(Cryo)preservation of lacrimal gland tissue and cells: towards curative regenerative medicine therapies for treatment of dry eye syndrome

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Scientific objective

Severe dry eye syndrome (DES) can cause blindness but despite being relatively prevalent, only palliative treatment options exist. *In vitro* lacrimal gland (LG) tissue reconstruction, ideally using autologous cells, could provide a curative treatment. Since just-in-time manufacture to GMP is logistically complex and inefficient (particularly for autologous therapies), ability to store lacrimal gland tissue or cells indefinitely until required, is desirable. We aimed to determine the feasibility of successful cryopreservation or hypothermic storage (HS) at 4°C of LG tissue and epithelial cells.

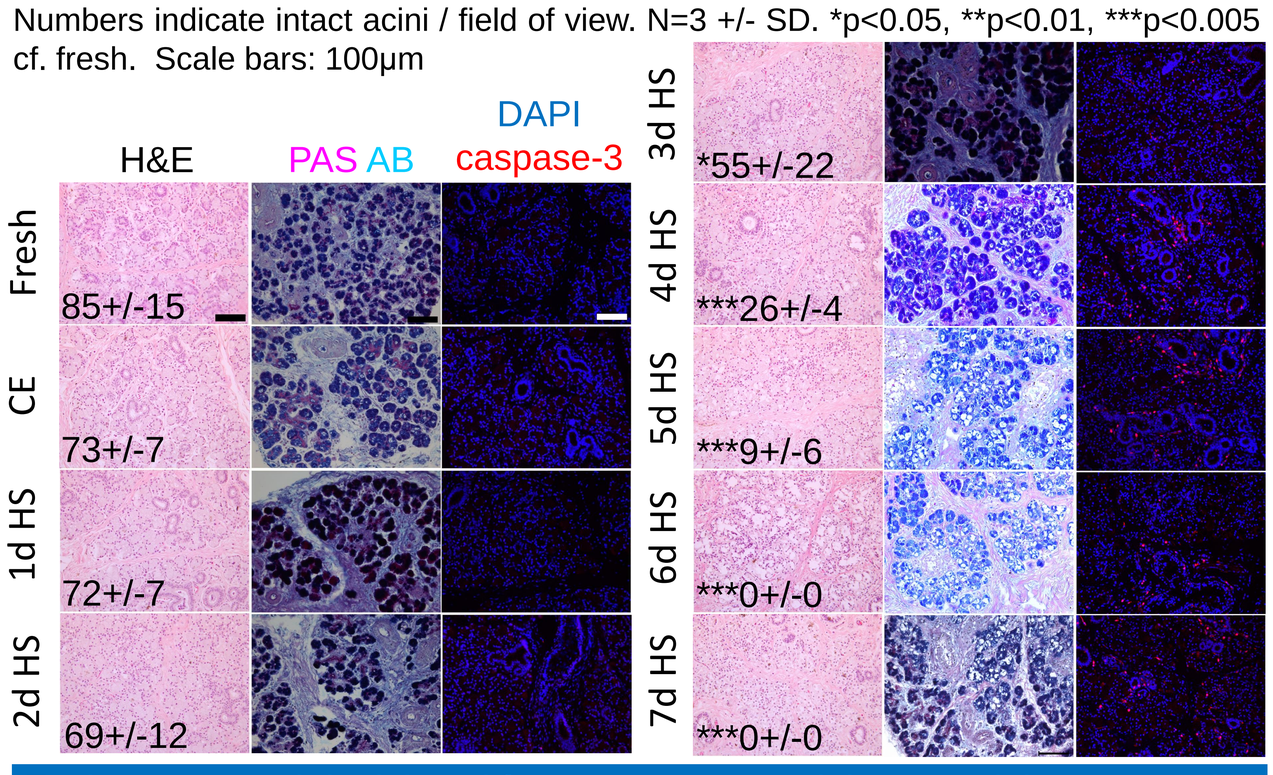
Method

Explant cultures were initiated from 1mm^3 pieces of fresh (F), hypothermically stored (HS) or cryopreserved (cooled at ~1°C/minute in 10% DMSO, 20% foetal calf serum) (CE) porcine LG tissue.
Tissue architecture was assessed using H&E, Periodic acid Schiff, alcian blue (mucins) and caspase-3 immunohistochemistry (apoptosis).
Timing and efficiency of outgrowth from, and cell yields per explant were assessed.
Cells obtained from fresh explants were also cryopreserved as above (CS).
In P1, proliferation (WST-1), colony forming efficiency, protein expression (Rab3D, HexA, lysozyme) and secretory activity in response to parasympathetic stimulation with carbachol (CCH) were evaluated.

