Identification of hazards in meat products manufactured from cultured animal cells: introduction

1.1 Background

The world's population is growing and along with it, the demand for meat and meat products is increasing. This is paving the way for food technology innovations with sustainability in mind. One such emerging innovation is cultured meat which is gaining traction from research stages to commercialization. Originally, cultured meat existed as a fringe science on the edge of the cell culturing space but started to gain popularity in the early 2000s (footnote 1). The first phase of cultured meat development confined itself to the academic landscape between 2000s to 2013 (footnote 2). At this stage, the medical technology traditionally used for growing cells for purposes such as drug testing and making antibodies was applied to generating meat. In this time, there were a series of notable milestones and experiments that ultimately built up to Mark Post's cultured meat burger, a burger made from bovine myosatellite cells in 2013 (footnote 3).

Subsequently, companies were formed to develop cultured meat from a variety of backgrounds including academia, cell culture companies and some government initiatives. As such, 2011 to 2020 saw the arrival of around 32+ start-ups investigating and working in this area (footnote 4) (footnote 5) (footnote 6). As the technology further advanced, it captured the attention of venture capitalists and large food conglomerates such as New Crop Capital, Bell Food Group, Tyson foods and Cargill who have been investing in cultured meat start-ups and placing in sums of money in the range of $2-17 million (footnote 7) (footnote 8) (footnote 9) (footnote 10). This indicates a seismic shift from the proof-of-concept phase to the start-up phase.

Consequently, there is need to have an appropriate regulatory framework to ensure that products from this technology are safe for human consumption. To date, only one authority, the Singapore Food Agency (SFA), has approved cell-cultured chicken (footnote 11). Also, in 2019 the United States Department of Agriculture's Food Safety and Inspection Services (USDA-FSIS) and the Food and Drug Administration (FDA) took an approach to collaborate in regulating cultured meat. Further to that, UPSIDE Foods successfully completed the FDA's pre-market safety review for a cultivated chicken product moving it one step closer to commercialisation in the U.S. (footnote 12)

At the time of writing (2022) in GB, the FSA mirrors the European Food Standards Agency (EFSA) regulatory framework for Novel Foods regime that oversees the authorisation of new food products that meet the criteria in the regulation. These include genetically engineered (GE) products (newly referred to Products of Genetic Technologies in the UK) where not subject to rules on genetically modified organisms or other specific legislation. The identity of this novel food falls into two classes of the Regulation 2015/2283/EU under article 3: item (vi) food consisting of, isolated from or produced from cell culture or tissue culture derived from animals, plants, micro-organisms, fungi or algae; and item (vii) food resulting from a production process not used for food production within the Union before 15 May 1997, which gives rise to significant changes in

the composition or structure of a food, affecting its nutritional value, metabolism or level of undesirable substances. It is for this purpose that this hazard identification report was commissioned (footnote 13).

1.2 Risk question and scope

The question to be addressed in this hazard identification was:

- What are the potential hazards to the consumer in the consumption of cultured meat?

Hazard identification is the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods. It is one of the first steps in risk assessment. The outcome of hazard identification is a scientific judgement as to whether the initial agents being evaluated could, under certain given exposure, cause an adverse effect in humans. Those hazards identified that have an expected increased exposure due to the consumption of cultured meat will be considered via specific risk assessment.

This work is aimed to highlight potential hazards associated with the production of cultured meat. The hazards identified include the generic hazards of production and composition such as the materials used: chemicals and biological materials used in the process are safe and do not cause harm to the end consumer, and that the product does not contain any microbiological or chemical components of concern. However, there are also more specific hazard considerations centred on the specifics of the bioprocess and the final product composition. For instance, ensuring that the stem cells differentiate correctly so that the product is composed of meat-related cells that have a composition comparable or better than that of meat and having processes for quality control. Another is ensuring that the final product does not lack any key nutritional components or limit the bioavailability of such components. A final example is ensuring that components that are not normally in meat, that are used in cell culturing such as scaffolds, and possibly some media ingredients, are absent or present at levels that do not have any unintended consequences when consumed.

There are many stages of development for producing cultured meat as outlined in section 1.3, from taking a cell line from a small vial (footnote 14) (footnote 15) or biopsy and increasing the culture volume stepwise (footnote 16) in stages (footnote 17) (footnote 18) (footnote 19) (proliferation), until a commercial sized bioreactor can be seeded, to differentiating the cells to final desired cell type (footnote 20) (footnote 21) (footnote 22) (footnote 23) (footnote 24), then maturing them, usually on a scaffold, to increase the protein content (footnote 25) (footnote 26) (footnote 27), and then detaching/grinding the cells with/from their scaffold to produce a final product that can be used to make meat like cells (footnote 28). At each stage, different chemicals (footnote 29) (footnote 30), biologics, media formulations (footnote 31) (footnote 32) (footnote 33) (footnote 34), additives (footnote 35) and supplements are used to ensure a successful culture.

