Dynamic inflammatory stimulation of hiPSC-endothelial cells in a multiplexed microfluidic chip

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Introduction

Immunotherapies such as chimeric antigen receptor (CAR) T-cell therapies are very promising cancer treatments. However, a frequent severe side effect is the cytokine release syndrome (CRS) which can lead to multi-organ failure and even death in patients. CRS is caused by the dysregulation of cytokine levels released by immune cells and the endothelium, whereby several of the underlying mechanisms are not yet understood. Understanding the response of the endothelium to the dynamics of cytokines released is a crucial challenge [1]. Here we use our previously reported multiplexed microfluidic chip (Fig. 1a, b) [2] to expose human induced pluripotent stem cell (hiPSC)-derived endothelial cells (ECs) to tumor necrosis factor (TNF)-α in a dynamic and automated manner. Specifically, we stimulate the cells at two different time points and analyze their ICAM-1 expression.

Experimental procedure

32 chambers were coated with 0.1 mg/mL rat tail collagen I. hiPSC-derived ECs were seeded at 1.5×10^6 cells per mL. The cells were exposed to a dynamic dosing regimen (Fig. 1d) in which they were first cultured for 3 days while EC growth medium (ECGM) was automatically refreshed every 3h, and then treated with 10 ng/mL of TNF-α for 24h, once (on D0 or D3), twice (on D0 and D3) or not at all ('None'). On day 4, the cells were fixed and fluorescently labeled using NucBlue, F-Actin Red and immunostained for ICAM-1 using AlexaFluor 488 as the secondary antibody (Fig. 1c).

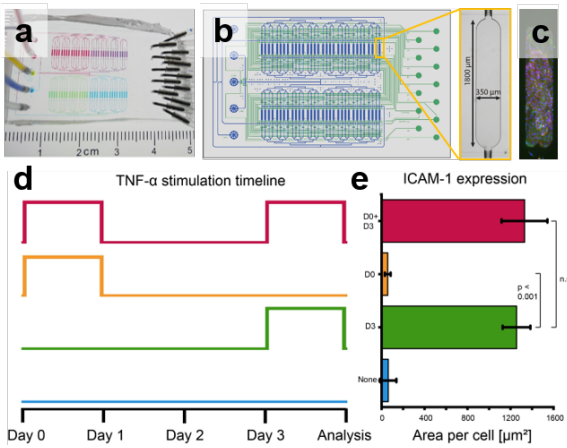

Figure 1: Dynamic dosing of TNF-α in a multiplexed microfluidic chip. a) photograph of chip, b) schematic of chip with culture chambers in blue and control valve channels in green [2]. Inset, single culture chamber. c) hiPSC-EC in chamber. d) Dynamic dosing regimens for TNF-α. e) ICAM-1 expression in dynamic dosing. n=4 for 'None', and n=8 for all others.

Results and Discussion

Fig. 1e shows the average ICAM-1 coverage per cell after thresholding. Both the D0 + D3 and D3 chambers showed significantly higher ICAM-1 expression ($p<0.001$) than the D0 and 'None' chambers. The D0 + D3 coverage was not significantly higher than of D3. The low D0 coverage indicates that the cells had recovered from the TNF-α treatment.

Conclusion and Outlook

Here we demonstrated that we can use a chip with highly parallelized cell culture chambers and discontinuous perfusion to treat hiPSC-ECs with different dosing regimens and subsequently analyze their ICAM-1 expression. The next step will be to use this chip to scan several different dosing regimens in long-term culture (weeks) and to use the technology to expose more complex 'vessels-on-chips' to such dynamic dosing regimens.

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