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Title: Substituting Animal Farming: *Current State and the Opportunities in Cultivating Meat*

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Abstract: Animal farming is in various aspects a great source of pollution and furthermore becoming a big part of the discussion of ethically derived products. The demand for meat products is increasing as a result of the growing population and increasing middle class. In response to this biotechnological solutions can be implemented to substitute current animal derived products. Substitutions for meats are challenging as taste, nutritional profile and texture is difficult to replicate. By cultivating meats, attempts are made to replicate the animal derived meats *in vitro*.

Various part of the production flow is described including: The type of animal and source of cell lines can differ and the strategy needed will depend on which is chosen; The growth media and subsequent growth factors vary depending on the animals from which the cells are harvested from; Anchorage dependence of cells which creates challenges for liquid cultivation in bioreactors; Scaffolding materials and techniques to replicate structured meat product.

Discussed herein is the strategy for cultivation of "sushi-grade" salmon by the company Wildtype. Based on a publicly available patent. Strategies include various methods for obtaining stem cells and various genetic strategies for proliferation and differentiation of these cells.

Outline

Introduction

The world is in transition where new sustainable solutions are a must, as the current exploitation of earth's resources is non-viable. Sustainability is relevant in all aspects of society. Livestock farming in particular is a significant contributor to environmental stress and climate change (Reiss, Robertson and Suzuki, 2021). The environmental footprint is larger compared to plant based products in terms of greenhouse gas (GHG) emissions, soil and water demand. The livestock sector emits 14.5% of GHG emissions, occupies 30% of Earth's terrain and utilizes 8% of freshwater globally (Ben-Arye and Levenberg, 2019). The production of animal derived food itself is inefficient, as up to 97% of the calories consumed by the animal is lost in the process of body maintenance and production of non-edible tissues (Ben-Arye and Levenberg, 2019). When produced intensive livestock farming can be the cause of illness, such as swine flu, avian flu, *salmonella*, *E. coli* and *campylobacter* (Ben-Arye and Levenberg, 2019). In the United States 70-80% of antibiotics are used on livestock, which increases the risk of overusing antibiotics and the subsequent risk of inducing a selection of antimicrobial resistant (AMR) strains (Ben-Arye and Levenberg, 2019). AMR strains could become a major concern for human health, as it is estimated that by 2050, AMR strains will cause more deaths than cancer and annually cost the OECD countries $2.9 trillion (Ben-Arye and Levenberg, 2019). Ethical concerns in the livestock industry, is another augment for substituting animal farming. Both public and scientific communities are starting to see farm animals as sentient beings, but the cost of a "humane" production of animal products is 2-3 higher, thus having an obvious economic constraint (Ben-Arye and Levenberg, 2019).

Current landscape of Alternative Proteins

By 2050 it is projected that there will be an increase in the world population of 60% and an increase in meat demand of 70% (Reiss, Robertson and Suzuki, 2021). This will only contribute more to the concerns of sustainability and ethics, of meat production. This has given rise to the production of alternatives to animal derived protein.

Many companies produce different plant based substitutes to animal derived products. With precision fermentation, it possible to produce recombinant animal compounds such as proteins (eg. whey and egg proteins) or lipids (eg. omega-3s) (Gyr, 2022). Cultivated products could replicate the same nutritional profile or taste as the animal products they are supposed to replace (Swartz, 2019), this could be cultivated meats or even cultivated lactic glands for subsequent milk production. The industries of plant-based, fermentation-derived and cultivated products are interlinked in various ways, resulting in various hybrid products and illustrated by Figure 1 (Gyr, 2022).
Alternative proteins, including cultivated products, have seen an increase in recent years, as depicted in Figure 2 investments in this industry are increasing year after year

(Gyr, 2022). Cultivated products especially for its potential to be both an ethical, sustainable alternative to conventional meat and have the potential to maintain the taste and nutritional profile of meat.

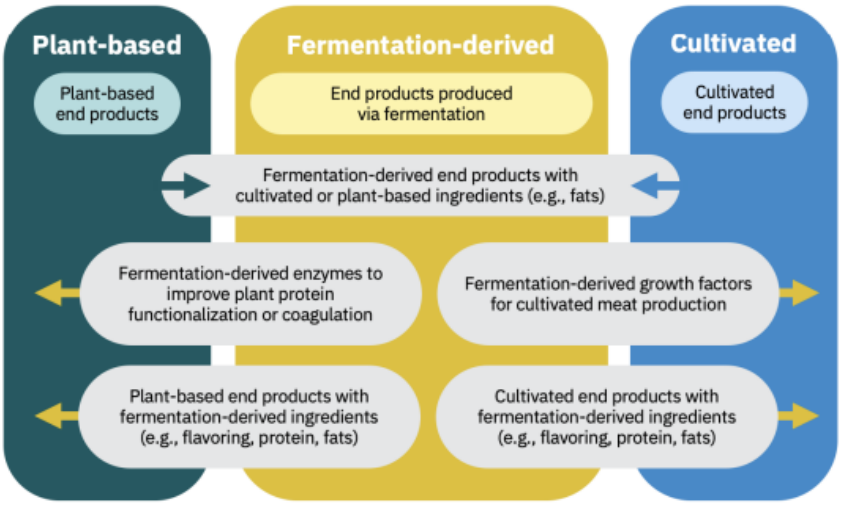

Figure 1 (Gyr, 2022). Depiction of fermentation-derived, plant based and cultivated products and how these products and techniques can be interlinked as hybrid products.

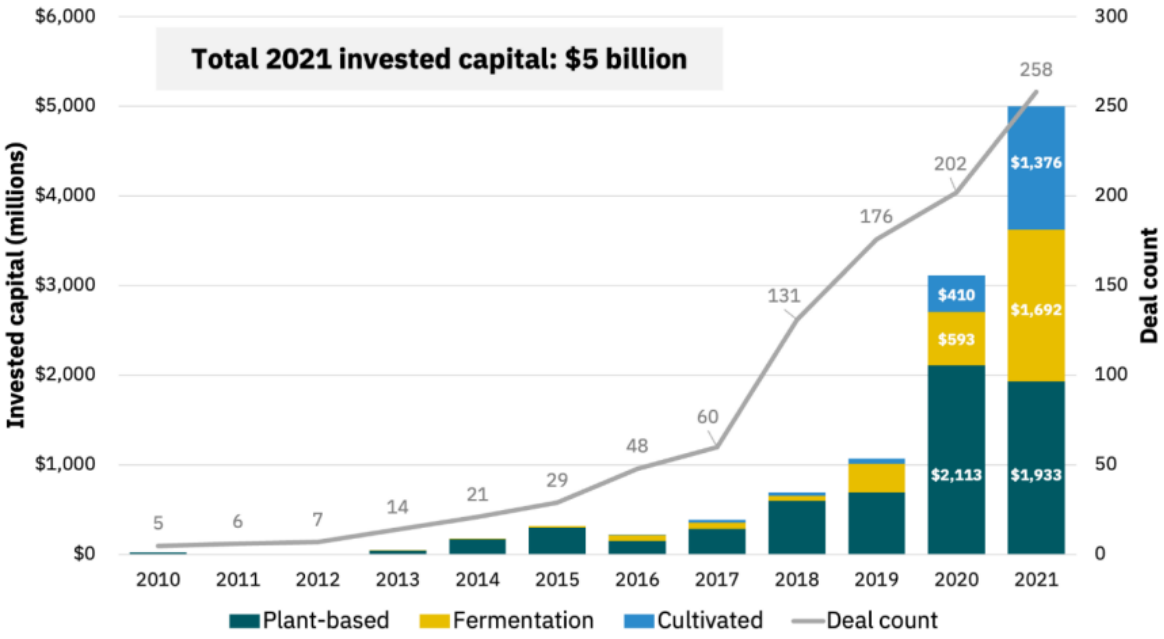

Figure 2 (Gyr, 2022). The chart shows the yearly capital investments made within the alternative proteins industry and the yearly deal counts made. Colors within the bars represent the amount (in US$ millions) in following subcategories: Green; Plant based, Yellow; Fermentation, Blue; Cultivated. The line graph depicts the total yearly deal counts made within the alternative proteins industry.