1.3 Cultured meat production process

1.3.1 Selection of the starting cell lines

Cultured meat is currently defined as a cell-derived meat-like product that is produced for consumption by humans and animals (footnote 36). Cells are that are derived from an animal in an ex vivo environment are grown using a cell culturing processes (footnote 37) (footnote 38). Cultured meat describes the generation of skeletal muscle cells (myocytes) and/or with/or without associated cell types including, fibroblasts, adipocytes, stromal cells, vascular cells and nerve cells. Stem cells are obtained by isolation from an animal (footnote 39), but the cells can also be obtained from a cell bank once a stable cell line has been established and safely stored

(footnote 40). Different types of cells can be used for the generation of cultured meat including adult stem cells, adult progenitor cells, embryonic stem cells, induced pluripotent stem cells and immortalised cell lines. Adult stem cells and progenitor cells are currently the most used due to their ease of differentiation, and being most analogous to the natural cell type (footnote 41) (footnote 42) (footnote 43) (footnote 44).

1.3.2 Culture environment

To commence culturing, the obtained cells are placed in an ex vivo environment that is used to maintain their growth (footnote 45). The culturing environment normally comprises three main components: a sterilized container such as a plastic culture flask or bioreactor, a nutrient medium containing nutritional components to support cellular growth by supplying nutrition needed to survive and the growth factors needed to control growth (footnote 46) (footnote 47) (footnote 48), a scaffold which is a biocompatible material used to provide 3D mechanical support to the cells. Additionally, controlling the culture conditions such a temperature, pressure, viscosity etc is necessary (footnote 49) (footnote 50) (footnote 51). The scaffold is important as most cell types associated to muscle are adherent in nature and would naturally die without it (footnote 52). There can be a diffusion limit placed on the cells by a scaffold that needs to be overcome by ensuring the cells have adequate supply of medium (footnote 53). The media is important as it supplies the cells with nutrition needed to survive and the growth factors needed to control growth (footnote 54) (footnote 55) (footnote 56) (footnote 57) (footnote 58) (footnote 59) (footnote 60).

The purpose of this ex vivo environment is to simulate what happens naturally under the conditions of homeostasis and to control growth of the cells (footnote 61). In this, there are many parameters that need to be controlled including the moisture level, dissolved O2 and CO2, temperature, waste removal, pressure, viscosity, hydrodynamic pressure, cell density, viable cells, glucose levels, flow rates, sparging rates, bubble size, waste removal, pH, and the mixing speed (footnote 62) (footnote 63) (footnote 64) (footnote 65). There are numerous ways of managing these, such as having perfusion pumps that continually remove waste and/or by using sensors that check the physio-chemical parameters of the culture that automatically rectify needed changes, such as adding more buffer, adding more O2 or increasing the flow rates.

This environment will ultimately ensure that the cells can be grown to produce a meat like product, and when using satellite cells, ensuring that all the stages of myogenesis are recapitulated (footnote 66). It also allows control over the process, as the parameters of the cell culturing can ultimately be monitored and changed to suit the production requirements (footnote 67). However, this growth environment will be different to the internal workings of the body, meaning there may be differences in the composition of the cells between the bioreactor and nature.

1.3.3 Main production stages

During the processing, the cells will need to go through three main stages of growth, namely proliferation, differentiation and maturation (footnote 68) (footnote 69). In proliferation the stem cells are stimulated to remain as stem cells by preventing differentiation to increase the cell numbers (footnote 70). In differentiation, the stem cells are triggered to differentiate, commencing conversion from stem cells to the desired cell type (footnote 71). In maturation, the cells are permitted to fully develop in their final form (footnote 72). These stages are managed by controlling the parameters of the culture to direct the growth phases of the cells and the differentiation to the final cell type. There are many ways to control the culture and the growth phases. For instance, use of a high amount of growth factors and/or serum is used to keep satellite cells undifferentiated, a change of culture medium from the proliferation medium to the differentiation medium, where the growth factor/serum level is severely reduced is used to trigger differentiation (footnote 73) (footnote 74). Alternatively, a genetically engineered cell line that contains a genetically inducible switch could be used, such that when one chemical is added the cells remain in a state

of proliferation, whilst adding another chemical and removing the first will induce differentiation57.

