Microwell arrays for monitoring phenotypic heterogeneity in vascular cell populations

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Significant remodeling 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 remodeling process is not fully understood and uncertainties remain over plaque cell origins and the plasticity of cells within the vascular 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 proliferative capacity of SMCs [2] and the presence of other highly proliferative cell types in vascular wall that readily interact with SMCs. To better understand vascular call fate, including characterizing the phenotype of cell subpopulations, we employed SU-8 microfabrication to create a series of addressable microwell arrays that enable screening at the single cell level of large numbers of freshly isolated vascular cells. By incorporating microwells of different areas (from 60x60 to 180x180) and seeding with a cell suspension of appropriate density (either a pure SMC population or a mixed vascular population), cells sedimented stochastically across the microwell arrays such that many wells contained single cells. These cells were characterized by imaging in situ prior to tracking them for >1 week as they were induced to de-differentiate in culture. To validate this approach, variation in the proliferation of individual cells was tracked and the expression of SMC markers (e.g. SMA) following phenotypic modulation quantified. This microwell array approach, which is amenable to drug screening applications, will enable detailed characterization of phenotypic changes in vascular cell sub-populations, providing new insights to inform tissue engineering applications.

Keywords: cardiovascular disease, vascular cell population, smooth muscle cells, vascular wall remodelling

References

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