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

E. Smith,[a,c], P. Pridham-Field[b], A. Sim[b], M. Zagnoni[a], M.E. Sandison[c]

a Electronic & Electrical Engineering, University of Strathclyde, Glasgow, UK

b AMS Biotechnology (Europe) Ltd, Abingdon, UK

c Biomedical Engineering, University of Strathclyde, Glasgow, UK

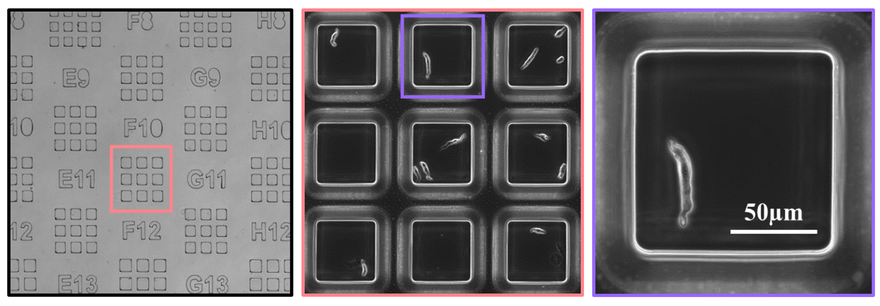
INTRODUCTION

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 and is thought to be driven by a sub-population of vSMCs. Microarray technologies are inherently suited to offer large-scale solutions for single cell analysis 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.

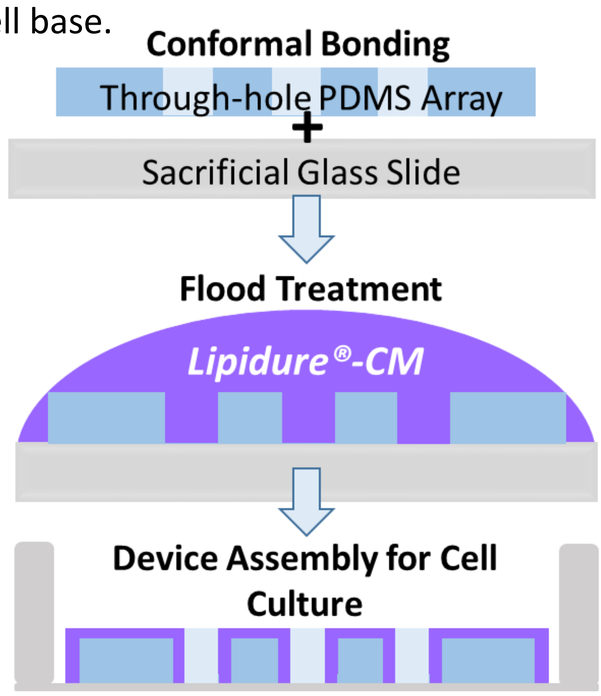
METHODS

In this work, we assess the effects of three cryopreservation reagents (Cellbanker® 1, Cellbanker 2® and commonly used 10% DMSO) on the viability and proliferative capacity of rat aortic vSMCs.

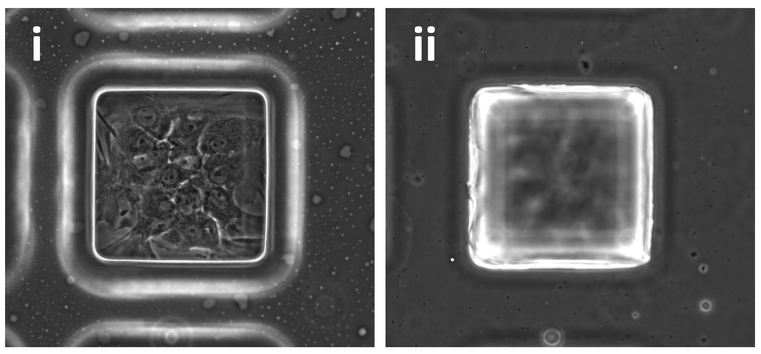
Microfabricated cellular arrays: addressable microwells were produced by first creating a thin (100µm) poly(dimethylsiloxane) (PDMS) membrane containing square through-holes (from 100x100 to 140x140 µm), method adapted from Hsu *et al* [3].



Surface functionalisation: flood exposure of the PDMS with Lipidure®-CM was employed to achieve highly cell repellent walls. Bonding the membrane to a glass coverslip-bottomed dish provided a cell-adherent microwell base.

