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Optimisation of a 3D cell culture system for growing human cells on a satellite in space

1. 3D cell culture for space research

Following the return of astronaut Scott Kelly after a year in space comparisons with his twin showed large pathological changes in many organs, it is still not clear why this occurs; this is a growing concern with sending astronauts to Mars and on longer space flights.

Understanding the morphology of cells using cell-culture and the physiological and pathophysiological changes that a cell undergoes when subjected to stress, like when exposed to radiation or in reduced gravity, is beneficial to human health and can be applied to many conditions including osteoporosis, muscle atrophy and organ damage (Biolo *et al*., 2003; Honda *et al*., 2014).

There are still many limitations with traditional two-dimensional monolayer culture, including distortion of shape due to growth area restraints. This has led to the introduction of 3D cell-culturing; the resulting cultures more closely mimic the pattern of cell growth *in vivo* (Antoni *et al*., 2015). This enables a far more accurate representation of the morphology of these cells and their interactions with their microenvironment, providing invaluable information for development of treatments and progression of healthcare. 3D culture allows for experiments which generate data that would otherwise require an animal model which is costly and time consuming (Pampoloni, Reynaud and Stelzer, 2007).

The aim of this study was to optimise a 3D cell culture model that can be used to grow mammalian cells on the BAMMSat that is currently being developed by Professor David Cullen at Cranfield University.

2. Materials and methods

2.1 Cell culture

HeLa (epithelial cells from a malignant cervical tumour) and C2C12 (mouse muscle cells) were grown in complete growth medium. This contains all of the necessary nutrients to support growth of the cells in the cell culture laboratory.

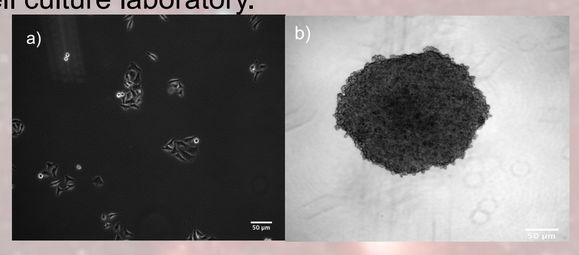

Figure 1: HeLa cells in **a)** 2D; cells are epithelial in shape and **b)** in 3D. Both images are at a x40 magnification.

The HeLa cells were previously transfected with live green-fluorescent protein and red fluorescent protein for confocal imaging.

2.2 Feeding cells

The cell culture medium was changed every 24-48 hours. Cells were grown and imaged for between 16 and 21 days.

2.3 Growing 3D spheroids

The cells were grown in Matrigel®, 1.5% agarose gel with DPBS and on a commercial plate (Nunclon™ Sphera™ 96-well U-bottomed plate) at a range of concentrations to determine the optimum concentration with reproducible spheroids.

2.4 Imaging cells

Cells were imaged using a light microscope and confocal microscopy.

2.5 Measuring cell health in spheroid form using Alamar Blue assay

High purity resazurin sodium salt (Sigma-Aldrich®) was dissolved in DPBS (Gibco®, pH 7.4) to a concentration of 0.15 mg/mL, in an unlit hood. This was filter-sterilised through a 0.22 µm filter in to a light protected and sterile tube. This solution was added to the wells to fill 1/10th of the total volume and left to incubate for 3 hours. Measurements were taken using a plate reader.

3. Results

3.1 Long-term culture of HeLa spheroids is possible on commercial plates.

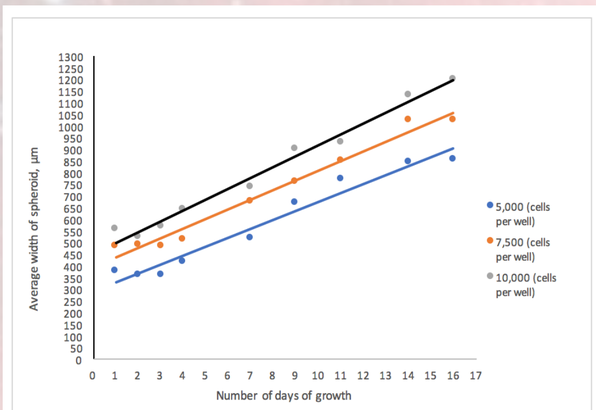

Figure 2: Average width of spheroids at concentrations of 5,000, 7,500 and 10,000 cells per well on a Nunclon™ Sphera™ cell culture plate over a period of 16 days