Within the cultivated meat industry, efforts have focused on substituting beef. But in order to substitute animal products there will also be a need to look into aquatic animals, which have other concerns in regards to the seafood industry and the following consequences for aquatic ecosystems, heavy metals accumulation in wild fish, overfishing and the nutritional quality of farmed fish.

Specific Problems of the Fishing Industry

Industrialized fishing is estimated to have decreased the oceanic biomass by up to 80%, posing a huge threat to wild populations of fish (Rubio *et al.*, 2019). Heavy metals also bioaccumulate in the food chain due to industrial pollution, consumption of seafood contaminated with heavy metals can result in health problems in both humans and animals(Zeitoun and Mehana, 2014). Depletion of many marine fish has shown to affect especially ecosystems related to coastal systems and coral reefs. One example is the overfishing of cod in Canada in the 1980s-90s which seems to have resulted in a cascading effect, resulting in depletion of nitrate in these waters (Scheffer, Carpenter and de Young, 2005).

Aquaculture, on the other hand, can not completely solve problems related to fishing, as the farming of carnivorous fish often involves feeding with wild fish (Rubio *et al.*, 2019). Even though it is possible to control toxic contamination in farmed fish and maintenance of nutritional quality is possible, this requires feeding the fish with feed containing the right fatty acid composition (Cahu, Salen and de Lorgeril, 2004). Diseases in aquaculture can emerge, and are affected by: The dense populations of fish, number of species farmed, international trade and climate change (Murray and Peeler, 2005). The exchange of pathogens from farmed fish into wild populations is difficult to avoid. The high densities of fish in farming result in rapid transmission of infections. Thus the environment in aquafarming facilitates not only transmission but also emergence and establishment of new pathogens (Murray and Peeler, 2005).

Problem formulation

Substituting animal farming by implementation of cultivated animal products solves many of the ethical issues of the current industry. It reduces the waste, less energy of the cells are used for maintenance, and the nutritional profile can be controlled while the production environment ensures no pollution with pesticides, antibiotics and reduces the risk of pathogens (Ben-Arye and Levenberg, 2019). It is predicted that industrial production of cultivated meat will approximately lower the usage of land by 99%, decrease emission of greenhouse gasses by 96% and minimize water usage by 89% in comparison to conventional meat production (Reiss, Robertson and Suzuki, 2021). While beef has been the main focus in the cultivated meat industry, there is a need for research in the field of cultivated seafood. Cultivated fish would not pose the same risks

to ecosystems or human health and nutrition as aquaculture and conventional fishing. This makes cultivated fish a potential candidate for an ethical, sustainable and healthy alternative to these industries.

This report will focus on the general methods for cultivated meat production, and use a patent on the production of cultivated salmon by the company Wild Type, as a case to discuss their cultivation strategies for "sushi-grade" salmon (Elfenbein and Kolbeck, 2018). Specifically this report will be working with the following problem formulation:

How is cultivated meat generally produced and how are different strategies implemented in the production of cultivated salmon by the company Wildtype?

Background

A description of the general workflow of cultivated meat production is needed in order to discuss the opportunities of this industry. It will furthermore be relevant for the case and discussion in order to discuss Wildtype's production strategies. Figure 3 shows the main steps in the production workflow. This starts with cell extraction where considerations include the species, the origin of cells and even race differences depending on the desired final product. Cell line establishment, where desired cells are isolated from the tissue and improved for cultivation purposes. The established cell lines can then be grown in bioreactors for proliferation and subsequent differentiation of cells into eg. muscle fibers or adipocytes. A final step can be added if it is desired to produce a textured product by growing cells within a scaffolding structure.

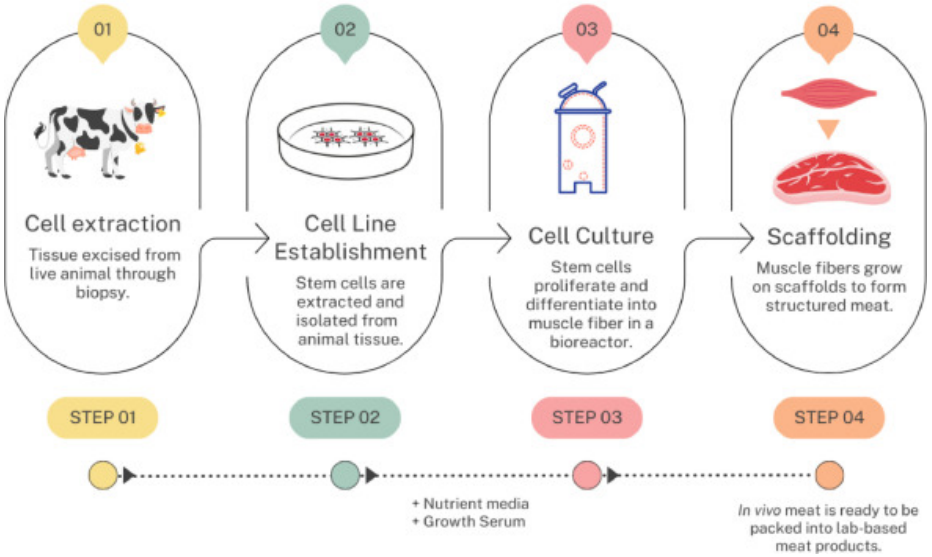

Figure 3 (Ching et al., 2022). A simple visualization of the general steps in the production workflow of cultivated meat production. Cell extraction (Biopsy or embryonic origin), Cell line establishment (generation of cell lines suitable cultivation), Cell culturing (proliferation, differentiation and bioreactor design), Scaffolding (growth of cells within matrix to mimic textured meat).

Cell lines

This section will focus on the different cell types used for the production of cultivated meat and how they are obtained and maintained.

Pluripotent stem cells

Pluripotent stem cells (PSC) are self-renewable cells with the potential to become all cell types of the body. Thus having the potential to differentiate into all the cell types needed for cultivated meat. PSCs can be divided into embryonic pluripotent stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Embryonic Pluripotent Stem Cells (ESCs) are found within the inner cell mass of blastocyst and can be obtained from an early stage embryo (The Good Food Institute, 2022c). ESC-lines have been obtained for various species, but challenges arise when it comes to obtaining a stable ESC-line due to their sensitivity to growth media requirements, stress and potential of spontaneous differentiation, all of which differ from species to species (The Good Food Institute, 2022c).

