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Tracking Phenotypic Heterogeneity at the Single Cell Level in Populations of Vascular Cells Through the Development of Microwell Arrays

E. Smith[1], M. Zagnoni[1], **M.E. Sandison**[2]

[1] Electronic and Electrical Engineering, Royal College Building, University of Strathclyde, G1 1XW, Glasgow
[2] Biomedical Engineering, Graham Hills Building, University of Strathclyde, G1 1XQ, Glasgow

Introduction

Significant remodelling of the vascular wall underlies cardiovascular disease resulting in the formation of atherosclerotic plaques populated with macrophage and smooth muscle cells (SMCs). These SMCs are thought to arise from the vessel wall, as mature SMCs de-differentiate from a contractile to a migratory, proliferate phenotype. However, the remodelling process is not fully understood and uncertainties remain over the plasticity of cells within the wall. Both drug development and regenerative medicine have been restricted by these uncertainties. Recently, through a combination of time-lapse, high-speed fluorescence and 3D reconstruction microscopy, we demonstrated unambiguously [1] that freshly isolated mature, contractile SMCs can rapidly transform into not only a migratory but a phagocytic phenotype, a characteristic behaviour of macrophage. Results also showed strong heterogeneity in the proliferation of SMCs [2] and the presence of other highly proliferative cell types in vascular wall that readily interact with SMCs. To better understand vascular cell fate, including characterizing cell-cell phenotypic heterogeneity, we are developing strategies that enable screening at the single cell level of large numbers of freshly isolated vascular cells.

Methods

We have employed two different microfabrication strategies to create a series of addressable microwell arrays: firstly using the epoxy-based negative photoresist SU-8 3050 to form 50 μm deep wells patterned over a glass substrate; and secondly adapting a soft lithography approach [3] to produce a 100 μm thick poly(dimethylsiloxane) (PDMS) membrane with through-holes (to act as wells) that is oxygen plasma bonded to a glass substrate. By incorporating individual microwells of different areas (from 60x60 to 180x180 μm) and seeding with a suspension of freshly isolated cells of appropriate density, cells are sedimented stochastically across the microwell arrays such that many wells contain single cells. These cells are imaged *in situ* prior to inducing de-differentiation by exposure to serum-containing culture media (10% FBS) and subsequent changes in their phenotype are tracked (morphology, proliferation, end-point immunocytochemistry).

Results & Discussion

Single native SMCs have been successfully seeded into microwells and tracked for 1 week in culture. Time-lapse microscopy was used for validation and clear variation in the proliferation of individual cells was observed (from a single division to reaching the 4th generation over 7 days), as well as heterogeneity in the expression of SMC markers (e.g. SMA) in the resulting daughter cells. In this work, we aim to investigate phenotypic changes in single-cells from pure populations of SMCs, which we obtain by a multi-step enzymatic digestion process, as well as in mixed vascular cell populations (e.g. including “non-SMC” from the adventitia) where clusters of 2-4 cells are seeded per well. This will enable us to investigate how cell-cell interactions affect the resulting phenotype of both SMC and other cells in the vascular wall (e.g. progenitor-like cells). Unexpectedly, in mixed populations, non-SMC did not stay confined within their wells and were able to migrate along SU-8 walls (previously studies have reported no cell attachment to SU-8 [4]). SMCs did stay confined but often adhered to and spread along the sidewalls, hindering image analysis. Therefore, our ongoing work is focused on the use of PDMS-based arrays and the capture of expanded data sets where phenotype is quantified by live-cell imaging of proliferation, microbead uptake and Ca^{2+} responses to pharmacological stimulation.

Conclusion

This microwell array approach, which is amenable to drug screening applications, will enable detailed characterization of phenotypic changes in vascular cell sub-population and will provide new insights to inform tissue engineering applications.

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References

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