

Review Article

White Paper Cell Cultured Meat

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Abstract

FAO forecasts that the global meat production needs to increase by 50% to provide quality protein for the ten billion people living on planet earth by 2050. Meat fits in a healthy diet and is a good source of essential proteins, B-vitamins and several minerals (i.e. selenium and iron). A growing world population will require in 2050 about twice more food, produced on twice less arable land. Good digestible proteins may be a limiting factor in the future. Meat production is seen as less sustainable with negatives impacts on animal welfare. There is a search for alternative proteins to replace part of the animal proteins that are currently used in abundance in the diet of most developed and affluent countries.

Keywords: Cultured meat; Protein; Stem cell

Introduction

To meet the 2050 challenge of feeding a rapidly growing human population, it is essential to carefully consider how to produce enough protein. Cultured meat forms part of the emerging field of cellular agriculture. It seeks to deliver products traditionally made through livestock rearing in novel forms that require no, or significantly reduced, animal involvement. Key examples include cultured meat, milk, egg white and leather. Making meat with minimal involvement of animals is a rapidly emerging new concept. Cell-cultured or lab-grown meat production directly originated from cell and gene therapy, a science that is increasingly used in the field of regenerative medicine. Cultured meat is meat grown from animal cells in culture and the first step is to make them grow in a cell proliferation bioreactor where a nutrient solution or medium allows the cells to quickly grow and multiply. The isolated animal cells regenerate with external support like oxygen and nutrient-rich broth that enables the cells to grow and multiply [1]. Meat without animals is the new notion of cellular biotechnology using stem cells and bioreactors as the basic platform to “brew” nutritious meat.

Cell-based meat should not be confused with meat substitutes such as “soy burgers”. Prof Dr Mark Post from University of Maastricht produced the first lab grown hamburger in 2013. Memphis Meat in California USA, debuted the world’s first chicken strip from animal cells in March 2017, following their animal-free meatball introduction in 2016. Memphis Meat received financial backing from Cargill and individuals like Sir Richard Branson and Bill Gates. Since the first cultured meat hamburger in 2013 a number of start-up companies have emerged (see Table 1; [2]). Besides cell cultured proteins for meat production these also involve cell based dairy proteins, seafood, leather and egg albumin. In this white paper the current state of the art of the technical production process will be described and both the pros and cons of this alternative protein production will be discussed.

What is (are) the current technical process (es)?

Edible meat consists of skeletal muscles along with adipocytes, fibroblasts, endothelial cells and leucocytes, which gives the flavour and texture and finally, make it palatable.

The process involved in production of cultured meat is

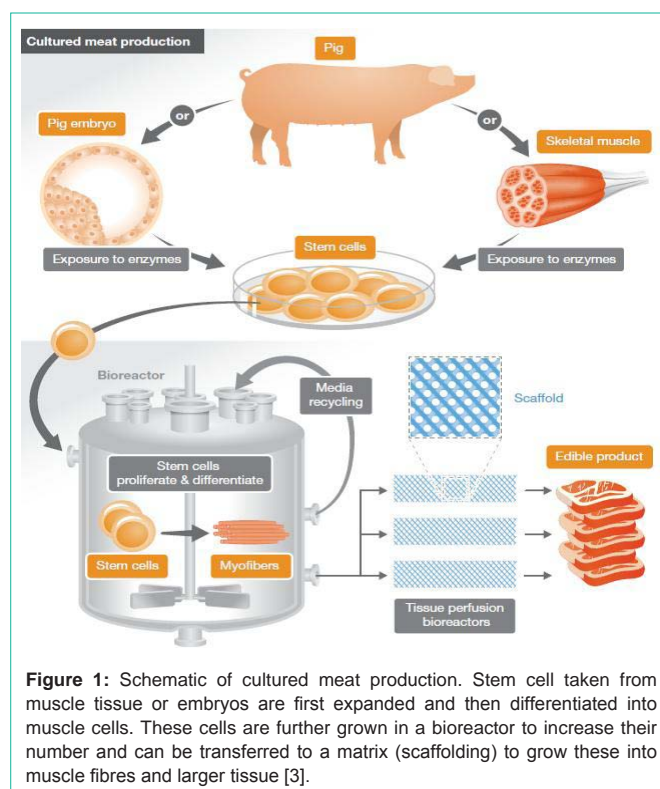


Figure 1: Schematic of cultured meat production. Stem cell taken from muscle tissue or embryos are first expanded and then differentiated into muscle cells. These cells are further grown in a bioreactor to increase their number and can be transferred to a matrix (scaffolding) to grow these into muscle fibres and larger tissue [3].