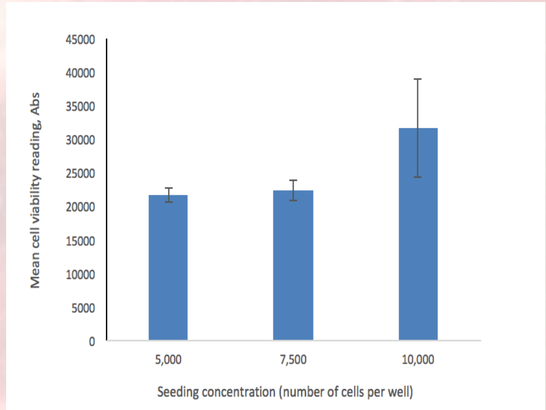

Figure 3: The mean cell viability reading for spheroids grown on a Nunclon™ Sphera™ plate on day 11 of growth.

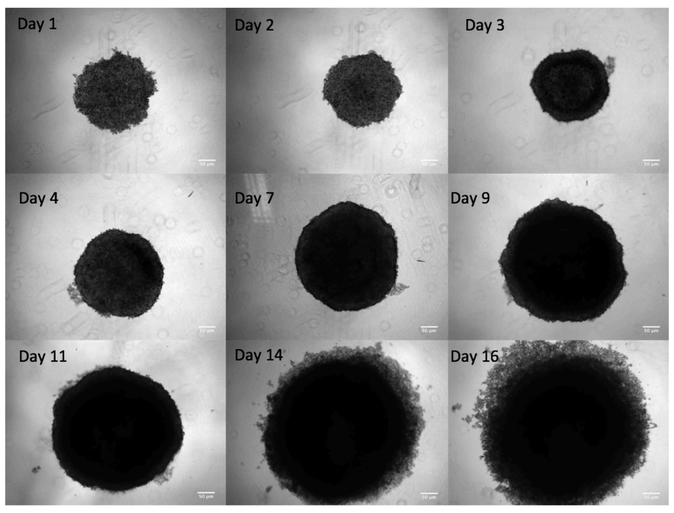

Figure 4: Growth of spheroid at a seeding concentration of 10,000 cells per well over 16 days.

3.2 Agarose-DPBS allows spheroid growth over a seeding concentration of 5,000 cells per well.

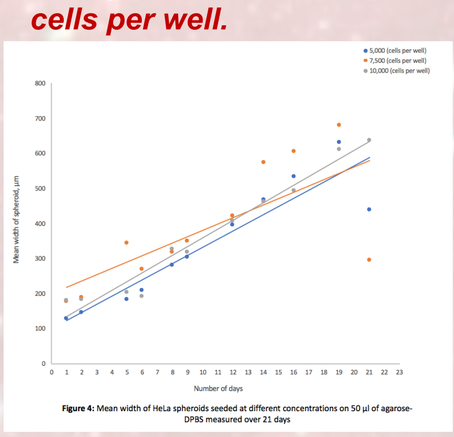

Figure 4: Mean width of HeLa spheroids seeded at different concentrations on 50 µl of agarose-DPBS measured over 21 days

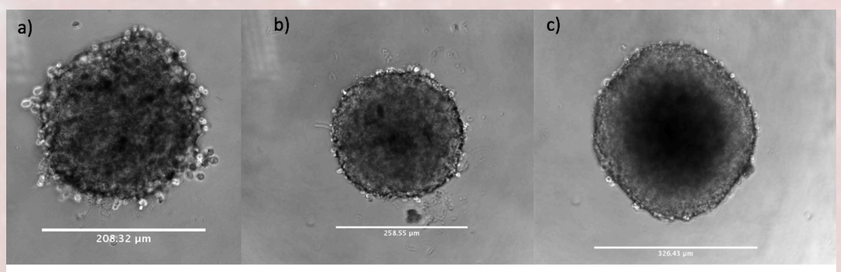

Figure 5: HeLa spheroids grown on agarose-DPBS at concentrations of **a)** 5,000 **b)** 7,500 and **c)** 10,000 cells per well.

