Identification of Immunomodulatory Topographies to Regulate Myofibroblast Differentiation and Influence Fibrous Encapsulation

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Introduction

Macrophages and fibroblasts are known to be key contributors of foreign body response. Macrophages can change their phenotype and secrete cytokines that trigger fibroblast differentiation[1]. Previous studies prove that the macrophages can be modulated using physical cues like topographies and can change their immune profile[2]. In this study, we took a combinational approach to find topographies that can alter macrophage secretion profile as well as fibroblast differentiation to modulate FBR. This was tested in a condition called glaucoma which is treated using drainage devices to maintain IOP in the eye.

Methods

A poly (styrene-block-isobutylene-block- styrene) (SIBS) Topochip containing 2176 distinct topographies was fabricated using hot embossing. Three screens were performed, one with primary tenon fibroblasts where we quantified expression of the trans-differentiation marker alpha-smooth muscle actin (α-SMA) and a second where proliferation was quantified through EdU staining by automated image analysis. A third screen was done using primary macrophages for differential attachment. Surfaces were ranked and hits from the screen were chosen and validated for the same readouts and multiplex ELISA was done where macrophage's secretion profile was quantified for pro- and anti-inflammatory cytokines.

Results and discussion

Topographies showed strong effect on tenon fibroblast morphology and stress fiber formation relative to flat control. α-SMA and EdU show a 4-fold differences between top and bottom hits. We opted to go for a dynamic three- pronged approach in selection of the hits for validation work that will be used for *in vivo* model, one for tissue integration, other for pro-encapsulation and lastly for anti-fouling. Chosen hits were fabricated and validated. Among them, three topographies were chosen, Topography 1153 was chosen for pro-encapsulation, topography 79 for tissue integration and topography 509 for anti-fouling. Chosen hits were correlated with cytokine analysis where we found topography 1153 with high levels of pro-inflammatory cytokines IL-6, IL-1β whereas topography 79 showed higher levels of arginase, IL-1Ra with low levels of IL-6, IL- 1β compared to other hits.

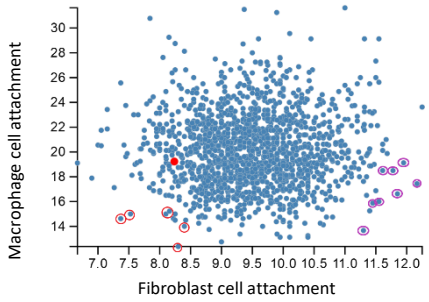

Fig: Scatter plot showing the screening results from which hits chosen were rounded. Plotted with macrophage and fibroblast attachment on X-axis and Y-axis respectively shows hits chosen for antifouling (red) and tissue integration fibroblasts (purple). Red dot represents flat surface.

Future Work

Based on the screening and validation results, we chose three topographies which were used to fabricate devices for the animal trials to test which can induce a better bleb survival and low encapsulation in *in vivo* rabbit model.

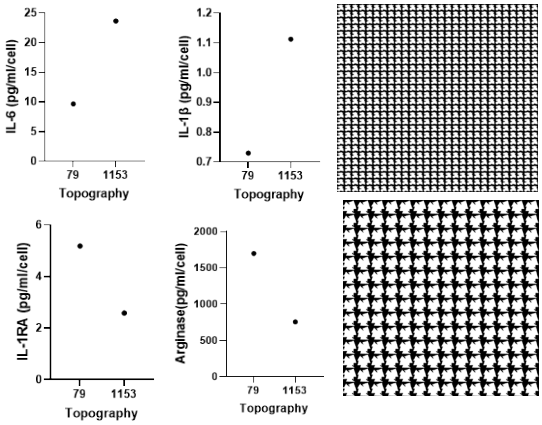

Fig: Cytokine analysis for the selected topographies Feature Idx 79 (top), 1153 (bottom) normalized per cell for Il-6, Il- 1β and IL-1 Ra and Arginase showed differences.

References

1. Anderson, J. M. *BIOLOGICAL RESPONSES TO MATERIALS*. www.annualreviews.org (2001).
2. Vassey, M. J. *et al.* Immune Modulation by Design: Using Topography to Control Human Monocyte Attachment and Macrophage Differentiation. *Advanced Science* **7**, (2020).