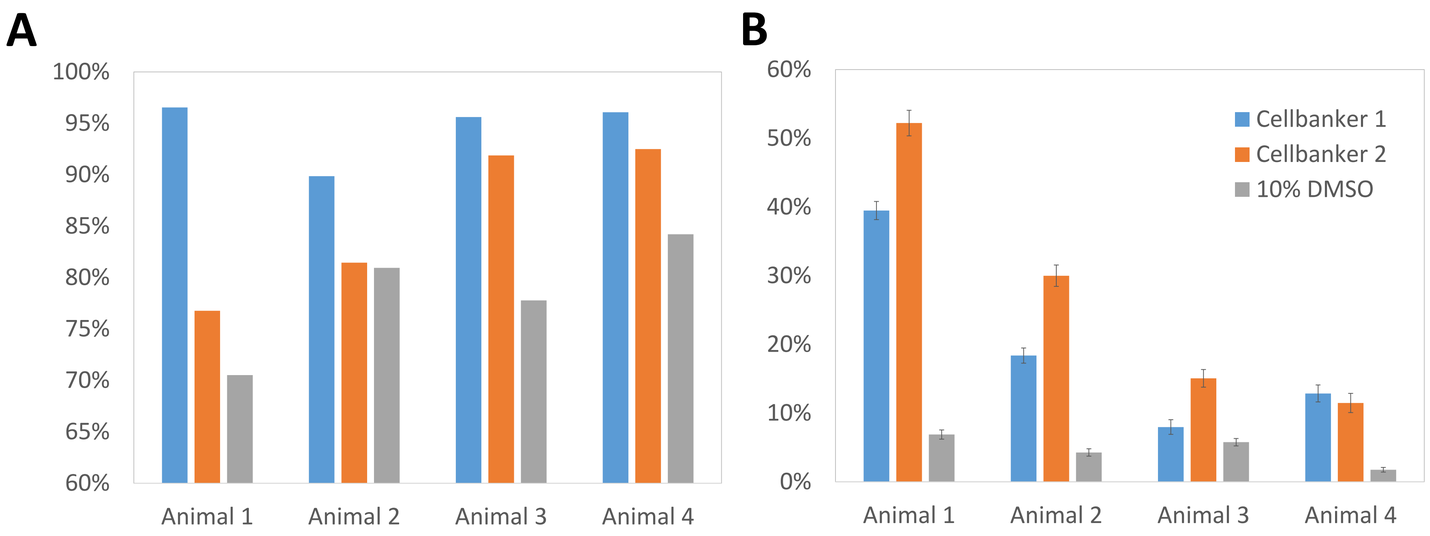


Cell viability: Trypan blue and MTT assays were used to quantify cell viability and proliferation, post-thaw and after 1 week in culture, respectively.



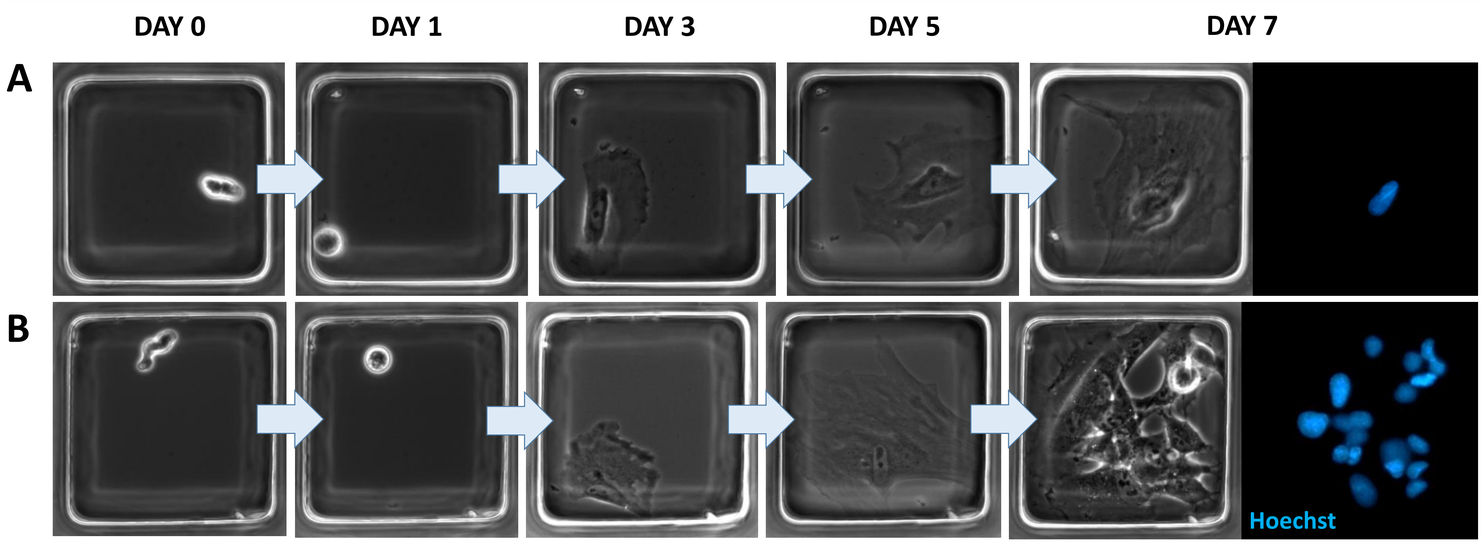
Cell tracking: Hundreds of single vSMCs were monitored at fixed time points (day 0, 1, 3, 5, 7) to study phenotypic diversity and proliferation over >1 week. Cells were imaged *in situ* prior to inducing phenotypic modulation (by addition of serum). Robust cell confinement was maintained following freeze-thaw of a Lipidure®-CM coated array: i) highly proliferative microwell after 8 days in culture; ii) top surface of same microwell showing no cell migration out of well.