Induced pluripotent stem cells (iPSCs) is another way of obtaining PSC, by reprogramming already differentiated cells in a way that enables them to return to a pluripotent state with the similar properties of ESC (The Good Food Institute, 2022c). The reprogramming of the cells is monitored by their expression of defined genes (typically transcription factors (TFs)) related to the specific cell types (Rackham *et al.,* 2016). Reprogramming is possible via eg. overexpression of TFs: by the use of virus (Fujie *et al.,* 2014; Schlaeger *et al.*, 2014), episomal or mRNA gene delivery (Schlaeger *et al.*, 2014), proteins (Cho *et al.*, 2010) or small molecules (Zhang *et al.*, 2012). iPSCs are generally possible to obtain from any adult stem cell (ASC) by overexpression of the Yamanaka factors (TF genes: *Oct4, Klf4, c-Myc,* and *Sox2*) (Takahashi and Yamanaka 2006). This makes iPCS more easily obtainable than ESCs, while gaining similar functions (Choi *et al.*, 2015).

Transdifferentiation is also a possibility, avoiding initial conversion to iPSC e.g., fibroblasts differentiation directly into muscle (Ito *et al.*, 2017) or fat (Wu, Jin, and Gao 2017). This approach is limited in its efficiency and they are limited in their expansion as they result in a post mitotic population (Prasad *et al.*, 2017). Proliferation of cells needs to be done before transdifferentiation in order to produce cultivated meat on a large scale. Furthermore, there would be a waste of the cells that do not convert into the desired cell type (The Good Food Institute, 2022).

Adult stem cells

ASCs are stem cells that have differentiated into specific tissue types, that replace existing cells that have worn out or died (The Good Food Institute, 2022c). One of the most abundant ASC found in muscle tissue are myosatellite cells, these cells can be taken through a small biopsy, and can be performed on a live animal, avoiding unnecessary slaughter (The Good Food Institute, 2022c). Further purification Is possible via cell sorting based on known surface markers (Liu *et al.*, 2015).

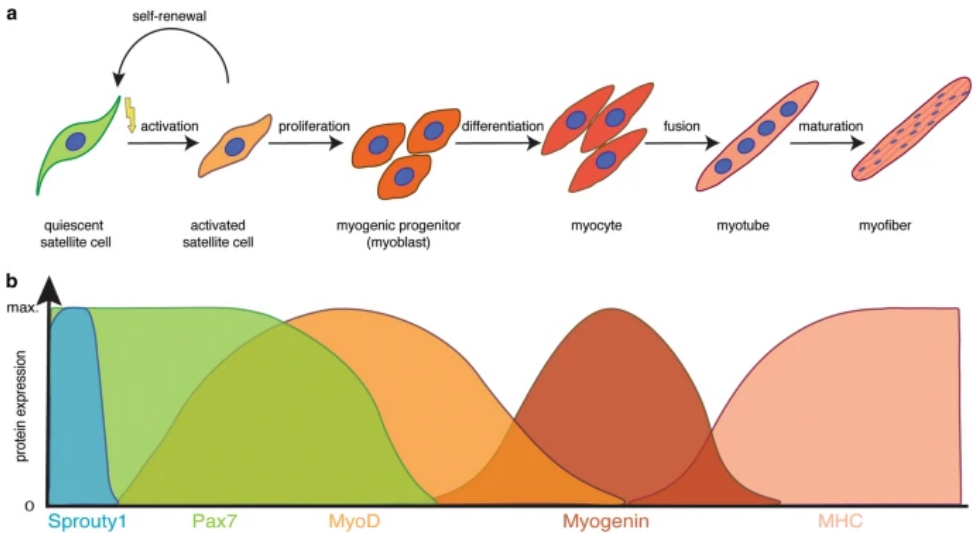

Figure 4 (Schmidt et al., 2019). Progression of myogenic lineage and the expression pattern of key myogenic regulators .
a. Visual illustration of myogenic progression lineage. Proliferation starts by activation of myosatellite cells to generate myogenic progenitor cells, which by differentiation turn into myocytes. These fuse and become myotubes that lastly mature into myofibers.
b. The key modulators and their expression profile during the respective stages of myogenic lineage progression.

Myosatellite cells are found under the basal lamina of muscle tissue along the myofibers. They are quiescent until they are activated by stress or injury (Schmidt *et al.*, 2019). When activated they differentiate into myoblasts which differentiate into myocytes. The myocytes fuse together to form multinucleated myotubes that subsequently form the final myofibers (see figure 4)(Schmidt *et al.*, 2019), each of these express different key TFs (Chal and Pourquié 2017). In muscle of adult tissue, all myosatellite cells express the paired box transcription factor Pax7 (essential for their cell function), while some also express Pax3 or myogenic regulatory factor 5 (Myf5) (Schmidt *et al.*, 2019). Activation of the cells will lead to co-expression of Pax7 and myoblast determination protein 1 (MyoD) from here they can differentiate further into myocytes followed by maturation into myofibers (Schmidt *et al.*, 2019). Some cells will down regulate MyoD and stay in an inactive state dependent on the expression of Sprouty1 (Schmidt *et al.*, 2019). The myogenic potential of myosatellite cells depend mostly on the expression of

Pax genes followed by expression of the myogenic regulatory factors: MyoD, Myf5, Myogenin, and MRF4 (Schmidt *et al.*, 2019). Fish myogenesis stages are similar to that of other vertebrates, but can to some extent differ in the expression pattern (Bomkamp *et al.*, 2022).

Some myosatellite cell markers are found in the plasma membrane, making it possible to isolate cells via flow cytometry (Schmidt *et al*., 2019). Some of these markers are: C-X-C Chemokine Receptor type-4 (CXCR4), Vascular Cell Adhesion Molecule 1 (VCAM1), Calcitonin-Receptor (CALCR), M-Cadherin, Syndecan-4, CD34, α7-Integrin and β1-Integrin (Schmidt *et al.*, 2019).

In fish various identification markers for slow and fast muscle fibers are used. For slow muscle fibers these include: Prospero homeobox 1 (Prox1), myocyte enhancer factor 2ca (Mef2ca), the transcription factors PR domain containing 1 (Prdm1/U-boot) and slow myosin heavy chain (Bomkamp *et al.*, 2022). Fast muscle fiber identification markers on the other hand include: troponin T (tnnt), troponin C (tnnc), muscle α-actin (acta1), parvalbumin (pvalb), α-tropomyosin (tpma) and fast myosin heavy and light chains (Bomkamp *et al.*, 2022). MyoD in gilthead seabream has been found in two different isoforms and might be expressed differently in each muscle fiber type (Bomkamp *et al.*, 2022).

ASCs besides myosatellite cells, include mesenchymal stem cells (MSCs), which is among the most studied types of stem cells. They can be obtained from various tissue types such as: adipose tissue, bone marrow, dental pulp, umbilical cord and placenta. The diverse sources of MSCs have challenged their definition as stem cells (Sipp, Robey, and Turner, 2018). MSCs are to some degree defined by their ability to form adipocytes, chondrocytes and osteoblasts (The Good Food Institute, 2022c). They have a limited potency toward skeletal muscle cells depending on their original tissue source (The Good Food Institute, 2022c). This makes it possible to use MSCs as a starting population for the main cell types of meat. It should be noted that Myogenic differentiation of MSC in fish has not been reported so far (Bomkamp *et al.*, 2022).

