Perfusion of Oxygen in 3D Plastic Compressed Collagen Constructs

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Introduction

The development of 3D connective tissues *in vitro* is heavily dependent upon remodelling of the matrix, in particular collagen, by resident cells. We have developed a novel plastic compression (PC) technique, for the fabrication of dense cell-collagen based bio-mimetic tissues (Brown *et al.* 2005).

Cell survival in these PC collagen constructs is critical for successful tissue modelling and so the aim here is to understand, quantitatively their dynamic perfusion. This is important for the development of tissue bioreactors for the culture of PC constructs. We have used a fibre-optic oxygen sensor to measure changing oxygen levels in the core of such constructs. This effectively measures O_2 consumption by cells, and by extrapolation, gradients and diffusion properties in the model tissues, which can be correlated with cell death.

Materials and Methods

Acellular and cell-seeded type I collagen gels were made, as previously described, and routinely compacted by a combination of compression and blotting. The rate of compaction was controlled by the force applied and the extent of fluid removal to a porous 'sink'. Cellular collagen gels were made, using Human Dermal Fibroblasts (HDF), at densities from 0.5. million-2 million cells per construct. These constructs were compressed using standardised protocol and rolled to form tight spiral rods (final construct = 21mm length, 2.31mm diameter). pO_2 oxygen probes were inserted into the centre of gels, and oxygen monitored over a period of up to 5 days in static culture.

Results and Discussion

The consumption of oxygen by cells was statistically significantly different between 0.5 million, 1 million and 2 million cells per construct (figure 1, *= $P<0.001$). However even after 5 days of this culture, the level of cell death was modest, using analysis with live/dead staining and confocal microscopy.

Pulmonary Arterial Smooth Muscle cells were also studied to establish cell specific rates of oxygen consumption. These cells consume higher amounts of oxygen in comparison to Dermal fibroblasts. Oxygen diffusion gradients have been established to test the role of increasing collagen density in single and double compressed constructs. Marked differences in the oxygen diffusion gradient were seen when collagen was approximately twice as dense.

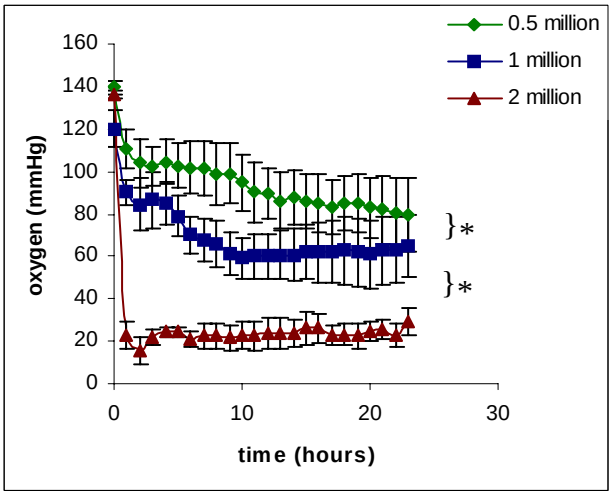

Figure 1. Oxygen partial pressure measured in the centre of HDF-seeded constructs.

Conclusions

This is a highly effective model of perfusion in 3D connective tissues. Gradients were cell type, cell density, collagen density and time dependent. PC collagen material and its laminated 3D structure, allows relatively rapid O_2 equilibrium across extended gradients (~1mm) even at high cell densities. Fibroblasts are able to tolerate and grow within these constructs at relatively low oxygen tensions for extended periods.

We have established a novel method for the monitoring of oxygen in the centre of model PC collagen tissue cultures, and shown that the high density PC collagen material allows good perfusion of oxygen. This model tissue offers unique insights to cell physiology in 3D through its extreme simplicity and controllability. Rapid cell death in this matrix-rich system does not seem to be such a major problem.

References

Brown, R.A. Wiseman, M. Chuo, C.B. **Cheema, U.** Nazhat, S.N. 'Ultrarapid Engineering of Biomimetic Materials and Tissues: Fabrication of Nano- and Microstructures by Plastic Compression.' 2005. *Adv. Funct. Mater*. 15 (11): 1762-1770

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