SPOTLIGHT feature

Food & Beverage Analysis

Incubating the World's First Synthetic Burger

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The creation of a synthetic burger in a laboratory in 2013 was a world first. By producing edible meat from bovine stem cells, the 'in vitro meat project' is prompting a rethink of the way we produce and develop food, delivering a crucial first step in the creation of a sustainable method for meat production. To achieve this feat, the team at the University of Maastricht in the Netherlands had to ensure that their laboratory equipment, and in particular their incubation systems, matched up to their own pioneering standards.

Food for the Future

The need for alternative meat production techniques is becoming increasingly pressing. A study conducted by the Food and Agriculture Organization of the United Nations in 2006 estimated that 70% of all agricultural land, around 30% of the Earth's surface, is currently dedicated to livestock production for the needs of seven billion people. The Food and Agriculture Organization of the United Nations states that by 2050 [1], the global meat demand will have increased by approximately more than two-thirds, thanks to an estimated global population of 9 billion.

As human population and demand for agricultural products increases, partly driven by rapid economic development in Africa and the Middle East [2], traditional methods of meat production become increasingly unsustainable. Rearing cattle for meat production requires a lot of input, both financially and in natural resources required. For example, producing 1 kg of meat requires around 15,000 litres of water [3]. This was one of the issues that prompted the research at the University of Maastricht.

In October 2011, the 'in vitro meat project' was started, with a team led by Professor Mark Post alongside laboratory technicians Anon van Essen and Sanne Verbruggen. The final project aim was to create edible meat in the form of a hamburger without the direct use of bovine tissue. In this way future production could become more sustainable and efficient. This was a unique project that, to the University's knowledge, was not being replicated anywhere else in the world.

Professor Mark Post with a burger made from Cultured Beef. (Credit: David Parry / PA Wire)

Building a Burger

The burger was first shown, cooked, and tasted in London on 5th August 2013, and was the result of months of hard work. The physical burger itself took three months to create.

During production, stem cells (satellite cells) were harvested from a sample of muscle tissue taken from a cow's shoulder. These cells are often described as dormant myoblasts (muscle cells), and although they play a vital role during the repair and maintenance of muscle within the body, they have a very limited ability to replicate. However, satellite cells can be activated when exposed to stimuli, such as injury or high mechanical load, and billions of cells were cultured from the original samples.

Myoblast cells naturally merge in a process known as myogenesis – the formation of muscle tissue particularly seen during embryonic development. In this process, cells fuse into multi-nucleated fibres called myotubes to form muscle tissue. The myoblasts were placed around circular gel hubs, leading to the formation of rings of tissue. The contraction and relaxation of the cells caused them to put on bulk. In the end, 20,000 rings were painstakingly layered together to create the final burger.

This method heavily involved the successful incubation and growth of robust, healthy cells, and therefore the exact requirements of the incubation system needed to be carefully considered. Only in this way could the team at the University of Maastricht be confident in producing the best possible final product.

Guaranteeing Quality Cell Lines

An initial consideration when selecting incubators for the in vitro meat project was the volume of cells to be cultured. The large quantity meant that the incubators had to hold high numbers of samples, with absolutely no compromise of reliability and security throughout. In addition, the cells were cultured using 10 layer bottles, which were very heavy, and so the robust design of incubators was crucial.

Efficient environmental and temperature control was also crucial for effective incubation. The optimal environments for stem cell growth are those that most resemble the natural environment within the organism. For muscle stem cells, this means consistently low oxygen and high carbon dioxide concentrations (3% O_2, 5% CO_2) and stable temperatures of around 37°C. These conditions increase the proliferation ability of satellite cells compared to standard cell culture conditions of 21% oxygen, as this is a closer match to the in vivo oxygen levels in the intact myofibre [4]. Fluctuations in temperature also impact cultures, with consequences ranging from the total failure of the cell line to the expression of abnormal phenotypes and altered cell metabolism [5]. Tight controls therefore needed maintaining, as cell cultures exposed to variable temperatures would not be viable for the project.

Finally, when the satellite cells were isolated from the animals there was a small chance of impurity in the initial sample. Any possibility of contaminants being transferred to the cultured cell line had to be completely eliminated to ensure the burger's success and safety. Consequently, reliable decontamination systems within the incubators were vital. If decontamination did not take place successfully, the whole process would need to restart. As the physical burger took 3 months to produce, this would place a huge financial and time burden on the team.

These three concerns, capacity, control and decontamination, were major considerations that took place when deciding on the equipment for the burgers production. The selected incubation system, produced by Panasonic, comprised several technologies to guarantee the needed levels of quality and control.