Other ASCs residing in muscle tissue such as fibro adipogenic progenitor cells (FAPs), , fibroblasts, and myofibroblasts, are responsible for the extracellular matrix (ECM) (The Good Food Institute, 2022c). FAPs are non-myogenic multipotent MSCs (Bomkamp *et al.*, 2022) and can differentiate into fibroblasts and adipocytes (Biferali *et al.*, 2019). Myogenesis and myogenic differentiation is influenced by growth factors and cytokines, secreted by FAPs (The Good Food Institute, 2022c). FAPs can be recognized by expression of: stem cells antigen 1 (Sca1), preadipocyte factor 1 (Pref1), delta like non-canonical Notch ligand 1 (Dlk1), platelet-derived growth factor receptor-α (PDGFRα or CD140a) or vimentin (Bomkamp *et al.*, 2022).

Preadipocytes in mice indicate expression of makers such as: CD29, CD34, SCA1 and zinc finger protein 423 (ZFP423) (Bomkamp *et al.*, 2022). In fish, markers can differ between species but can in Atlantic salmon include: fatty acid synthase (fas), CCAAT/enhancer-binding protein (specifically c/ebpα and c/ebpβ), peroxisome proliferator-activated receptor gamma (pparγ) and transgelin (Bomkamp *et al.*, 2022). To distinguish preadipocytes from cells undergoing adipogenesis are the expression markers: *c/ebpα*, *c/ebpγ*, *fatp1*, *fas* and *bmp4* (during differentiation) and *fabp11* (during maturation) (Bomkamp *et al.*, 2022). Differentiation in fish FAPs and MSCs into mature adipose and the related genes expressed at the respective stages of lineage progression are shown in figure 5.

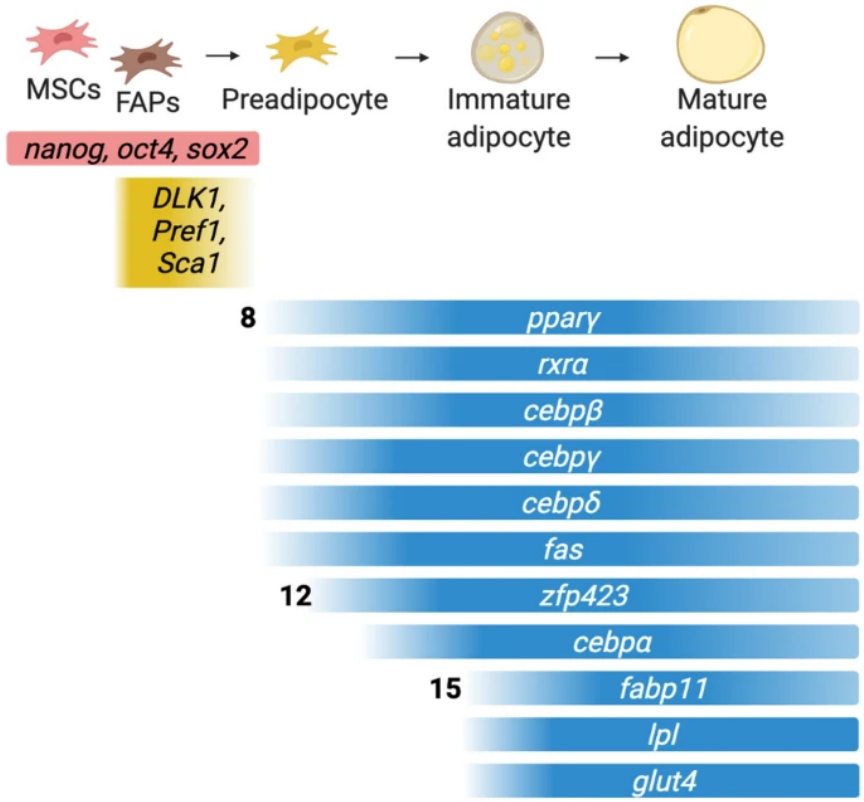

Figure 5 (Bomkamp et al., 2022). Involved genes during differentiation and maturation of fish FAPs and MSCs to mature adipocytes. The order of the genes are presented corresponding to their approximate activation. Numbers denote the timing of activation in hours after fertilization in zebrafish somite. Red: Genes related to MSCs. Yellow: Genes related to FAPs. Blue: Adipogenesis related genes from preadipocytes to mature adipocytes. Intensity of color indicates the strength of expression of each gene.

Cell Line Considerations

The majority of studies on stem cells have been conducted on mice and humans, this creates a need to establish accessible literature and protocols for other species relevant to cultivated meat. Much of the current information is held by private companies (The Good Food Institute, 2022c). Differences in cell lines may also differ between location of the biopsy, the specific breeds and even the sex of the animal, and can be considered in regards to desired traits in the final product (The Good Food Institute, 2022c).

After the selection of a cell line to work with, comes the challenge of proliferation and differentiation. Proliferation can be a challenge when working with ASCs, as there is a limit to the number of times the cells can divide, called the Hayflick Limit (The Good Food Institute, 2022c), which could be overcome by spontaneous immortalization via spontaneous mutations *in vitro,* but these mutations can also influence the cells in unpredictable ways. PSCs can to some extent bypass the Hayflick Limit by preventing the degradation of telomeres through epigenetic changes (Hochedlinger and Jaenisch, 2015) and up-regulation of telomerase (Huang *et al.*, 2014). Thus PSC can be useful in initial scaling, but also has challenges regarding genetic drift, which in turn can cause apoptosis and senescence (The Good Food Institute, 2022c).

Immortalization can be achieved by recombinant methods, by over-expression of telomerase and inhibition of eg. p16, which has a role in tumor suppression of the cell (Tsutsui *et al.*, 2002). Others have utilized viral elements for immortalization (Jha *et al.*, 1998, Sieber and Dobner, 2007). Immortalization can also be turned on and off by the use of Cre-*lox* (Robin *et al.*, 2015; Westerman and Leboulch, 1996), Flp-*FRT* (Westerman and Leboulch, 1996) or *piggyBac* transposon (Xie *et al.*, 2016) systems. Genetic engineering strategies however, might meet obstacles when it comes to regulatory standards.

Growth Media

For a successful production of cultured meat, media composition is essential. Growth media formulations for cultivation of ASCs such as myosatellite cells, MSCs and FAPs generally include some type of basal media, non essential amino acids, L-glutamine and low concentration of fibroblast growth-factor-2 (FGF-2) (Reiss, Robertson and Suzuki, 2021). For PSCs it is similar but might have additional GFs such a: Transforming growth factor (TGF-β), epidermal growth factor (EGF), serum/serum-replacement, extracellular matrix components, heparin (Reiss, Robertson and Suzuki, 2021). A basal media is typically a buffered solution with glucose, amino acids, water-soluble vitamins and inorganic salts. The basal media can then be added specific factors (eg. growth factors or hormones) that enable proliferation, long term maintenance or differentiation (Swartz

and Bomkamp, 2022). Other ingredients such as lipids and antioxidants are also added occasionally.

Glucose: Most commonly glucose is used as the energy source in culture media (The Good Food Institute, 2022b). The concentrations of glucose will vary, depending on cell type (The Good Food Institute, 2022b). The demand from the cells will be higher when they are in their exponential growth phase and can lead to lactic acid being produced and thus potentially disturb the pH level (Zagari *et al.*, 2013).