summarized in (Figure 1). Stem cells are separated from muscle cells (or embryonic cells) and under suitable growth conditions such as temperature, oxygen, nutrients and growth factors, cells grow and proliferate to form multinuclear myotubes. Maturation of myotubes results in the formation of muscle fibres and further growth of muscle fibres ends up into a product, which mimics meat.

The production of cell cultured meat proteins can be divided into the following processing steps:

- Selection of stem cells
- Cell culture processes
 - o Proliferation of the stem cells

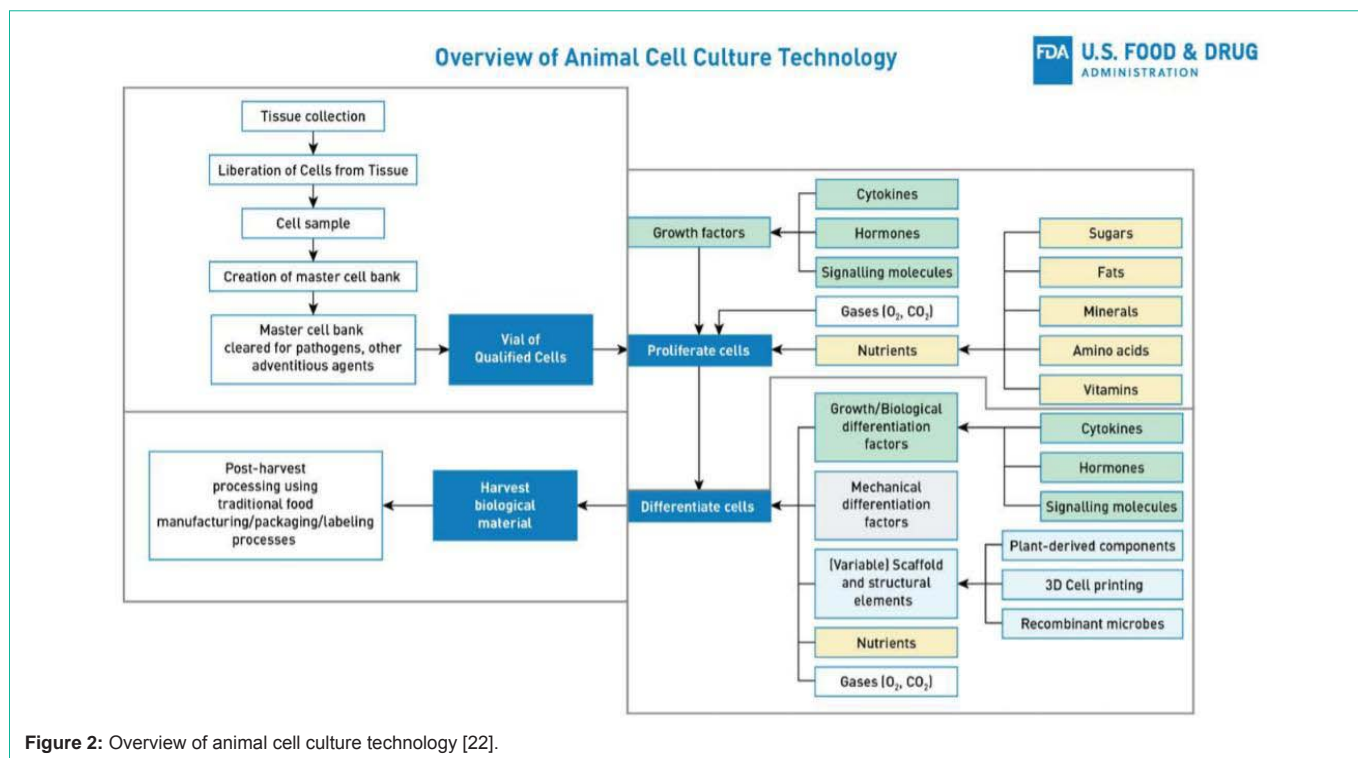


Figure 2: Overview of animal cell culture technology [22].

