Monitoring Real-Time Glucose Consumption of Intervertebral Disc Cells in 3D alginate and in Organ Culture

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INTRODUCTION

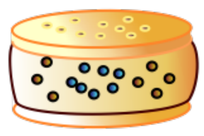
- The intervertebral disc (IVD) consists of three different tissues: the gelatinous core [nucleus pulposus (NP)], the surrounding lamellar annulus fibrosus (AF) and the cartilaginous endplates (CEP) on top and bottom of the IVD.
- The IVD is an avascular organ and provides its cells a hypoxic and nutrient-deficient environment.
- There is only little knowledge on glucose consumption of IVD cells and mainly in *in vitro* animal models.[1, 2]

Study Aim

Glucose consumption of human IVD cells is not yet studied in real-time. We use new glucose sensors that allow investigation of real-time glucose concentration of different IVD cell types in 3d culture.

MATERIALS and METHODS

IVD Cell Isolation



Separation of human IVD into AF, NP and CEP tissue.

Two step isolation of cells with pronase (0.19%) and overnight digestion with collagenase II (NP: 1600 U/ml, AF: 3200 U/ml, CEP: 39000 U/ml and g tissue)

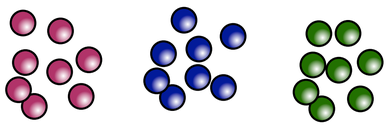
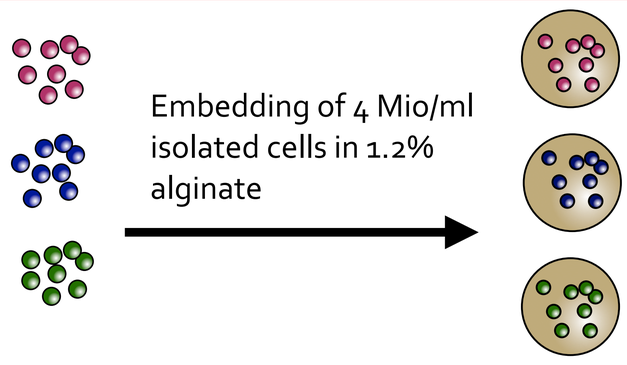


Figure 1: Two step isolation of human IVD cells. Tissue was collected from patients undergoing spinal surgery and with written consent.

3D culture and real-time Glucose Measurement



Embedding of 4 Mio/ml isolated cells in 1.2% alginate

30 beads (~2.4 Mio cells) were placed into T25 culture flasks and covered with 9ml HG-DMEM

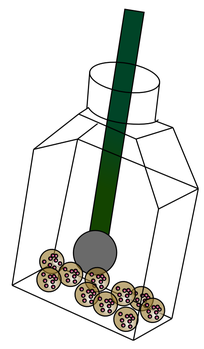


Figure 2: Isolated IVD cells were embedded in 1.2% alginate at a density of 4Mio cells/ml. Circa 30 beads of each cell type were added into respective T25 culture flask and 9ml of HG-DMEM with 5% FCS. Glucose sensors (C-CITSens) were incorporated by supplier to guarantee sterility. Flasks were put tilted (~30°) on an orbital shaker (10rpm) to ensue that sensor is covered by media and to facilitate glucose measurement.

Cells were cultured for 7 days under agitation (10 rpm) and media was changed after 3, 4 days respectively.

RESULTS

- Recording of glucose concentration in real-time was possible.
- Comparison between different IVD cells was obtained by analysing slope of raw data (current in nA) before and after media change, Fig. 3.
- A clear difference was seen between CEP and AF/NP cells with higher glucose consumption.