Amino Acids: The requirement of essential amino acids (EAAs) and Non-essential amino acids (NEAAs) *in vitro* can differ from the ones known *in vivo* as some are dependent on specific organs or cells. As an example *in vivo* in human cells, Arginine, Cysteine, Glutamine and Tyrosine are NEAAs, but become EEAs when growing human cells *in vitro*(The Good Food Institute, 2022b). The testing of amino acid requirements has shown thirteen to be indispensable amino acids (Harry eagle media). This media furthermore consisted of glucose, eight water-soluble vitamins, six organic salts and dialysed serum (The Good Food Institute, 2022b).

Inorganic salts: Salts are important for osmolarity, to serve as cofactors for enzymes and as components for different proteins. Excitable cells such as muscle cells are sensitive to change in ionic concentrations (The Good Food Institute, 2022b). Earles minimal media for example, include: magnesium sulfate, calcium chloride, sodium phosphate, potassium chloride, sodium benzoate and sodium chloride. Other formulations have included iron, zinc and copper (The Good Food Institute, 2022b).

Vitamins: Vitamins work as hormones, cofactors for enzymes and antioxidants. Water soluble vitamins that are included in media formulations are: B2, B3, B5, B6, B7, B8, B9, B12 and choline (The Good Food Institute, 2022b). Additionally, fat soluble vitamins A, D, E and K are also included but which fat soluble vitamins that are essential, can depend on the specific cell types (The Good Food Institute, 2022b). For serum-free media, extra considerations of the stability of vitamins must be taken into consideration as serum contains stabilizing proteins (The Good Food Institute, 2022b).

Serum: The most common serum used for cell culturing is fetal bovine serum (FBS), as it can supplement growth for cells derived from humans, animals and insects (The Good Food Institute, 2022b). Serum contains among others: many proteins, hormones, lipids, antioxidants, attachment- and growth factors. Even though serum free formulations are used to a higher degree than serum based formulations, FBS is still used for some routine cultures both in academia and to some extent industrially (The Good Food Institute, 2022b). FBS is obtained from fetal calves, which are discovered in the process of slaughtering pregnant cows. There are various problems with the use of serum. It

contradicts the purpose of cultivated meat from an ethical perspective as it is a byproduct from slaughter, making it a non-viable solution in the long term. Furthermore, is it an undefined component that can vary between countries and species of cows (The Good Food Institute, 2022b). Serum is a limited product and use of it for cultivated meat would compete with more profitable and mature industries such as pharma. Lastly, serum is also a potential source of various forms of contamination by eg. bacteria, vira and prions (The Good Food Institute, 2022b). This makes serum replacement an essential part of cultivated meat production. One example of serum free replacement is Knockout Serum Replacement which include: 12 amino acids, 3 antioxidants, 19 trace elements, transferrin, insulin, lipid rich albumin (Price, Goldsborough, and Tilkins, 1998). This replacement can maintain pluripotency and self renewal of ESCs in a similar fashion to that of FBS, when used in a basal media with added growth factors (The Good Food Institute, 2022b).

Benjaminson, Gilchriest and Lorenz (2002) looked into the use of various extracts as FBS replacement for fish explants. They saw an increase in tissue growth of explants grown on maitake mushroom extract, similar to the growth increase of explants grown on FBS, see Figure 6 (Benjaminson, Gilchriest and Lorenz, 2002). Further research on the potential of mushroom extracts in serum replacement is needed. If it is indeed possible to utilize mushroom extracts in serum replacement, it could potentially reduce cost of media significantly, as various mushrooms can be produced via low-tech methods.

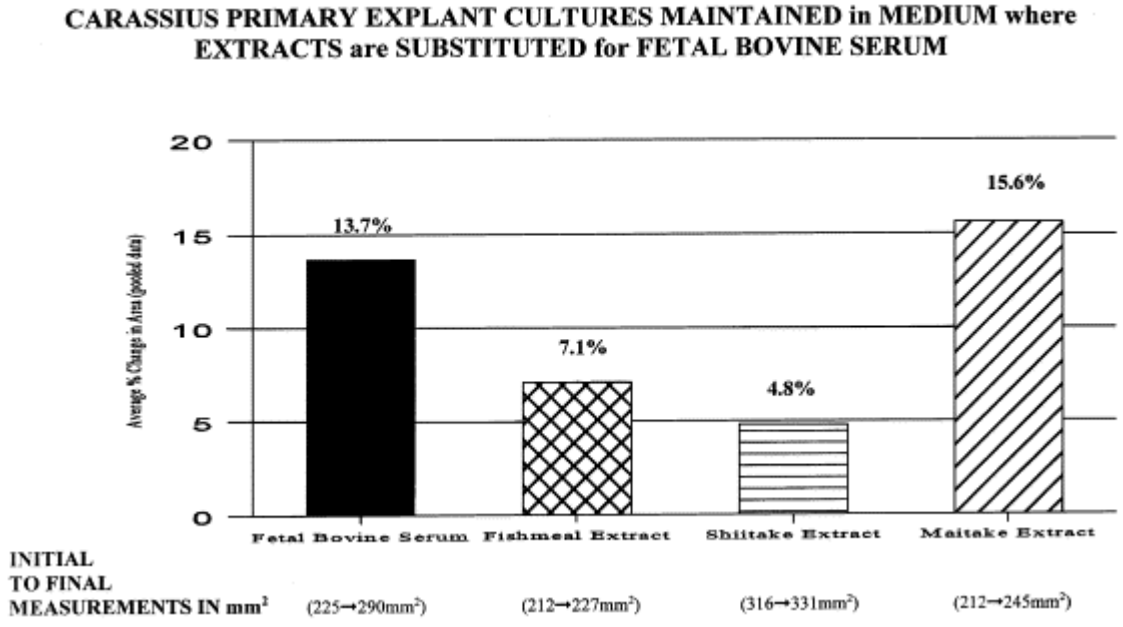

Figure 6 (Benjaminson, Gilchriest and Lorenz, 2002). Results from Benjaminson, Gilchriest and Lorenz (2002) experiment, show in the percentage increase in explant growth on media containing substitutes for FBS: Black: FBS (control); grid: Fishmeal extract; horizontal lines: Shiitake extract and angled lines: Maitake extract.

Albumin, fetuin, transferrin: FBS has a high concentration of proteins with transferrin, fetuin and albumin being the most important for cell culturing. Transferrins assist the transport, control and delivery of ferric iron to cells (Baker, Anderson and Baker, 2003). Regulation of transferrins is important, as high levels can be toxic and low levels can cause apoptosis (The Good Food Institute, 2022b). In serum free formulations recombinant transferrin is added for iron homeostasis, and prevents oxidative damage from free iron (The Good Food Institute, 2022b). Fetuin-A is a factor relevant in cell attachment and spreading *in vitro*, while functioning as an antagonist in the transforming growth factor beta (TGFβ) signaling (Szweras *et al*., 2002). It is not a necessity in serum free formulations, but is attractive, as it can assist induction of attachment and proliferation. Other attachment factor found in FBS are commonly used in cell culture (eg. fibronectin, vitronectin, and collagen) (The Good Food Institute, 2022b). Albumin can stabilize and carry various small molecules, shows antioxidant properties and can reduce shear stress effects (Francis, 2010). It is not essential for serum free formulations but is frequently added as a recombinant protein (The Good Food Institute, 2022b).