- o Differentiation into muscle cells
- o Scaffolding
- Harvesting cells or muscle structures

A detailed overview of these processing steps is given in (Figure 2).

Stem cell selection

Stem cells are undifferentiated cells that can become different types of tissue as they mature (and can form healthy new muscle in a living organism to replace what has been lost). The muscle forming cells will join together in long chains and become multicellular myotubes. Since these myotubes are living tissues, at some point they will start spontaneously contracting. Figure 3 gives an overview of the stem cell selection and preparation process. In order to isolate the stem cells enzymes are used [3].

Embryonic Stem (ES) cells, myo-satellite cells and specialized cells in muscle tissue are preferred as a source of muscle cells [4]. ES cell lines have unlimited regenerative potential but mutations can accumulate with generations and they need specific induction to produce muscle cells. Currently, however, no bovine, porcine, caprine or ovine ES cells have been established. Myo-satellite cells are limited in regeneration but can more closely mimic myogenesis. These satellite cells easily differentiate into myotubes and more mature myofibrils and are therefore selected as the preferred cell source for tissue engineering of skeletal muscle [5].

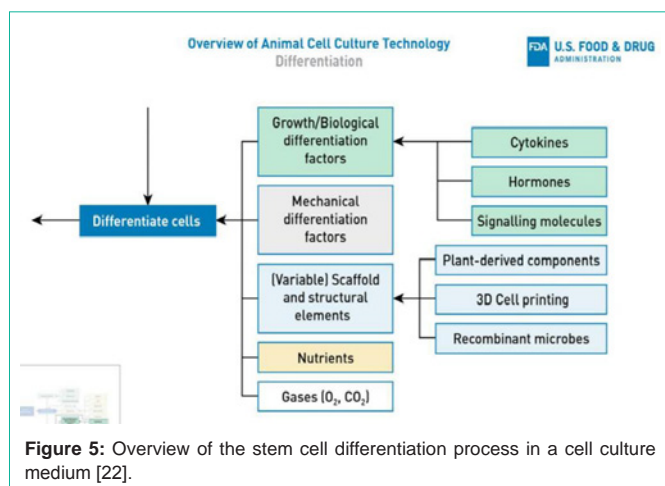
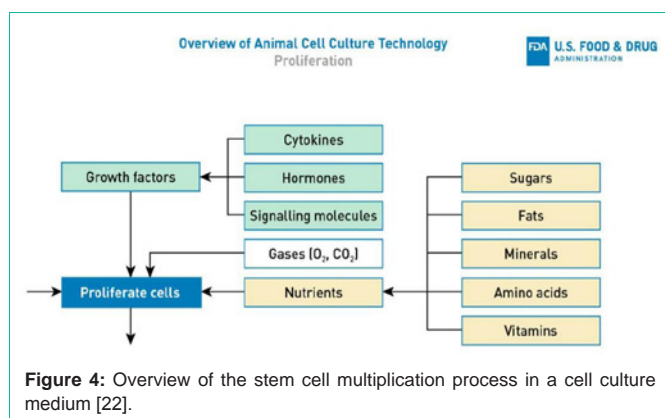
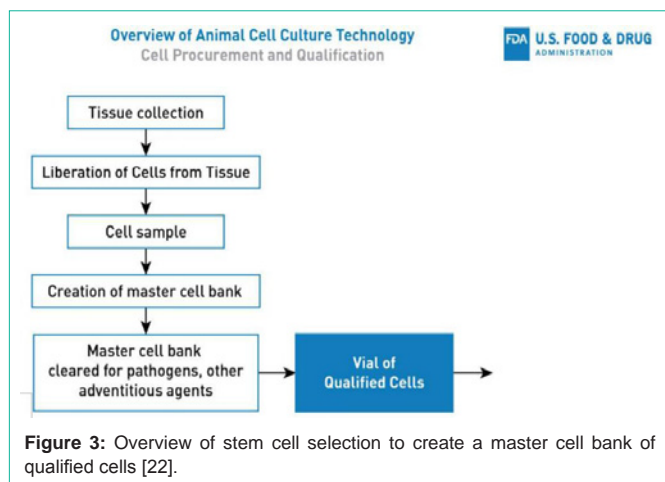
Fermentation-based cellular agriculture contrasts to the above mentioned tissue engineering-based systems in that it does not use any tissue from a living animal. Instead products are manufactured by fermentation using bacteria, algae or yeast that have typically been

