We are developing a 3D tissue engineered model of oral lichen planus

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What is OLP and why developing the model is important?

Oral lichen planus (OLP) is a common T cell immune-mediated mucocutaneous disease of unknown aetiology.

. Investigations into this condition are hampered by the lack of effective experimental models to study the disease (1).

Our aim is to develop a tissue-engineered oral mucosal model containing activated T cells to replicate OLP for use in the development of novel treatment strategies.

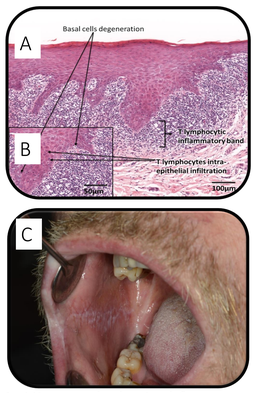
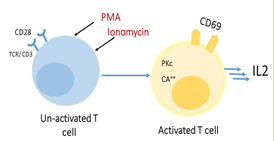


Fig 1: Histopathological features of oral lichen planus. (A) 20x magnification, and (B) 40x magnification. (C)Clinical presentations of oral lichen planus on buccal mucosa

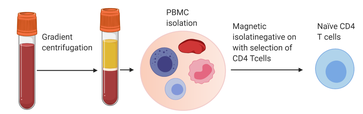
What have we done?

1 Jurkat cells activation



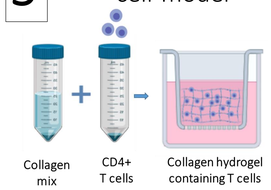
2 Jurkat cells were transferred from RPMI into Green's medium and their proliferation was determined with cell trace.

3 T cells isolation from blood

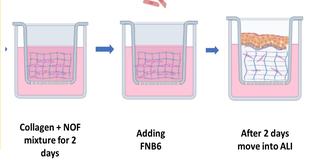


4 Polarising CD4+ into Th1 cells. Differentiation confirmed with flow cytometry, ELISA, q-PCR, and western blot

5 An experimental T cell-model



6 Tissue engineered Oral Mucosa



Summary

- We have optimised our protocols for the activation and transferred T cells from RPMI into Green's media.
- We have polarised CD4+ T cells isolated from buffy coat into Th1 which are the main T helper phenotype responsible for the OLP pathogenesis (2).
- We have assessed viability of Jurkat cells in 3D collagen hydrogel and created Full thickness oral mucosal model.

Results

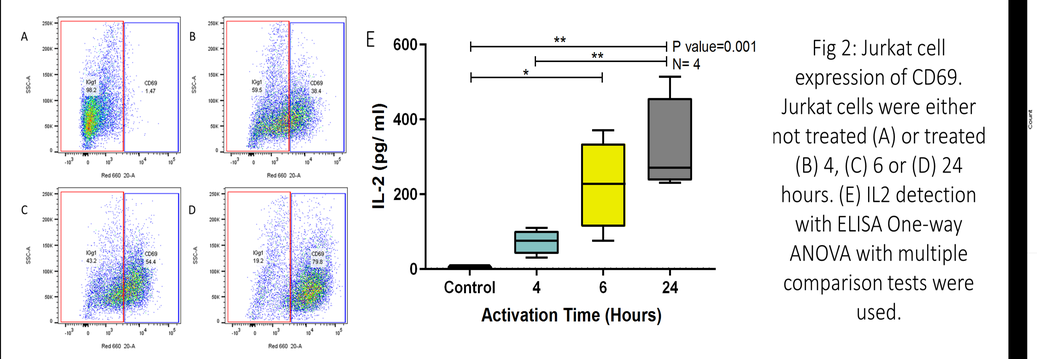


Fig 2: Jurkat cell expression of CD69. Jurkat cells were either not treated (A) or treated (B) 4, (C) 6 or (D) 24 hours. (E) IL2 detection with ELISA One-way ANOVA with multiple comparison tests were used.