Hormones: FBS contains various hormones (endocrine signaling molecules) both protein based and steroid based. These hormones regulate gene expression, growth and metabolism by signaling cascades, and some might only affect certain cell types (The Good Food Institute, 2022b). Insulin is a hormone that has shown to be an essential part of FBS, when used for cell culture. Among all the hormones found in FBS, insulin is the only one commonly added to serum free media (The Good Food Institute, 2022b). The effect of other hormones is typically achieved by addition of the specific growth factors regulated by the respective hormones (The Good Food Institute, 2022b).

Growth factors: 55-95% of costs for cultivated meat production has been estimated to be due to media use (Ben-Arye and Levenberg, 2019). Whereas growth factors (GF) make out 99% of the total media cost . It is possible to replace purified GFs with GF-producing cells, but this makes it difficult to control the concentrations of GFs (Ben-Arye and Levenberg, 2019). Some approaches to this challenge, is to produce GFs at lower grade or through bioreactor design, where recycling of the media components is possible (Ben-Arye and Levenberg, 2019). The GFs are a wide range of different signaling molecules. They initiate signaling cascades involved in growth, proliferation or differentiation. FBS contains various growth factors typically in the fibroblast growth factor family, insulin growth factor family, neuregulin family and transforming growth factor family (Zheng *et al*., 2006). GFs are found in much smaller concentrations in FBS than eg. proteins; fetuin and albumin, thus making GFs difficult to detect and quantify in an accurate manner (The Good Food Institute, 2022b).

Bioreactor design

Replacing the industrial production of meat, will require a production of cultivated meat on an industrial scale to ensure an efficient and affordable production. The change from lab scale to production in bioreactors adds considerations such as mixing, shears stress, foaming, heat transfer and gas exchange. Generally bioreactors for cultivated meat can be divided into batch, fed-batch, continuous and perfusion (The Good Food Institute, 2022a).

When growing cultures in a batch, cells are grown in a vessel with a fixed volume until the maximum density of cells has been reached. In fed-batch fed media is added during the process. A continuous process there is both an inflow of fresh media and an outflow of cells, product and media simultaneously. Whereas perfusion is a type of continuous process, where cells are kept in the culture in a way that allows for the recycling of media and high cell densities (The Good Food Institute, 2022a). Table 1 shows soma achieved cell densities for cultivated cells in different types of bioreactors.

Bioreactor Type	Cell Density	Cell Type	Microcarriers
Stirred tank reactor	2x10^6	human MSC	Yes
Stirred tank reactor: single use	1x10^7	CHO	No
Unknown	4x10^7	human stem cells	No
Rocking Platform: Perfusion WAVE bioreactor with cell separation devices	1x10^8	CHO	No
Hollow fiber	1x10^8 to 1x10^9	human MSC	No
Air-lift bioreactor (theoretical)	2x10^8	"animal cells"	No
Core-shell microtubes	5x10^8	hiPSCs	No

Table 1 (The Good Food Institute, 2022a). Some examples of achieved cell densities of stem cells cultivated in different bioreactor systems. Chinese hamster ovary (CHO), human iPSC (hiPSC).

In continuous stirred reactors, cells are grown in suspension and mixed by mechanical stirring, this allows high degree of oxygen mass transfer, and can be combined with the use of microcarriers (The Good Food Institute, 2022a). It is also possible to use air lift reactors for suspension growth, which is advantageous at large scales, as the mixing with the use of air does not require moving parts, resulting in reduction of power used for mixing, increased homogeneity and reduced shear stress (Merchuk, 1990).

Shear stress

As animal cells lack a cell wall they are much more susceptible to shear stress than other microbes. The shear stress influences both the differentiation (Stolberg and McCloskey, 2009) and the viability of the cells (Hu, Berdugo and Chalmers, 2011). The shear stress can be caused by the turbulence in the liquid caused by the impeller's speed, these effects can be mitigated by cell adaptation, use of flow breakers or adding poloxamers to

the culture media (Chang *et al.*, 2017). Rupturing of bubbles in the liquid can also contribute to the shear stress, eg. bobbles of less than 1 mm in diameter can result in increased shear stress and cytotoxicity rates in the cells (Nienow, 2006). One solution to the problem of shear stress is to decrease turbulence to create a laminar flow. Some methods include novel design of impellers to create an upward flow of liquid that is expelled to the sides, allowing the fluid to mix by itself (The Good Food Institute, 2022a).

Anchorage dependance

Most stem cells are anchorage dependent. Even though PSCs are able to grow as aggregates in suspension in an anchorage independent manner. In order to prevent spontaneous differentiation of PSCs, they have to be dissociated into single cells (The Good Food Institute, 2022a). The single cell will typically need to be treated with Rho kinase to prevent cell death (The Good Food Institute, 2022a). The cultures grown as aggregates might be more resistant to shear stress, but this decreases cell viability and densities, compared to single cell suspension (Lipsitz *et al.*, 2018). To overcome these challenges, adaptation directed evolution (Tizei *et al.,* 2016), assisted or natural selection or genetic engineering strategies could be used.

Microcarriers

Microcarriers are small beads (typically 100-400µm in diameter), that can be used to grow anchorage-dependant cells in a bioreactor, by mimicking the characteristics of the extracellular matrix (ECM)(The Good Food Institute, 2022d). Cell expansion in this system can happen via bead to bead transfer (Verbruggen *et al.*, 2017) or by enzymatic dissociation of beads followed by transferring cells to larger vessels (Rafiq, Coopman and Hewitt, 2013). For anchorage-dependant cells there is a risk of anoikis which can be counteracted by adaption of the cells to suspension growth, growth in spheroids or achieving anchorage independence via eg. Rho Kinase inhibitor (The Good Food Institute, 2022d). The advantage of microcarriers is their large ratio of surface area to volume. It is also possible to have differentiation occur on the microcarriers themselves, by changing the components of the media.

Microcarriers can also be used for process optimisation eg. be designed with nooks, reducing the shear stress (Wu *et al.*, 2018). Microcarriers are typically constructed of eg. polyacrylamide, dextran, polystyrene, glass, plant-derived materials or materials that can be dissolved (The Good Food Institute, 2022d). Often microcarriers can be coated in ECM proteins, be made positively charged or made hydrophilic for facilitation of cell attachment (The Good Food Institute, 2022d). For unstructured blended products, microcarriers could consist of an edible polymer, for the possibility to incorporate it into the final product (The Good Food Institute, 2022d). In this way avoiding removal of cells from microcarriers and demonstration of safety for human consumption post-removal.

Scaffolding

Scaffolding is an important part of making an actual structured product capable of imitating the complex texture of meat cuts. *In vivo* the cells are found in a structured matrix of proteoglycans and proteins called the ECM (The Good Food Institute, 2022d). The stiffness of the ECM contains integrins that through mechanosensing, mediate effector proteins to form focal adhesion complexes. These connect the ECM to the actomyosin cytoskeleton (The Good Food Institute, 2022d). This mechanism leads to signaling downstream, which affects polarity of the cells, their migration and differentiation (The Good Food Institute, 2022d).