Capacity, Control and Decontamination

The team has been using Panasonic incubators for the last decade without any problems, and this positive experience prompted them to looking into purchasing similar equipment for this pioneering project. By enlisting an experienced provider of incubation systems, the 'in vitro meat project' team was able to implement specialist technology and knowledge to meet their requirements. The large range of incubators on offer meant that there was not a problem when selecting a model capable of holding the vast number of cell lines required. After this was established, it was only a matter of spotlighting the correct technology to ensure the needed levels of environmental control and decontamination.

The close monitoring and control of O_2 levels within the incubator was especially important for the stem cell cultures. At the University of Maastricht, this level of control was provided by a long-life zirconia oxygen sensor, especially designed to uphold sub-ambient oxygen levels from 1% to 18%. In addition, electronic PID controls maintained a high level of accuracy by preserving temperature and gas set points over the entire system range.

Temperature reliability was delivered by a patented Direct Heat and Air Jacket System combined with high density foam insulation and the construction of the incubators. This system, shown in *Figure 3*, protects against condensation and helps to reduce the impact of ambient temperature fluctuations on the interior chamber. Gentle fan circulation delivers uniform temperatures to all cultures in the chamber regardless of their position – a huge benefit during this project, when thousands of individual samples were being cultivated at any one time.

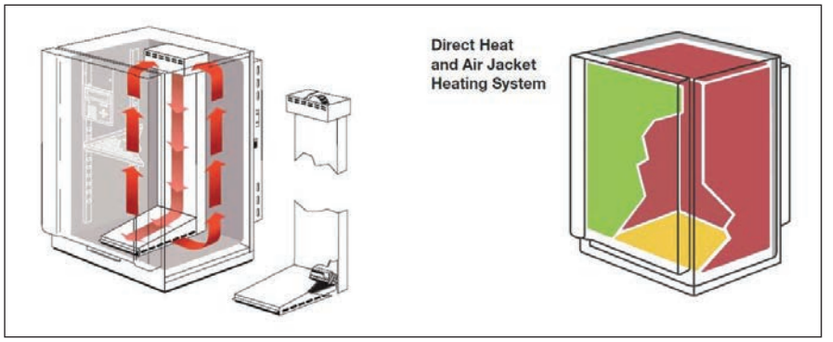
Finally, decontamination systems needed to be in place to safeguard the cell lines. In order to achieve this, the team used two types of Panasonic incubators when creating the synthetic burger. The first was used exclusively for isolation and decontamination, after which the isolated cells were moved over to a second incubator for culturing.

Professor Mark Post (standing) and Food Technician Peter Verstrate in the lab

This reduced the chance of cross contamination, as the cell lines already undergoing cultivation were not exposed to potential contaminants from the initial muscle sample.

In order to tackle and remove any airborne contaminants that may enter the chamber, as well as eliminate contaminants in the water pan, the chosen MCO-19M incubators included an isolated narrow-bandwidth, ozone-free UV lamp. This switches on automatically for a specified period after each incubator door opening, providing additional security to the cells in culture. In addition, all interior surfaces in the incubator are constructed from InCu saFe® copper-enriched stainless steel alloy, providing constant germicidal protection and, in combination with the SafeCell® UV lamp, preventing the growth of moulds, fungi and bacteria.

To remove any risk of contamination from incubators themselves, the University of Maastricht opted to use a H_2O_2 decontamination option for complete decontamination. After system check, this process starts with the vaporisation of hydrogen peroxide, which is then circulated throughout the chamber by the airflow system.



Direct Heat and Air Jacket system, ensuring cells are exposed to regular, controlled temperatures

After this, the ultraviolet (UV) lamp switches on and decomposes the H_2O_2 vapour into water vapour and oxygen. This system limits incubator downtime to less than three hours for total chamber decontamination, and proves not only to be quick but also easy to use and stays contamination free for a long time after each complete decontamination cycle. Thanks to this system, the myotubes could be cultivated not only securely, but without repeated interruptions for cleaning and decontamination, improving the projects efficiency.

Moving Forward

Made up of muscle strands grown exclusively in a laboratory, the burger was finished with a little egg powder, breadcrumbs and a few other common burger ingredients. When tasted, although lacking in seasoning and fat content, which took away from some of its juiciness, it had a definite taste of meat. As the first recognisable meat product created using this method, the project was vital proof of concept for meat culturing techniques, and provided a hopeful alternative for future meat production.

None of this would have been possible without a reliable, efficient and controlled incubation system in place. By implementing Panasonic's high levels of expert technology, the 'in vitro meat project' was able to create an optimum environment for its cells. In this way the 20,000 fibres required to construct the burger were produced efficiently, safely, and at very best quality.

www.biomedical.panasonic.eu

References

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