genetically modified, by adding recombinant DNA, so they produce organic molecules. These molecules can be used to bio-fabricate familiar animal products (e.g. gelatine, casein (used for milk), and collagen (used for leather)). Fermentation-based cellular agriculture draws upon commonplace industrial biotechnology and therefore may result in marketable products in a shorter timeframe compared to tissue cell culture based agriculture that relies on technology that has not been proven at large scales [6]. The company Modern Meadow, who were the first to produce steak chips, now primarily focus upon leather and have shifted away from a tissue engineering approach towards a fermentation-based system in which an undisclosed cell type is genetically instructed to produce a specific type of collagen for manipulation into leather. Other examples include start-ups Clara Foods (egg white), Pembient (rhinoceros horn), and Perfect Day (milk).

Proliferation and Differentiation of Stem Cells

Cell culture media

The multiplication of the stem cells and the subsequent differentiation in muscle cells takes place in a cell culture medium (for overview see Figures 4 and 5). One of the challenging tasks in *in vitro* meat production is the right formulation of culture medium. The medium should support and promote growth while being made of affordable, edible components available in large quantities. A myoblast is usually cultured in animal sera and foetal bovine serum is the standard supplement for cell culture media. However, this *in vivo* source is variable in composition and is a potential carrier for pathogenic agents [7]. The harvest of foetal bovine serum may also lead to ethical issues.



Commercially standardized media and serum-free media which are completely free of any animal-derived components would be more ideal. An example of a commercially available animal-free-serum is Ultrosor G. The composition of this serum mainly consists of growth factor, adhesion factor, binding protein, vitamins, minerals and hormones which are necessary for the growth of eukaryotic cells [8]. It contains only one-fifth of protein present in animal serum, yet growth and maturation of tissue is faster than that of medium consisting of foetal bovine serum. However, the major problem

is its high cost. Benjaminson et al. [9] reported serum-free media composed of mushroom extract that attains higher growth rate than foetal bovine serum and it is more cost-effective. Eventually, culture media should be completely synthetic and devoid of serum products. A limited number of such products have been developed for medical purposes and it is to be expected that more of these will become available [10].

Designing an appropriate composition of media with hormones, and growth factors is an important area of research work. Growth factors are necessary for cell growth and proliferation. Purified growth factors or hormones may be added to the culture media from plants, animals or transgenic bacterial species which generates recombinant proteins [11]. Co-cultured hepatocytes can produce insulin-like growth factors which promote myoblast proliferation and differentiation and also myo-satellite cell proliferation in some animals [12].

Upscaling bioreactors

Bioreactors have to be developed in order to scale up the production of cultured muscle cells and that allow for a proper medium perfusion with oxygen, nutrients, and different growth and differentiation factors. The design of a bioreactor should be such that it stimulates the growth of tissue. The success of scaled up cultured meat production depends on adequate perfusion of oxygen during cell seeding and cultivation on the scaffold. Adequate oxygen perfusion is mediated by bioreactors which increases mass transport between culture medium and cells. Rotating wall vessel bioreactors rotate in such a way that their speed balances the centrifugal force, drag force and gravitational force and allows the three-dimensional culture to be submerged in the medium, which helps in developing the structure of tissue similar to that of *in vivo*. This type of bioreactors provides high mass transfer with minimum shear stress.

Another type of bioreactor is direct perfusion bioreactor. They are more suitable for scaffold-based cultivation. Here, medium flows through a porous scaffold with gas exchange occurs in an external fluid loop. This type of bioreactors provides high mass transfer with significant shear stress [13]. Cell viability and density depend on the oxygen gradient in fully grown tissue cultures. Oxygen carriers can be added to the medium to retain high concentration of oxygen. There are two types of oxygen carriers, one is improved version of haemoglobin and another is artificially developed perfluoro chemicals. In a skeletal muscle cell culture for meat production, thousands of variables can be-and have to be-controlled to make the process reliable and efficient.

