Cryopreserving freshly isolated smooth muscle cells for large scale analysis at the single cell level

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Single-cell analysis of vascular smooth muscle cells (vSMCs) is central to advancing our understanding of cardiovascular diseases. The transition of vSMCs from a contractile to a migratory, proliferative phenotype is central to the remodelling of the vascular wall, which is thought to be driven by a sub-population of vSMCs. Microarray technologies are inherently suited to these studies, offering large-scale solutions for single cell confinement and tracking of cell fate. However, challenges are present when working with freshly isolated vSMCs, as these quickly deteriorate in buffer and there is a limited time before they lose their native phenotype in culture. To reduce animal use whilst maximising the number of viable cells, robust cryopreservation of native vSMCs is needed.

Here, we assess the three cryopreservation reagents (Cellbanker 1®, Cellbanker 2® and 10% DMSO) on the viability and proliferative capacity of rat aortic vSMCs. Trypan blue and MTT assays were used to quantify cell viability and proliferation in the short and long term. Hundreds of single vSMCs were also monitored by microscopy to study phenotypic diversity and proliferation over >1 week, using microfabricated cellular arrays comprising addressable microwells with cell-repellent walls (Lipidure®-CM coating) and a cell-adherent base. Results show that cryopreservation does not affect cell phenotypic plasticity, nor variation in proliferative capacity. However, the viability of primary cells was significantly reduced by cryopreservation in DMSO (78% of fresh) and to a lesser extent in Cellbanker2 (85%) but not in Cellbanker 1 (95%). Following 1 week culture, significantly reduced cell growth was obtained only when using DMSO (10% of that of freshly isolated cells), with clear improvement when using Cellbanker® 1&2 (40%-60%). These results highlight the potential advantages of adapting such cryopreservation methodologies for use with limited human tissue samples.