1.3.4. Large scale production

For the economical production of cultured meat, the cells would need to be scaled up from a small volume in plate culture up to a commercial sized bioreactor. This occurs by making stepwise increases in the size of the culture volume. At first, the cells will be seeded in a small culturing flask and grown for a specified time period or until confluence is reached (footnote 75). This flask is then used to seed another larger flask with more medium. This process is repeated until there are enough cells to seed a bioreactor (footnote 76) (footnote 77). For instance, a small 1L stirred tank reactor could be used to seed 1000L wave bioreactor, which could be used to seed a 10,000L airlift bioreactor (footnote 78). Using this process, a small 1ml vial of cells can be scaled up to a 25m3 tank, with volumes of 1000m3 potential being needed for cultured meat development (footnote 79).

Although many bioreactor designs exist, and sizes of up to 25m3 are used in industry, large industrial bioreactors used for stem cell culturing purposes are not common, and these need to be designed and made operational for cultured purposes (footnote 80). The most common bioreactors used are made of stainless steel. Additionally, as the bioreactor size increases so does everything else, including the media and reagents required, which may pose a challenge of being able to safely and sustainably source materials such as growth factors (footnote 81).

Scaffold materials also need to be produced at scale as the culture size increases. These materials are 3D biocompatible materials, that mimic the extra cellular matrix and provide a structure for the cells to grow on, directing their growth and providing mechanical support (footnote 82) (footnote 83) (footnote 84). They are required as most cultured meat cells are adherent in nature and there is a diffusion limit on growing cultured meat cells. In culture without blood vessels, cultured meat cells can only grow to around 0.5mm thick (footnote 85). Scaffolds can be made from natural or synthetic components, such as collagen, cross-linked pectin, agar, and alginate or poly-lactic-acid, polyacrylamide, poly-glycolic acid (footnote 86). There are number of designs proposed for use in manufacturing cultured meat (footnote 87) (footnote 88) (footnote 89), such as using a mesh network scaffold and perfusing media through the scaffold (footnote 90) (footnote 91), or by using microcarriers or aggregates (footnote 92) (footnote 93) (footnote 94) but these have to be designed in conjunction with the bioreactors (footnote 95) (footnote 96), made suitable for scalable production (footnote 97) (footnote 98), and to accommodate the proliferation, differentiation and maturation of the stem cells (footnote 99) (footnote 100) (footnote 101)35,69,70.

1.3.5 Cell harvest and detachment

Once a sufficient level of culture volume has been obtained and the cells have been differentiated and matured, they are harvested and processed to produce meat like products (footnote 102). Depending on the scaffolding used, the cells may need to be removed from the scaffold or the scaffold may be used along with the cellular biomass to produce a meat product (footnote 103) (footnote 104) (footnote 105). For instance, a collagen/gelatine scaffold could be used and incorporated into the final product (footnote 106), whilst a polystyrene scaffold would not be suitable for edible consumption (footnote 107). Detachment can occur in several ways, such as mechanically removing the cells, enzymically separating the cells from the scaffold, or by using a scaffold material with designed properties such as thermal lift off or pH lift of where a temperature or pH change causes a reversible change in the structure of the scaffold that enables the cells to detach (footnote 108).

1.3.6 Final product formulation

The resulting mass can then be used to produce comminuted products, like burgers and mincemeat. Currently, comminuted products are the first products emerging for cultured meat as

these are simple to make and have lower structural requirements (footnote 109) (footnote 110). More complex tissue structures like whole-cut steak cannot currently be produced due to the biological complexity, their diffusion limit on the cells and may require the making of an intricate vascular network which is not yet possible for the formation of tissue or cultured meat. However, there are a few designs proposed to produce more structured tissues, such as using 3D printing technologies where the cells are placed in a hydrogel and cast into shapes (footnote 111). Finally, there will be the packaging and marketing of the product, but this may be preceded by additional product formulation and processing stages, such as using transglutaminase or binding proteins to cross link the meat and adding flavourings or additives to the cellular mixture, and these will depend on the final cell type produced and the components missing from the cells (footnote 112), for the desired nutritional, taste and texture profile of the product.

1.3.7. Good production practices

All culturing is required to be conducted under aseptic conditions to prevent contamination from cells that can outgrow the culture and spoil a batch (footnote 113). This requires that all laboratories, equipment and work surfaces are sterilized and maintained in this state throughout processing steps associated with the culture of the cells. Consequently, there are stringent cleaning/sterilization protocols with strict management practices such as documentation of all the cleaning. At the laboratory scale this means working in sterilized laboratories in laminar airflow cabinets. For production plants it means having regimes to clean and sterilize all the equipment, such as having inlets for hot steam and cleaning chemicals like sodium hydroxide and minimising any human contact with product lines.

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