Tracking Cells in vitro using Micro CT

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Aims

The aim of this work was to develop a technique that would allow cells to be tracked *in vitro* using Micro CT. Currently the main technique to track cells is completed by staining and then sectioning the scaffolds that the cells are cultured in to visualise them under optical/fluorescent microscopes. The sectioning can lead to movement of the fixed cells, especially when the scaffold is soft, not allowing a true position of the cells to be identified. The use of Micro CT allows non-destructive visualisation of cells *in situ*. Whilst osmium-tetroxide has previously been used[1] as a contrast agent for the micro-CT imaging of cells (ref) it is extremely hazardous and thus we consider less toxic options.

Method

Lyophilised collagen scaffolds were produced in a method modified from Davidenko et al[2]. Scaffolds were then subsequently sterilised through a process of washing in sterile 70% ethanol, sterile water and PBS. All samples were left in media for 24 hours before the experiment to allow protein adsorption onto the scaffold struts.

HT 1080 cells were cultured until 70% confluent. These cells were then passaged so that there were enough cells for the experiment. Barium Sulphate (Micropaque, Guerbet) was then added to the media at 35 μl per ml of media (DMEM, Sigma Aldrich) in the flask, based on a method previously described[3]. Cells were allowed to take up the contrast agents for just over 72 hours. Another flask of cells was cultured with just media present.

The cells were left to culture for 3 days before being seeded onto a collagen scaffold at 130,000 cells per scaffold in a bead of 15 μl. These were then left to stick to the collagen scaffold for 1 hour in a 48 well plate before being moved into a new 48 well plate and the well flooded with new plain media. Cells on the scaffolds were subsequently cultured for either 1 day, 3 days or 7 days. At each timepoint scaffolds were removed, washed in PBS before being fixed in ultrapure glutareldahyde. These scaffolds were subsequently freeze dried.

The dry scaffolds were scanned in their entirety using a Skyscan 1172 with a pixel size of 2.93 μm, an operating voltage of 25 kV, 0.2° stepsize with frame averaging of 2 and 180° rotation. The resulting projections were processed into 3D data sets using a full cone beam Feldkamp reconstruction algorithm with NRecon software. Images were viewed using Data Viewer.

Following Micro CT scaffolds were cut in half and one half was subsequently stained using DAPI. This stained the cell nuclei with a fluorescent (358 nm) stain. This was then viewed using a Zeiss Axio Observer Z1 phase contrast microscope fitted with a Zeiss Axiom 503 mono camera using a 10X objective lens.

Results

Before trypsinising, cells were viewed under the microscope and contrast agent uptake clearly observed.

The presence of cells and their penetration through the scaffold was more evident in the presence of contrast agent. *Figure 1* shows a collagen scaffold that has had HT1080 cells containing barium sulphate cultured in it with media for 24 hours. *Figure 1A* is the coronal image of the scaffold and the cells appear to be present near the top of the scaffold as expected as they will not have yet migrated into the scaffold. The red line shows where the transaxial image of the scaffold (Figure *1B*) was taken. This image shows how the cells are distributed.