During development of muscle *in vivo,* the stem cells will start to differentiate and in turn express the genes, as well as secrete, ECM components (The Good Food Institute, 2022d). What allows for further differentiation and migration of the cells, is the feedback mechanism from the ECM, known as dynamic reciprocity (The Good Food Institute, 2022d). Multiple studies on ECM have demonstrated its crucial role in differentiation and stemness of some relevant cell types for production of cultivated meat pluripotent stem cells (Wang, Luo and Leighton, 2015), mesenchymal stem cells (Engler *et al.*, 2006), myosatellite cells (Calve, Odelberg and Simon, 2010), and adipogenic stem cells (Guneta, Loh and Choong, 2016).

Scaffolding materials

The scaffold should ideally allow the attachment, differentiation and maturation while also being able to let perfusion of media occur, continuously. The material of the scaffold can be made from materials that are natural, synthetic or a combination (The Good Food Institute, 2022d). ECM material such as gelatin, fibrin, vitronectin, laminin, derived from vertebrate materials can function as native motifs for self adhesion (The Good Food Institute, 2022d). Other natural materials could be agarose, alginate, carrageenan, cellulose, chitosan, silk , fungal mycelium, or decellularized tissues (The Good Food Institute, 2022d). Some natural materials do not have the cell recognition motifs (eg. Arg-Gly-Asp (RGD) and Ile-Lys-Val-Ala-Val (IKVAV), motifs) these allow for the integrins on the cell surface to bind (The Good Food Institute, 2022d). Synthetic material on the other hand could be pluronic, polyglycolic acid (PGA), poly ethylene glycol (PEG), polyacrylamide, poly 2-hydroxyethyl methacrylate (PHEMA) (The Good Food Institute, 2022d). Materials that do not have an RGD motif can be mixed with material that do (eg, gelatin), be genetically engineered to express the RGD motif within the material (Widhe, Shalaly and Hedhammar, 2016) or the RGD peptides can be chemically crosslinked to the material itself (Tsai, Chen and Liu, 2013).

Hydrogels make up the majority of scaffolding material used in tissue engineering (The Good Food Institute, 2022d). They consist of a 3D polymer network with hydrophilic

properties , some able to absorb water 1000 times their own dry weight. Engineering can be done to control swelling and deswelling by stimuli such as pH, light, eclectic field and temperature and furthermore have various macromolecules incorporated (The Good Food Institute, 2022d). Stiffness of a scaffold can influence differentiation of stem cells eg. MSCs on soft hydrogels will favor differentiation into fat whereas hard hydrogels will favor differentiation into bone (The Good Food Institute, 2022d). Muscle satellite cells grown in a substrate with a stiffness similar to its niche, will self renew (The Good Food Institute, 2022d). One downside to hydrogels is their limitation in scaling due to the challenges of vascularisation.

One way of creating cultivated tissue is by constructing a scaffold then subsequently seeding cells into the scaffold, but that approach can not make a microstructure similar to the native tissue (The Good Food Institute, 2022d). Another way is by creation of smaller micromodules that can subsequently be combined and assembled into complex tissues. technologies such as polymer spinning, 3D bioprinting and decellularization are some of the technologies used to create these scaffolds (The Good Food Institute, 2022d). Decellularization of plant tissues for scaffolding has been demonstrated (Fontana *et al.* 2017; Modulevsky *et al.* 2014), and in the future, combining decellularization and genetic engineering could allow for expression of the binding motifs of ECM (such as RGD) directly in the tissue. It is also possible to utilize fungal mycelium as a scaffold, without having to perform decellularization (The Good Food Institute, 2022d).

Other considerations

In vivo, muscles are stimulated by neurons, similar effects can be achieved by stimulation via electrical pulses (The Good Food Institute, 2022d). Myotube can also be grown on conductive coatings, conductive polymers or in a magnetic field, to improve their alignment and maturation (The Good Food Institute, 2022d). Growth of myotubes together with motor neurons has also shown to improve maturation and formation of the myotubes (Kim *et al.* 2020).

Alternative solutions to transport limitations of nutrients, oxygen and waste, is to produce cells encapsulated in hydrogel tubes. This also shields the cells from shear stress and can result in high cell densities up to 5 x 10^8 cell/ml (The Good Food Institute, 2022d). Within these tubes the entire process of proliferation, differentiation and maturation can occur (see Figure 7).

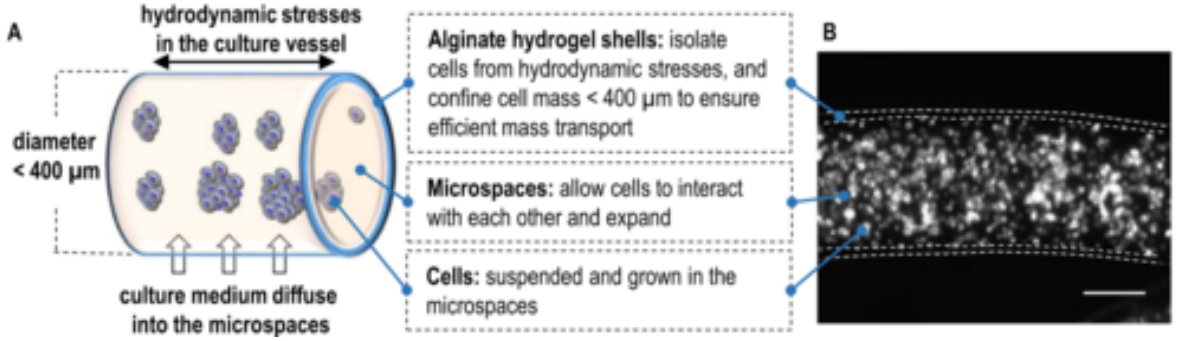

Figure 7 (The Good Food Institute, 2022d). Illustration of a microtube, its dimensions and principle.

These hydrogel tubes have robust structure and are flexible and can tolerate suction or ejection through the tubes. They can furthermore be woven or knitted in a similar fashion to that of the textile industry (Onoe *et al.,* 2013). If made of eg. alginate, it is possible to dissolve the hydrogel to obtain only the cellular structure (The Good Food Institute, 2022d).

Challenges arise when it comes to thick tissues, as oxygen and nutrients have to be able to be supplied to the cells (The Good Food Institute, 2022d). A top down approach to this challenge would be to fabricate a porous scaffold and seed it with cells. whereas a bottom-up approach would be to create small modular scaffold/cell-containing units to then construct and be combined into a final shape (The Good Food Institute, 2022d).

Comparing cultivated meat to plant based meat substitutes, it will have a taste and nutritional profile that compares better to traditional meats (McNamara, 2022). But until the industry matures the price will not be able to compare. Cultivated meat will have an improved environmental impact compared to traditional meat, but will, as of now, not be an improvement when compared to current plant based products. Creation of hybrid products could improve both cost and sustainability of cultivated meat. But consumer acceptance of alternatives to animal products is a challenge (McNamara, 2022).

The combination of plant based and cultivated meat hybrid products could also offer new possibilities for the plant based meat industry as cultivated fats can help improve the taste of current meat substitutes and contribute with flavors specific to different animal species. In 2020, Belgian based Peace of Meat surveyed their B2B customers within plant based production, where 68% of them responded that they would be either “likely” or “very likely” to use cultivated fats to improve taste and texture of their products (Retchin, Iacovelli, and Brandes, 2021). The hybrid market might be a way to facilitate the market entry for cultivated products, both in regards to lower cost and the slow introduction (McNamara, 2022).