3.3 Matrigel® is difficult to image, no single spheroids formed and may not be suitable for a fixed microscope on the satellite.

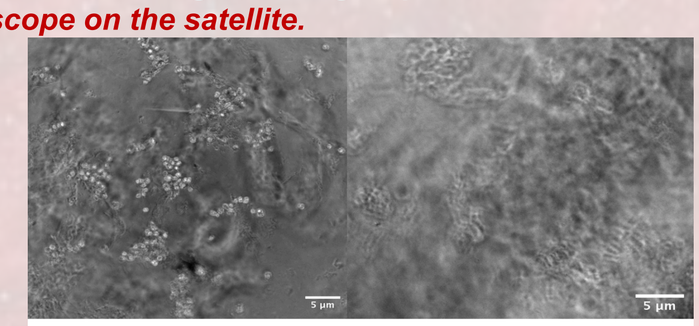

Figure 6: 10,000 cells per well on Matrigel®; difficult to focus using a light microscope for imaging.

3.4 Confocal and light microscope imaging of HeLa and C2C12 to compare 2D and 3D cell culture.

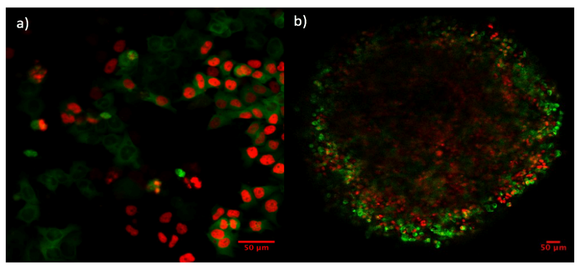

Figure 7: HeLa cells at a seeding concentration of 7,500 cells per well in **a)** 2D monolayer culture and **b)** 3D spheroid form (cytoskeleton in green and nuclei in red).

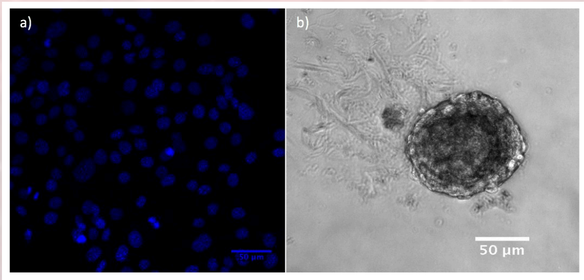

Figure 8: C2C12 cells at a seeding concentration of 3,000 cells per well in **a)** 2D monolayer culture (nuclei stained blue for confocal imaging) and **b)** 3D spheroid form.

4. Discussion and further studies

- The best method for growing reproducible spheroids is the commercial plate however, the spheroids on the agarose are healthier.
- Cell can be grown in culture for 16-21 days as a spheroid.
- C2C12 need to be tested on 1.5% agarose-DPBS to see if spheroid growth is reproducible with other mammalian cultures.
- It would be useful to see if a hybrid agarose with diluted media enables spheroid growth.
- Measurement of cell viability at regular intervals will give a better indication of cell health.

5. References

Antoni, D., Burckel, H., Josset, E. and Noel, G. (2015) 'Three-dimensional cell culture: a breakthrough in vivo', *International Journal of Molecular Science,* 16(3), pp. 5517-27.

Biolo, G., Heer, M., Narici, M. and Strollo, F. (2003) 'Microgravity as a model of ageing', *Current Opinions in Clinical Nutrition and Metabolic Care,* 6(1), pp. 31-40.

Honda, Y., Honda, S., Narici, M. and Szewczyk, N. J. (2014) 'Spaceflight and ageing: reflecting on Caenorhabditis elegans in space', *Gerontology,* 60(2), pp. 138-42.

Pampaloni, F., Reynaud, E. G. and Stelzer, E. H. (2007) 'The third dimension bridges the gap between cell culture and live tissue', *Nature Reviews Molecular Cell Biology,* 8(10), pp. 839-45.