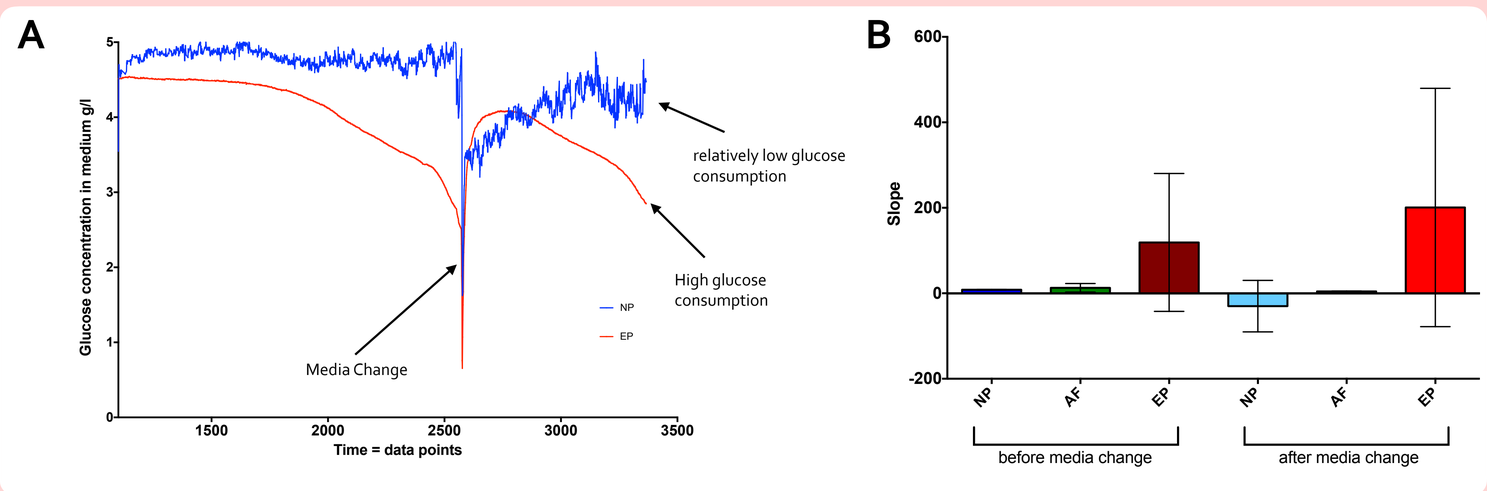


Figure 3: A Glucose concentration in g/l recorded for one experiment over 7 days of culture. Here AF cells are not included due to a problem with a sensor. **B** Slopes of raw data (nA) before and after media change (AF and NP n=3, CEP n=2).

OUTLOOK

- Recording real-time glucose concentration in an organ culture set-up.
- Investigation of injury models of the IVD.
- Determine possible changes in glucose concentration in IVD when different loading schemes are applied (C) e.g. free swelling, static diurnal load (A) or complex load in a bioreactor (B).

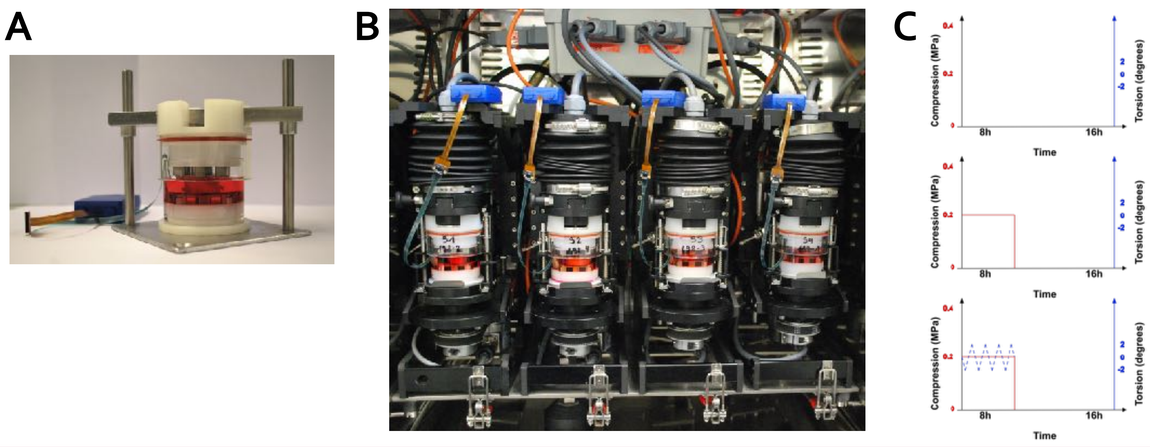


Figure 4: Experimental set-up for real-time glucose concentration measurement in organ culture. A IVD in station for application of static load **B** Bioreactor allowing application of torsion and compression either dynamically or statically **C** Different loading regimes used i.e. free swelling, static load and complex loading (top-down).

DISCUSSION

- Based on this preliminary study we suspect a true difference in glucose consumption between the CEP and the NP cells (more repeats are ongoing).
- The higher consumption by CEP cells could be explained by their location in the IVD. As CEPC are closer to the nutrient transport route, i.e. the capillary system of the vertebrae they should be adapted to higher glucose levels than cells in the centre of the disc, which rely on pure diffusion.
- It should be noted that this study was done on one IVD donor and one sensor per cell population. In the near future we plan to repeat this experiment and to vary glucose concentration.

REFERENCES

[1] Fernando et al. (2011), J Orthop Res **29**:11, 1634-41
[2] Bibby et al. (2005), Spine **30**:5, 487-96

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