compared to untreated control 6097.98±1674.52) whilst ET-1 and prostacyclin were unaffected. EDA treatment significantly reduced CA, indicating enhanced wettability. TPS-modified-PCL films and scaffolds were able to specifically induce the adhesion of BOECs ($p<0.05$,n=3, 193.67±38.19 compared to unmodified PCL 36.14±5.33)

Conclusion: Viability of BOECs and their cytokine release in response to TPS indicates that this peptide is a good candidate for biofunctionalisation. In this study, we were able to implement a controlled highly-specific crosslinking reaction through sulfhydryl-crosslinking of TPS to PCL, which ensures the high functionality of the resulting biocomposite. The results showed that this biocomposite is able to specifically attract BOECs, which is a promising step towards the development of an acellular instructive implant for *in situ* tissue engineering applications. We are working on investigating the ability of this biocomposite to recruit EPCs from PBMS in a bioreactor mimicking physiological shear conditions.

References:

1- Veleva AN *et al*. (2007). Biotechnol Bioeng **98(1)**: 306-312.

2- Sohier J *et al.* (2014). Biomaterials. **35(6)**: 1833-44.