Structuring differentiated muscle cells - Scaffolding

Scaffolding is an essential part of the cultured meat process because it provides heterogeneity structures that influence variables like texture. Scaffolding, which actually provides a support structure for cellular adherence, and develops – if needed – into the various component cells of the integral meat composition. This means when edible scaffolding materials are used they could help to create a more “steak” like cell cultured meat product experience.

Generally, collagen-based meshwork or microcarrier beads are used as a scaffold since they are biocompatible and biodegradable. The cells cultured on scaffolds are introduced into a stationary or rotating

Table 1: Start-up companies that produce cultured animal proteins and their location.

Company	Location	Product
BlueNalu	San Diego CA, USA	cell-based seafood
Bolt Threads	Emeryville CA, USA	cell-based fibers (textile)
Cellular Agriculture Society	Cambridge MA, USA	cell-based agriculture
Clara Foods Redwood	Redwood City CA, USA	cell-based egg albumen
Finless Foods	San Francisco CA, USA	cell-based seafood
Just	San Francisco CA, USA	cell-based meat
Geltor	San Leandro CA, USA	cell-based collagen
Good Food Institute	Washington DC, USA	promoting plant and cell
Higher Steaks	Bristol, UK	cell-based meat
Integriculture	Tokyo, Japan	cell-based meat
Kiran Meats	San Francisco CA, USA	cell-based meat
Meatable	Amsterdam, NL	cell-based meat
Memphis Meat	San Francisco CA, USA	cell-based meat
Mission Barns	Berkeley CA, USA	cell-based meat
Modern Meadow	Nutley NJ, USA	cell-based leather
Mesa Meat	Maastricht, NL	cell-based meat
New Age Meats	San Francisco CA, USA	cell-based meat
New Harvest	New York NY, USA	incubator
outmost House	Berkeley CA, USA	incubator plant and cell
Perfect Day Foods	Berkeley CA, USA	cell-based dairy protein
Seafuture Sustainable Biotech	Calgary, Canada	cell-seafood
Super Meat	Tel Aviv, Israel	cell-based meat
Future Meat Technologies	Tel Aviv, Israel	cell-based meat
Aleph Farms	Ashdod, Israel	cell-based meat
Wild Earth	San Francisco CA, USA	cell-based pet food
Wild Type	San Francisco CA, USA	cell-based salmon

nutrient filled bioreactor. The cells get fused to form myotubes which differentiate into myofibers with the help of differentiation media. This technique produces soft consistency meat or boneless meat, which can be used to prepare hamburger and sausages etc. The main drawback associated with this technique is it cannot produce highly structured or 3D structured meat for e.g. steaks. Co-culture of myoblast and fibroblast is the potential technique for *in vitro* meat production [4].

In order to create more structures cultured meat products 3D printing methods could be used to print scaffolding structures and or the actual cells. 3D Printing involves the spraying of live cells or balls of cells in layers over the gel that acts as a printing paper. Later, the cells get fused to create 3D structure of any shape.

However, it should be noted that successful scaffolds for 3D skeletal muscle formation are all currently animal-derived due to factors such as cell adhesion, fibre alignment and comparability to an *in vivo* environment [14]. The additional consideration is whether the scaffold should be part of the product and therefore edible or

degrade during the culture process to leave 'just' the cultured meat; or, whether the cells are removed from the scaffold so it can be reused to save material. Cost is also important and it is expected that new scaffolds will continue to be developed for as long as cultured meat products are themselves developed and re-developed.

For cultured meat, thin 3D cultures can be utilised to form processed meats (burgers, sausages) whereas whole meats would need the optimisation of thicker 3D structures with a nutrient and oxygen supply and waste removal to sustain the inner core of cells. This is an advanced technique which can take care of meat consistency, vascularization, fat marbling and other elements of conventional meat like taste and appearance [15]. The only drawback with this technique is it is expensive and under development.

