Cells and Tissues

The old fashioned way of growing tissue cultures in a laboratory is to spread a few cells over a petri dish of nutrient and let them grow across the surface of the plate – which is what they will do in a very flat and in a very 2-dimensional way - because of, you know, *gravity*.

However, when you look around the world there aren't a lot of flat organisms out there. Well, OK there are flatworms and even flatfish – but by and large most Earth-based organisms, along with their internal organs and tissues, fill out all three dimensions. This is partly because complex animals first evolved in water, where gravity is partially-counteracted by buoyancy. This tradition has been continued on land, where embryos still grow in a fluid environment, be it in an egg or a womb. But, in a laboratory, even if we try growing tissue in oxygenated, nutrient-filled flasks, those cells still tend to fall to the bottom and then grow flat.

So, overall, it's clear that gravity is an issue in cell and tissue growth, although we don't fully understand why it is *less* of an issue inside eggs and wombs.

Wouldn't it be great if there was a laboratory where all the effects of gravity could be eliminated, so that you could watch what happens when tissue cultures grow under these conditions?

Fortunately, the wealth of great science gear already aboard the ISS includes facilities for growing cell and tissue cultures. However, the history of this technology has taken some unusual turns. Back in the 70s when people were preparing for the dawn of the Space Shuttle era, building a space-based culture system that would support 3d tissue growth was high on the list. The concept of a bioreactor was developed, which was essentially a soup can with a system of membranes that worked a bit like lungs and kidneys, to maintain a constant supply of oxygen and nutrients and remove waste products, meaning the cells could float happily and homeostatically in a sealed container of fluid – and hopefully grow into a 3d tissue.

Of course, the envisioned system would only work in space, since on Earth all the cells would just sink to the bottom. However – so the story goes – just as the bioreactor was about to be launched, there was the 1986 Space Shuttle Challenger disaster and suddenly NASA's new bioreactor was all dressed up with nowhere to go. Frustrated, the bioreactor's developers figured that there might be a way to at least mimic the effects of microgravity, by keeping the fluid in motion so that the cells wouldn't all settle on the bottom. They figured this might allow them to test a few more features of the system before the Space Shuttle was back and flying again.

Pretty soon, they figured out that if you just put the soup can on its side and rotated it, then the cells within it would remain in a constant state of *trying* to settle on the bottom, but never quite managing to – instead just going around and around in a continuously-circulating current – which was a bit like being in free-fall, even if it wasn't *really* free-fall. And, to everyone's surprise, this space-prototype started growing 3d tissues. In a soup can made to rotate on its side – we were suddenly achieving what we had assumed would need a multi-billion dollar space transportation system to achieve.

So, it seems, sometimes even just *the thought* of sending something into space is enough to stimulate a technological breakthrough, even if you never actually manage to get it into space.

Nonetheless, a bioreactor was eventually launched aboard Space Shuttle STS 70 in 1995 and increasingly sophisticated bioreactors were launched regularly thereafter. The bioreactor system, which has seen a lot more use on Earth than it has ever seen in space, is now commemorated in the NASA Space Technology Hall Of Fame.

Even today, we are not sure whether the space-going cultures have it over the Earth-bound cultures as the best way to investigate 3d tissue cultures. Despite the success of the rotating soup cans on Earth, we think that space-borne bioreactors should be able to grow tissues of higher quality and size than are achievable on Earth – although this has yet to be fully demonstrated.

What's exciting about 3d tissue culture systems is that they can support cell *differentiation*. For example, if you grow intestinal cells in a petri dish on Earth, you will just grow a collection of flat undifferentiated cells. But, if you grow these intestinal cells in a 3d tissue culture system, the cells develop microvilli, which are finger-like protrusions of the cell membrane. In living organisms, these microvilli are needed to increase the surface area of intestinal cells, ensuring the efficient absorption of nutrients from the gut. So it seems there is something about being suspended in 3 dimensional space that activates and sustains specialised gene expression in cells.

We are still a long way from being able to grow a functioning kidney in an artificial environment, but that's something we are aiming for. And 3d tissue cultures may also offer an experimental window to help us better understand how cancers develop and grow – so that we might *stop them* developing and growing.

The latest attempt at growing 3d tissue cultures in space is the Cellular Biotechnology Operations Support System or CBOSS, which has been aboard the ISS since 2001. Like most tissue culture systems, CBOSS is designed to support the growth of particular cell lines which grow, reproduce and ideally differentiate into the particular tissue that the cells come from.

These cell lines are often from rats or mice, but some are also human. They are generally cancer cells because cancer cells have the capacity to grow without the genetic restraints that are imposed on normal cells. Unrestrained growth is generally what cancer is all about – and it's also what you want in a tissue culture. With a rapidly-growing and reproducing culture of cells, you can see how those cells grow and differentiate and how they respond to changes in their environment, over time and over generations.

But CBOSS's success to date has been, at best, mixed. Out of a set of seven experiments conducted in recent years, the one investigating ovarian cancer cell lines was inconclusive, because the culture died. The one investigating ovarian colon cell lines was inconclusive, because the culture died. The one growing a cell line of kidney cortical cells to produce erythropoietin and Vitamin D, might have *commercial* applications. So, whatever happened in that experiment, the last place you are going to hear about it is on a cheap podcast.

A fourth experiment growing pheochromocytoma cells, which are like embryonic neuronal cells, was intended to study how damaged neuronal cells might regenerate. This is a really exciting area, which might have all sorts of *medicinal* applications – except… the culture died. A fifth experiment growing erythroleukemia cells, which are cancerous human red blood cells, was set up to investigate how red blood cells are maintained and sustained in the body. And… nope, died.

But the sixth experiment, exploring the immune function of Human Lymphoid Tissue, got results that even a cheap podcast can talk about. The HLT experiment positively demonstrated impaired immune responses in microgravity. It's as though microgravity can suppress the immune system, giving you symptoms that are like a mild case of AIDS, which could explain some of temporary immune dysfunction that is commonly seen in orbiting astronauts. So there you go – amidst all that death and despair we still got some great science on the ISS!

The seventh and last CBOSS experiment is looking at the fluid dynamics of the CBOSS system itself. The reason why the cultures died in the other CBOSS experiments was poor mixing of the cells within their nutrient medium, as well as unexpected bubble formation, which could also have killed a lot of the cells. Hopefully, the FDI, the fluid dynamics investigation, will find a solution to these problems and show the way forward to making future ISS tissue cultures more viable and more scientifically-valuable.

So, while space-based cell and tissue cultures are yet to *really* get off the ground, they are a growing concern.