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## Development and validation of a 3D lymph node-adipocyte co-culture system

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**INTRODUCTION:** White adipose tissue is involved in localised paracrine interactions with lymph node cells. Localised immune challenges with lipopolysaccharide stimulate the release of Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and interleukins 4, 6, 8 and 10 from lymph node cells. This in turn directs adipocytes to respond by releasing fatty acids from their triacylglycerol stores. This mechanism is believed to be of great importance as it provides energy and metabolic precursors to the lymph node cells during the immune response.

We have developed a long term 3-dimensional co-culture system with adipocytes and lymph node cells for the purpose of investigating interactions between these cells *in vitro*. (Patent application number 0606764.9).

**METHODS:** Preadipocytes were isolated from the popliteal adipose tissue depot. The cells were expanded then seeded into a type I collagen gel. The preadipocytes were induced to differentiate over a 14 day period. The lymph node cells were isolated by teasing apart the node and releasing the cells into a petri dish containing culture medium. Present experimental work with the 3D system is aimed at introducing lymph node cells, in proportions similar to those found in intact lymph nodes, among differentiated adipocytes.

**RESULTS:** Differentiating preadipocytes *in vitro* showed multiple lipid droplet accumulation and similar protein expression patterns to those of mature adipocytes *in vivo*.

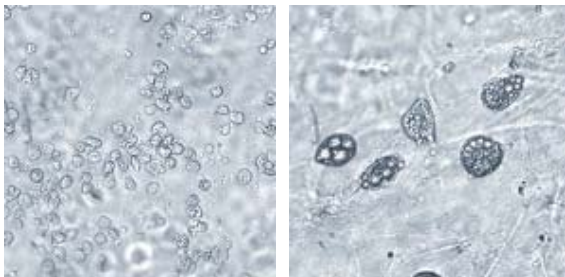
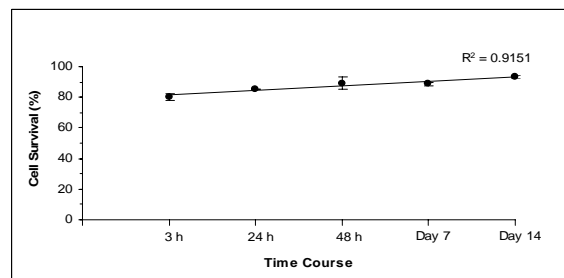


Fig. 1: Differentiation of preadipocytes in 3D culture (phase): Day 0 (left), Day 14 (right).

Differentiated adipocytes expressed and showed upregulation of the S100 protein, insulin receptor and caveolin-1 and TNF- $\alpha$  receptors (TNFRI and

TNFR2) and the chemokine receptor CCR5 during differentiation, when assessed by immunofluorescence and western blot (1). Prior to the induction of differentiation, preadipocytes showed good proliferation, assessed by BrdU incorporation and maintained high cell viability during differentiation (Graph 1).

Graph 1. Cell survival of differentiating preadipocytes in a collagen gel.



Over 80% (n=10000) of lymph node cells were viable after isolation from the popliteal node and over 75% (n $\geq$ 5000) of these cells remained viable in the system. T cells were the major cell type (52.1%) among the mixed cell population isolated from the popliteal lymph node (assessed by flow cytometry), followed by B cells, dendritic cells and macrophages.

**DISCUSSION & CONCLUSIONS:** Present experimental work with the culture system is aimed at introducing lymph node cells, in proportions similar to those found in intact lymph nodes, among differentiated adipocytes and observing interactions and the establishment of a spatial relationship between them. Co-cultures will be used to investigate the lymph node adipocyte interactions following immune stimulation (lipopolysaccharide treatment) measuring production of inflammatory mediators (cytokines) and lipolytic activity.

**REFERENCES:** <sup>1</sup> S.Daya, A.J. Loughlin, H.A. MacQueen (2006) Culture and differentiation of preadipocytes in 2-dimensional and 3-dimensional *in vitro* systems. Submitted to Differentiation.

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