Potential benefits of cell cultured meat proteins

Cultured meat could deliver reduced water use, greenhouse gas emissions, and land use compared to conventional livestock meat production. This potential has been assessed in a number of Life Cycle Assessments, although all are based upon hypothetical models of what form cultured meat production might take.

Tuomisto et al. [16] compared cultured meat to conventionally produced beef, sheep, pork and poultry, and found it involves approximately 78-96 % less greenhouse gas emissions, 99% less land use, 82-96 % less water use, and 7-45 % less energy use, depending upon what meat product is it compared to (although poultry uses less energy). Mattick et al. [17] used a different model for cultured meat production, with the most notable differences being the media production method used and inclusion of a cleaning phase. These results suggest cultured meat could involve some trade-offs, with significant energy use leading to cultured meat having greater global warming potential than pork or poultry, but lower than beef, while retaining significant gains in land use. Smetana et al. [18] conducted a cradle-to-plate assessment to compare cultured meat to a range of meat alternatives plant-based, mycoprotein-based, and dairy-based - and chicken, as the least environmentally problematic conventional meat. Across a set of environmental categories they found that cultured meat had the highest impact, mostly due to its high energy level requirements, with the only exceptions being land and water use. The overall picture is that cultured meat could have less environmental impact than beef, and possibly pork, but more than chicken and plant based proteins. However, all three Life Cycle Assessments note that cultured meat technology has significant scope for innovation that could reduce the energy requirements below those used in these assessments, and subsequently could deliver better environmental outcomes than these models predict.

Another potential benefit is that cultured meat could be less prone to biological risk and disease through standardised production methods, and through tailored production could contribute to improved nutrition, health and wellbeing [1]. However, there are some areas to address around genetic instability of multiple cell divisions (Hocquette, 2016) and the components used in the cell culture media (Dilworth & McGregor, 2015).

Cultured meat could enable more of the global population to have consistent access to protein because it is less reliant on climate, land quality and area. While the precise economic value of harvested cells has yet to be determined, the potential to harvest large numbers of

cells from a small number of donor animals gives rise to the possibility of considerably higher returns per animal than traditional agriculture [19].

Cultured meat aims to use considerably fewer animals than conventional agriculture. From an animal protection perspective this could appeal to vegetarians and flexitarians that are interested in reducing their meat intake on ethical grounds.

When considering food waste, traditional carcass utilisation within the commercial meat industry is a problem in the context of waste management. Even though, nothing is wasted from a carcass. Cultured meat provides a new opportunity, whereby the prime cut alone is produced for consumption or processing rather than the whole carcass. There is also opportunity for each producer to create their own version of the product (much like craft brewers, farmhouse cheesemakers and charcuterie producers now), therefore giving them diversity and competitiveness in the market, as well as engaging in higher skilled jobs in a new knowledge economy.

Challenges for cultured meat protein production

The major hurdles to cross are both in terms of the core science of growing meat, and developing a manufacturing process that will enable “cell based meat farms” to produce at a larger scale and at a cost that can compete with animal-reared meat. At the moment most science and cell culture based systems focus on the production of muscle cells. However, in order to produce “real” muscle tissue that has the potential to fully replicate meat, multiple cell types are required (collagen, fat and bone). The current economics of cell cultured meat is a long way from competing with the current meat production of animals.

Muscle cell culture media are expensive, in fact prohibitive on the large scale, therefore, the manufacture of a sustainable, animal-free, affordable media is a major challenge. The same challenge applies to scaffold manufacture. Myogenic cells prefer to reside in animal-derived materials as would be expected as these materials more closely mimic their natural physiological environment. The majority of successful bio-artificial muscle has been grown on scaffolds made from collagen [20].

However, going forward, it is expected that cultured meat can be more cost-competitive with conventional raised meat (prices have come down to more than 1:50 compared to 2013). All signs are clear that the first bioreactors to produce small quantities cell-based cultured meat that can be sold commercially will be ready sometime in 2020 or slightly thereafter. Memphis Meat claims to be able to sell cell cultured meat products to selected restaurants. The introduction of cell-cultured meat for mass consumer introduction at retail level is probably sometime 2028 [2]. When and if these products will be sold, they will initially all be further processed type of products based on cell cultured meat proteins.