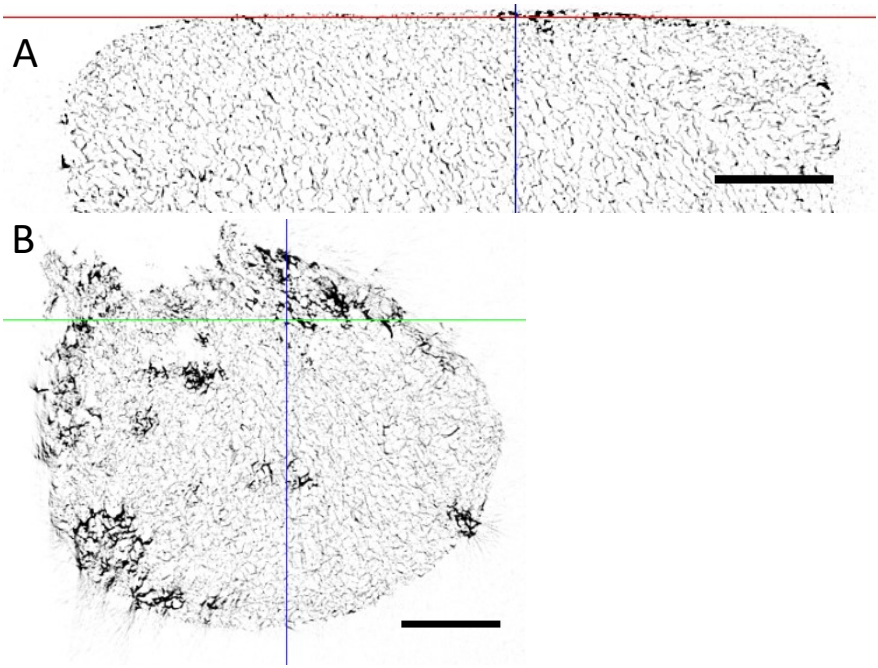

Figure 9: Micro CT images (Coronal (A) and Transaxial (B)) of a collagen scaffold seed with HT1080 cells (precultured in barium sulphate and media) and cultured for 1 day (Scale bar is 250 µm)

Figure 2 shows a collagen scaffold that has had HT1080 cells, pre-cultured in barium sulphate, cultured on them for 3 days. By comparing *Figure 1* and *Figure 2* it is possible to determine that the cells have spread across the scaffold but have also migrated through the collagen scaffold.

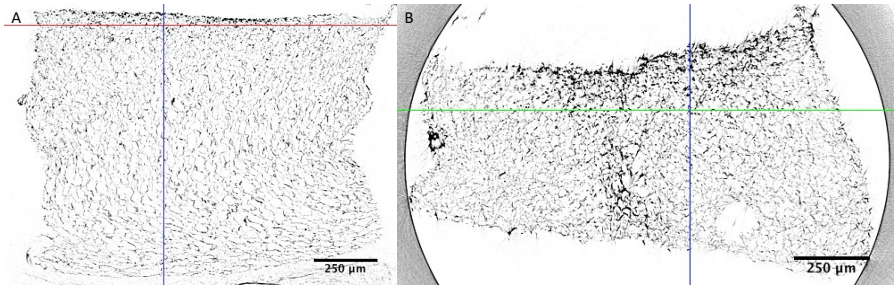

Figure 10: Micro CT image of collagen scaffold (Coronal (A) and Transaxial (B)) seeded with HT1080 cells (precultured in barium sulphate) after culture for 3 days

To check that the cells are in the position observed using Micro CT, the samples were sectioned and then half was stained with DAPI. *Figure 3* shows the HT1080 cells were located in the same place in the scaffold as they were using Micro CT.

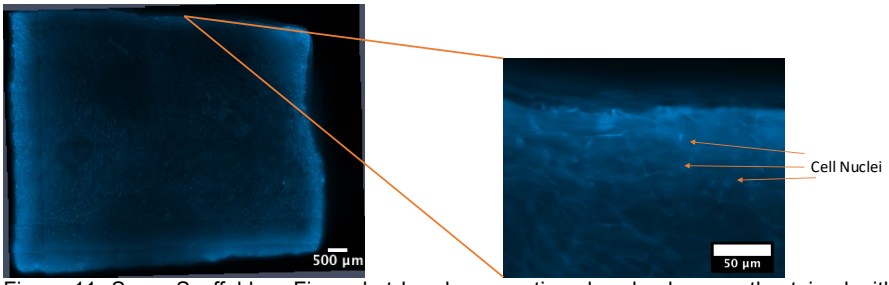

Figure 11: Same Scaffold as Figure but has been sectioned and subsequently stained with DAPI. The left image shows the entire scaffold whilst on the right is a closeup of the scaffold clearly showing the cell nuclei.

Conclusion

From the work presented in this scaffold we have found that it is possible to use desktop Micro CT to visualize cells within a collagen scaffold. With adaptations of this technique using scaffolds with varying pore structures it will be possible to track cell movement within collagen structures.

References:

1. Silva, M.M.C.G., *et al*. Biomaterials 27 (35) (2006): 5909–5917
2. Davidenko, N., *et al*. Acta biomaterialia 25 (2015): 131-142
3. Dullin, C., *et al*. Journal of synchrotron radiation, *22*(1) (2015), 143-155