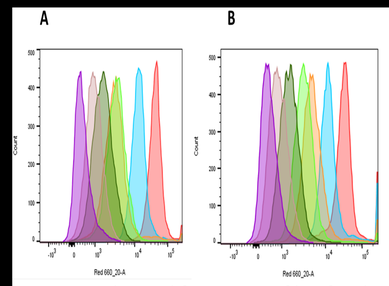


Fig 3: The proliferation profile of Jurkat was measured in (A)RPMI and (B) Green's medium using a Far-Red CellTrace dye.

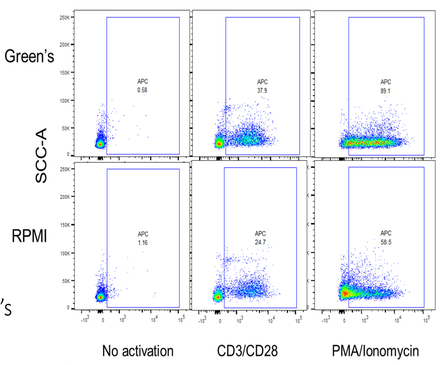


Fig 4: CD4+ T cells were either not activated, activated with CD3/CD28, or with PMA and Ionomycin in both Green's and RPMI. CD69 was detected with Flow cytometry

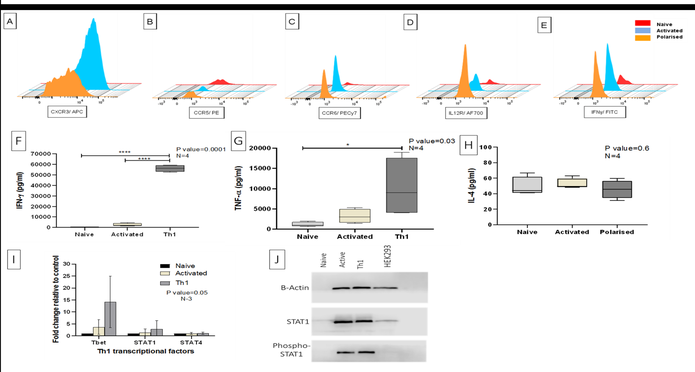


Fig 5: Th1 phenotype measured with multicolour Flow cytometry LSRII: (A)CXCR3, (B) CCR5, (C) CCR6, (D)IL12R, (E)IFNγR. Th1 related cytokines measured with ELISA (F) IFNγ and (G)TNFα. Transcriptional factors measured with (H)qPCR and (I)Western Blot.

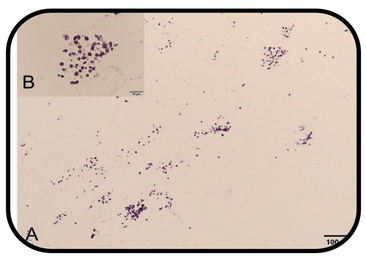


Fig 6: Jurkat cells seeded in collagen hydrogel at different concentrations. Models were stained with H&E (A) 20x, (B) 40x magnification

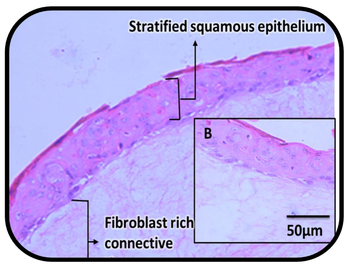


Fig 7: Tissue Engineered Oral Mucosa of FNB6 epithelial cells grown on top of fibroblast embedded in collagen cultured for 14 days at ALI. H&E stained (A) 20x, (B) 40x magnification

What is next

Incorporate polarised T cells into TEOM

Perform cytotoxicity drug testing

Characterise the model against OLP markers

Thesis submission

Citation

1. Odell, E. W. (2017) *Cawson's essentials of oral pathology and oral medicine e-book.* Elsevier Health Sciences.
2. Khan, A., Farah, C. S., Savage, N. W., Walsh, L. J., Harbrow, D. J. and Sugerman, P. B. (2003) 'Th1 cytokines in oral lichen planus', *Journal of oral pathology & medicine,* 32(2), pp. 77-83.

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