Before cell cultured products can actually be sold, though, there needs to be a regulatory approval by the relevant food safety agencies in different countries. As food technology advances, it will be necessary how to inspect and regulate in order to ensure food safety and labelling, regardless of the production method. Global and country-specific regulations for many cellular biotechnology products, including cultured meat, are still in the early stages of

debate and implementation.

It is still unclear if regulators will handle cell-cultured meat with the same rules as traditionally slaughtered meat. One of the issues in the US that still needs to be solved is the regulatory standards and definitions. For the US, this means that the Food and Drug Administration (FDA) and not the United States Department of Agriculture (USDA) will likely be given the authority to decide on issues like standards and labelling since no life animals are involved. The verdict is still out yet. For the US it is suggested that the FDA should have oversight over premarket safety evaluations for cell cultured meat and poultry products, with the USDA providing input to FDA as part of this process. Collaboration between the FDA and USDA will be necessary to accept a new definition of meat produced from animal cells.

There are a large number of potential hazards and health concerns that can or should be defined for cell cultured meat production. Cell culture methods commonly incorporate antibiotics and cell growth modulators, which are currently under scrutiny in normal animal production. There are potential contaminants in cell culture such as *Mycoplasma* spp that could result in health risks. Undifferentiated cell lines resemble cancer and could cause disease in immune suppressed people.

Other questions remaining are: Could potential harmful agents be introduced into culture from seed cells or cell culture materials that might pose risks to human health from a finished food product? What kinds of substances used in cell culture media would be present in meaningful amounts in the finished food product, and are ordinary food ingredient evaluation procedures sufficient to ensure safety? What kinds of structural materials might be used to culture tissues, e.g. scaffolding, and are there any that could not be addressed by ordinary food ingredient safety assessment? How likely is it that cultured animal cells could produce harmful substances as a result of errors in the culture process?

Finally, when a cost effective cell culture based product can be developed, it still has to be accepted by the consumer. It will be important to come up with a marketable name and a good tasting product with high nutritional quality. It has been suggested to use the term “clean meat”, but this would imply ready to eat meat products, that could result in improper heat treatment by the consumer. Current evidence suggests that, whilst consumers most readily perceive benefits to animal welfare and the environment, these issues are unlikely to be central to their buying decisions. It is likely that the picture of consumer acceptance of cultured meat will continue to change over the coming years as the concept nears commercialisation. Increased familiarity, increased perceived feasibility, regulation, commercial availability, media coverage, and the ability to try cultured meat are all factors which are likely to drive consumer acceptance in the future [21,22].

Conclusion

- Cultured meat is a promising, but early stage, technology with key technical challenges including cell source, culture media, and mimicking the *in vivo* myogenesis environment. At the moment the focus is on muscle cell production. For a real meat experience also other type of cell should be cultured, preferably in combination with

the muscle cell culture. Texture and taste will be inferior compared with animal based meat.

- The upscaling of laboratory based cell culture production into a cost effective commercial-scale growth and differentiation of stem cells in bioreactors is a major challenge.

- Animal-derived components in the culture media (i.e. foetal calf serum) and scaffolding matrixes (i.e. collagen) should be replaced by plant based or synthetic materials. These could be less efficient and or involve other issues like GMO derived materials.

- The cell culture media have to contain oxygen, nutrients, but also growth and differentiation factors that include hormones and antibiotics.

- There are a large number of potential hazards and health concerns that can or should be defined for cell cultured meat production. Potential contaminants in cell culture such as *Mycoplasma* spp or other harmful agents could result in health risks. Undifferentiated cell lines resemble cancer and could cause disease in immune suppressed people.

- Major benefits include reduced land and water usage and the limited use of live animals for meat production. The actual effect on CO₂ footprint will be limited or even negative compared to regular pork and poultry meat production.

- Earliest availability of commercial products ranges from 2020 to 2028. These will all be cell cultured meat protein based products (further processed), no “steak” like structures.

- Food safety regulations and official approval by the different local authorities still has to happen in both the US and Europe. HACCP and GRASS type of audit systems have to be defined for cultured tissue engineering-based production systems. Fermentation-based cellular agriculture has been approved for some products, but this contrasts to the above mentioned systems in that it does not use any tissue from a living animal.

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