Results 1. vSMC viability following cryopreservation



The effects of three cryopreservation reagents (CELLBANKER® 1, CELLBANKER® 2 and 10% DMSO) on the viability of rat aortic vSMCs. Trypan blue (**A**) and MTT assays (**B**) were used to quantify post-thaw cell viability and subsequent proliferation over a 1 week period (normalised to fresh cells) respectively.

Results 2. Tracking single vSMC morphology & proliferative capacity

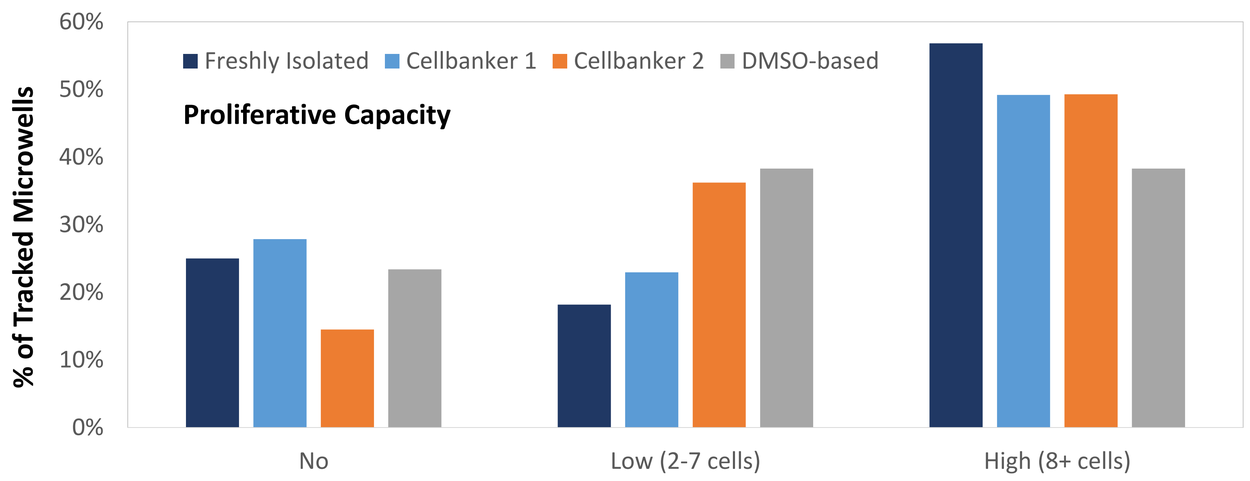


Examples of vSMC tracking in microfabricated cellular arrays achieved following cryopreservation in CELLBANKER® 2, phase contrast images illustrate changes in cell morphology and heterogeneity in proliferation over 1 week, with endpoint nuclear staining on day 7 (blue for cell nuclei)

A) A single starting cell which remains as a single undividing cell after 7 days in microarray culture.

B) A single starting cell which divides rapidly after day 5, producing a confluent well on day 7.

Results 3. Heterogeneity in the proliferative capacity of vSMCs



As single vSMCs were confined to their own microwell, all cells subsequently present in the microwell were the progeny of the original parent cell. Cells were counted on day 7 for wells that contained a single vSMC on day 0 or became a single-cell through apoptosis (*n* animals = 1). Similar trends in the distribution of single-cell proliferative capacities were observed with and without cryopreservation.

CONCLUSION

- Cryopreservation did not affect cell phenotypic plasticity nor proliferative capacity
- Cellbanker® 1&2 significantly outperformed DMSO-based protocols

References

[1] Sandison M.E. et al, *J Physiol,* 594(21):6189-6209 (2016); [2] Anggraini D. et al, *Lab on a Chip*, 22 1438-1468 (2022); [3] Hsu C.H., Chen C., Folch A. *Lab Chip,* 4:420-424 (2004)