Results

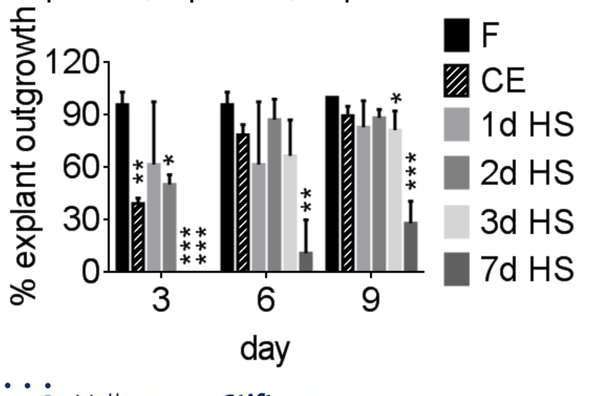
In fresh tissue and cryopreserved explants, acini are visible and there is no caspase activation. In tissue stored at 4°C, acini density decreases and caspase-3 is activated over time.

Numbers indicate intact acini / field of view. N=3 +/- SD. *p<0.05, **p<0.01, ***p<0.005 cf. fresh. Scale bars: 100µm



Explant outgrowth is delayed from cryopreserved tissue and tissue stored at 4°C compared with fresh tissue. Cell yields from all groups are equivalent (~1.25x10^5 / explant) except tissue stored for 7 days at 4°C.

*p<0.05, **p<0.01, ***p<0.005 cf. fresh



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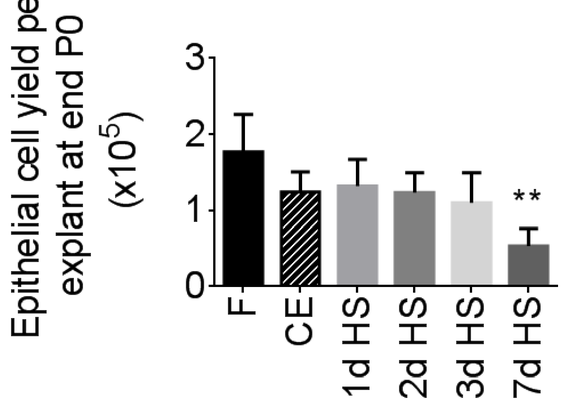
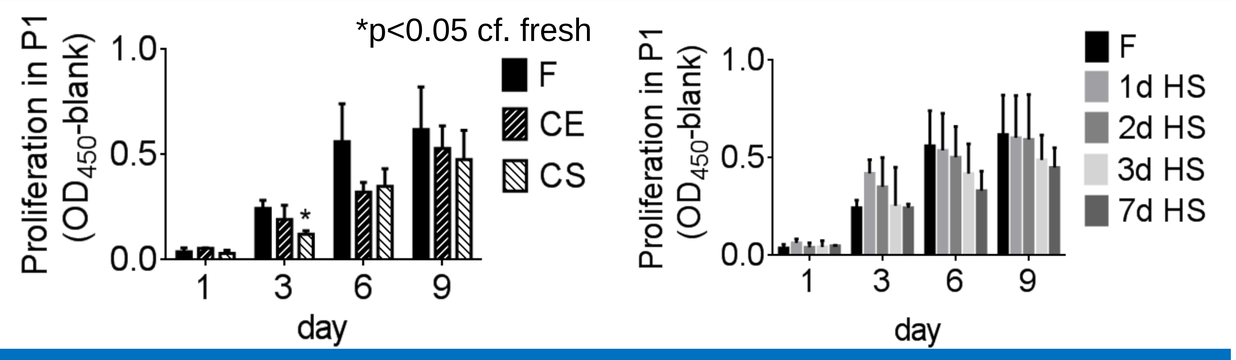
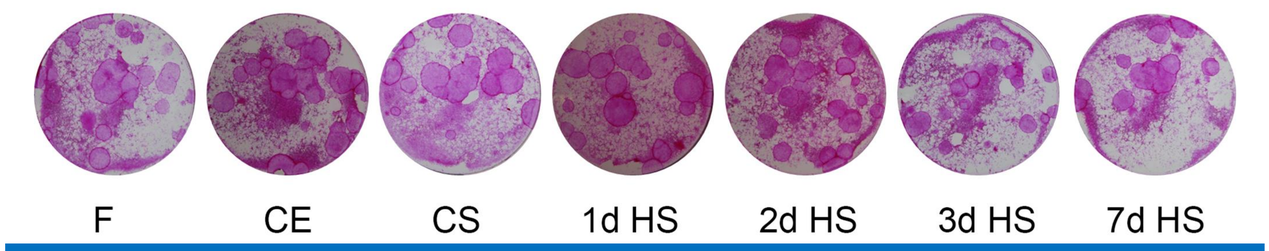


