Superficial and Deep Chondrocyte Subpopulations both Express the Crabtree Effect but Exhibit Differences in Oxygen Consumption Rate

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INTRODUCTION: Chondrocytes from full depth articular cartilage exhibit aerobic glycolysis, but demonstrate enhanced oxygen consumption during periods of low glucose availability[1]. *In vivo*, the superficial subpopulation experiences greater oxygen and glucose levels than the deep zone. We hypothesise that the superficial and deep subpopulations of articular cartilage exhibit different basal oxygen consumption rates and oxidative response to low glucose levels.

METHODS: *Cell isolation.* Bovine articular cartilage was harvested from metacarpalphalageal joints as a thin superficial layer, representing the uppermost 15-20% of uncalcified tissue depth, and the remaining deeper tissue. Chondrocytes were isolated from the extracellular matrix by serial digestion in pronase and collagenase. Viability was assessed to be >95% by trypan blue exclusion. *Oxygen measurement.* 10^6 cells were loaded into each well of a 96-well plate oxygen biosensor (BD Biosciences) and incubated in 310 µL DMEM+16% FCS containing 0.5, 1.3, 2.2, 3.0, 4.6 and 19.8 mM glucose. Additional samples were incubated with 0.5 mM glucose + 5 mM 2-deoxyglucose (Sigma, Poole, UK). The plate was sealed with film and the oxygen concentration [O_2] within the cell suspensions monitored fluometrically for 6h.

RESULTS: A monotonic depletion of oxygen was observed in each well, as illustrated in fig. 1.

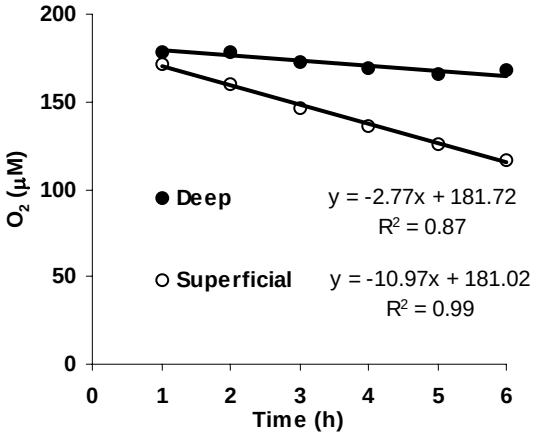

Fig. 1: The decreasing [O_2] in suspensions of either superficial or deep chondrocytes incubated in DMEM+16% FCS containing 0.5 mM glucose.

The oxygen consumption rate of each sample was calculated from the fitted linear relationship (fig 1) and normalised to cell number. The results summarised in figure 2 indicate that the superficial subpopulation, which represented 57-59% of the cell yield, has more than twice the per cell oxygen consumption rate of deep cells. Both the superficial and deep subpopulations exhibited a Crabtree effect, with a 2.2 and 2.1 fold respective increase in oxygen consumption rate as glucose was reduced from 4.6 mM to 0.5 mM. The addition of 5 mM 2-deoxyglucose to low glucose incubations did not suppress oxygen consumption, but instead stimulated it in the deep cells (data not shown).

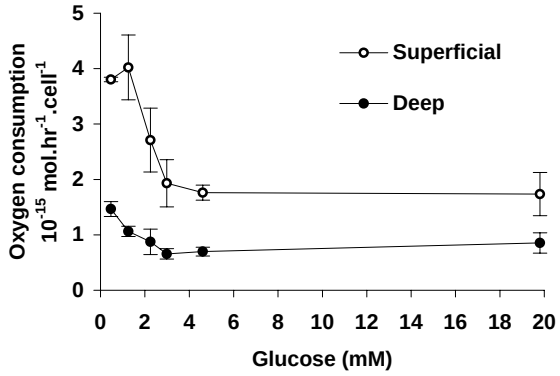

Fig. 2: The increasing oxygen consumption rate of superficial and deep chondrocytes with glucose deprivation. Data represents the mean ± StDev of 3 cell isolations, each tested in duplicate.

DISCUSSION & CONCLUSIONS: That the oxygen consumption of superficial cells is more than double that of deeps cells has important implications for the oxygen concentration within tissue engineered constructs formed using full-depth cells, compared to native tissue or constructs employing a layered approach. The present study has also demonstrated that the Crabtree effect is present in both subpopulations.

REFERENCES:

[1] H.K. Heywood et al (2004) *Transactions of the Orthopaedic Research Society*. **29**:0261

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