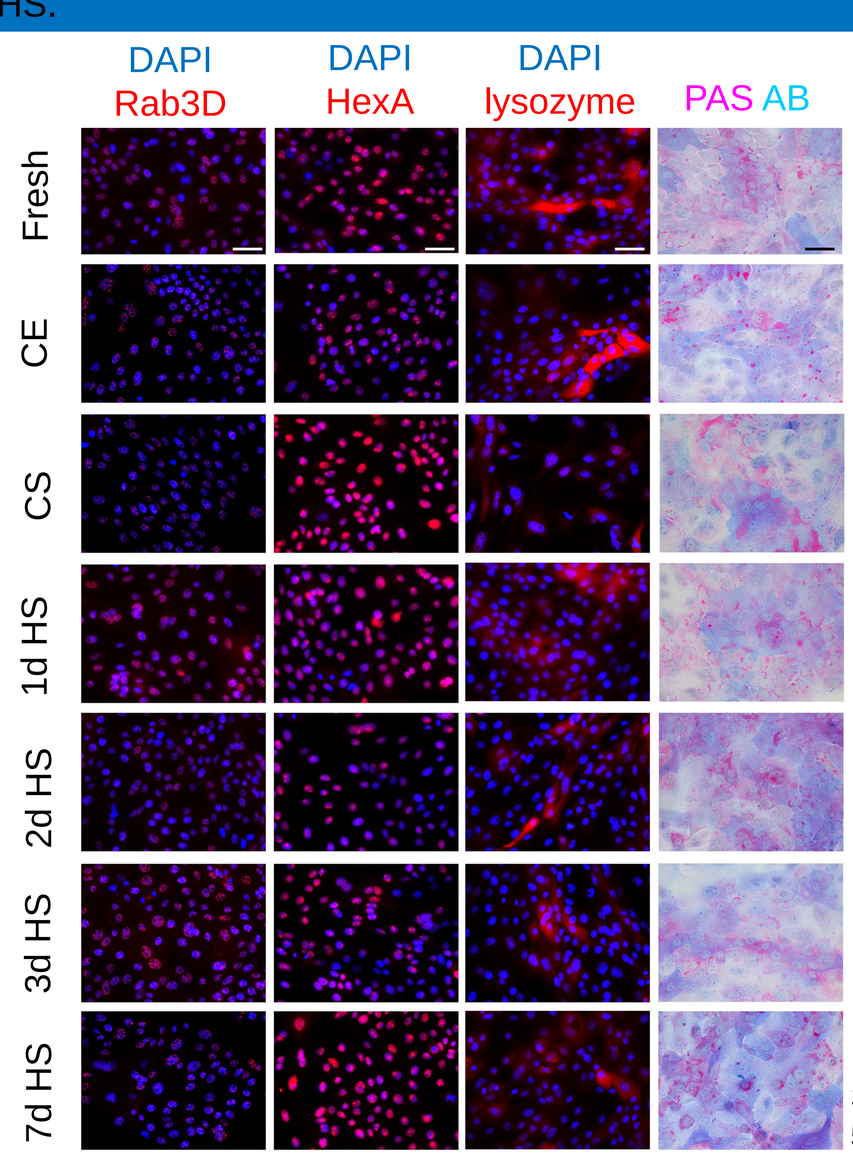
Proliferation is largely equivalent between cells isolated from fresh tissue, cryopreserved explants and tissue stored at 4°C.



CFEs are equivalent (~20%) in all groups



Expression of Rab3D, HexA and lysozyme in all groups is similar, as is mucin secretion. All groups show a significant response to CCH stimulation except CS and 7d HS.



Scale bars: 50µm

	Fresh	CE	CS	1d HS	2d HD	3d HS	7d HS
Baseline β-hex activity (FU 355/460)	1897 +/- 296	1431 +/- 229	1432 +/- 649	1216 +/- 1099	1326 +/- 165	1757 +/- 250	2037 +/- 385
CCH-stimulated β-hex activity (FU 355/460)	3751 +/- 295	2327 +/- 457	2402 +/- 417	2265 +/- 869	3019 +/- 797	2632 +/- 285	2208 +/- 597
Statistics	p<0.05	p<0.05	n.s.	p<0.05	p<0.05	p<0.05	n.s.

Conclusions

LG cells or tissue (in small portions) appear relatively robust to cryopreservation and so may be stored indefinitely until required. Storage of tissue at 4°C beyond a few days appears sub-optimal, although cells yielded from this tissue are similar to fresh. This is useful towards manufacture and delivery of curative regenerative medicine therapies for DES.

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