Case and Discussion

As fish is high in nutrients, a meat type of higher economical value, it could be one of the first cultivated meats to be considered both in regards to nutritional health and economical feasibility. This chapter will focus on the specifics of fish cultivation and use the company Wildtype as a case. Information on Wildtype is extracted from their parent (Elfenbein and Kolbeck, 2018), and might not be in detail but will be used together with the knowledge available for cultivated fish.

Wildtype Case

Wildtype suggests various strategies in regards to obtaining their cell lines. Figure 8 shows a visual overview of their different cell line strategies.

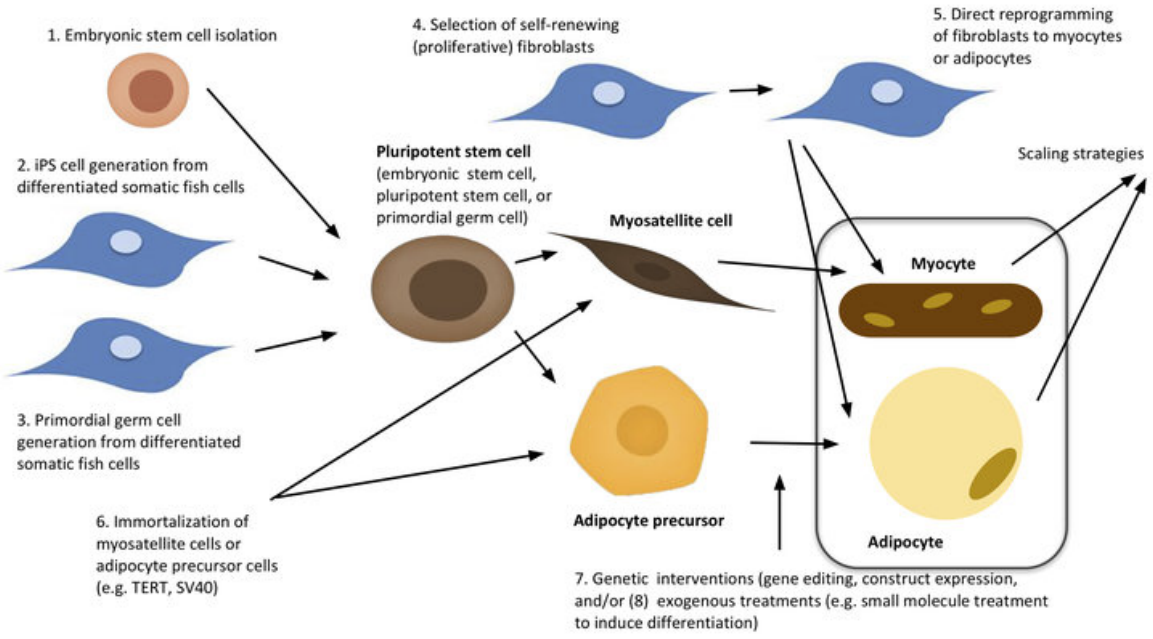

Figure 8 (Yaman, 2019). illustration of Wildtype cell line strategies from PSC or ASC starter cells to adipocytes and myocytes.

Wildtype include the strategy of utilizing different PSCs (ESC, iPSC and primordial germ cells), followed by differentiation into myosatellite cells and adipocyte precursors to then, either by genetic intervention or by exogenous treatment, differentiate into myocyte and adipocyte cells (Elfenbein and Kolbeck, 2018). They give various concrete examples of how they intend to produce fish and furthermore what they call, “sushi-grade” salmon.

They suggest ways to both produce fish meat from ESCs, iPSCs or by trans-differentiation. Their ESCs are isolated from salmon embryos, whereas the starting cells for their iPSC cell line are fibroblasts isolated from salmon (Elfenbein and Kolbeck, 2018). The fibroblasts are induced via an episomal reprogramming strategy (Elfenbein and Kolbeck, 2018), which is a strategy that utilizes episomal vectors to express pluripotency reprogramming factors such as: *Oct4, Sox2, Nanog, Lin28, Klf4* and *L-Myc* (Welch, 2012). The colonies are then grown on mouse embryonic fibroblast feeder cells (MEFs)(Elfenbein and Kolbeck, 2018), these types of cells contribute with ECM components and growth factors (Marchetto *et al.*, 2009). This strategy does not integrate the vector into the cells, avoiding the need for viral techniques. A similar strategy by Marchetto *et al.* (2009) is illustrated in Figure 9.

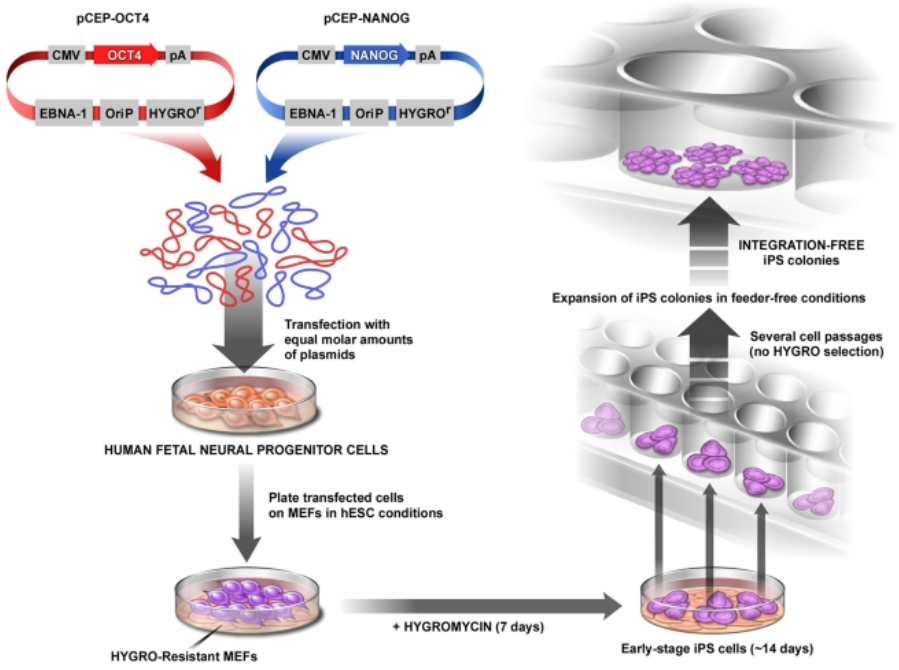

Figure 9 (Marchetto et al., 2009). Visual presentation of Marchetto et al. (2009) strategy for episomal reprogramming of stem cells. Episomal plasmids with reprogramming factors are introduced into stem cells, followed by growth on MEFs with hygromycin. After 7 days, colonies of iPSC will appear.

The general protocol to obtain MEFs is by euthanization of a pregnant mouse followed by harvesting of the mouse embryos, then cutting off and mincing desired cells before suspension into the MEF media, as shown in Figure 10. The use of MEFs in the production workflow would be an ethical problem for consumers such as vegans or vegetarians, and would be counterintuitive when they actively try to substitute FBS with non-animal derived extracts (Elfenbein and Kolbeck, 2018).

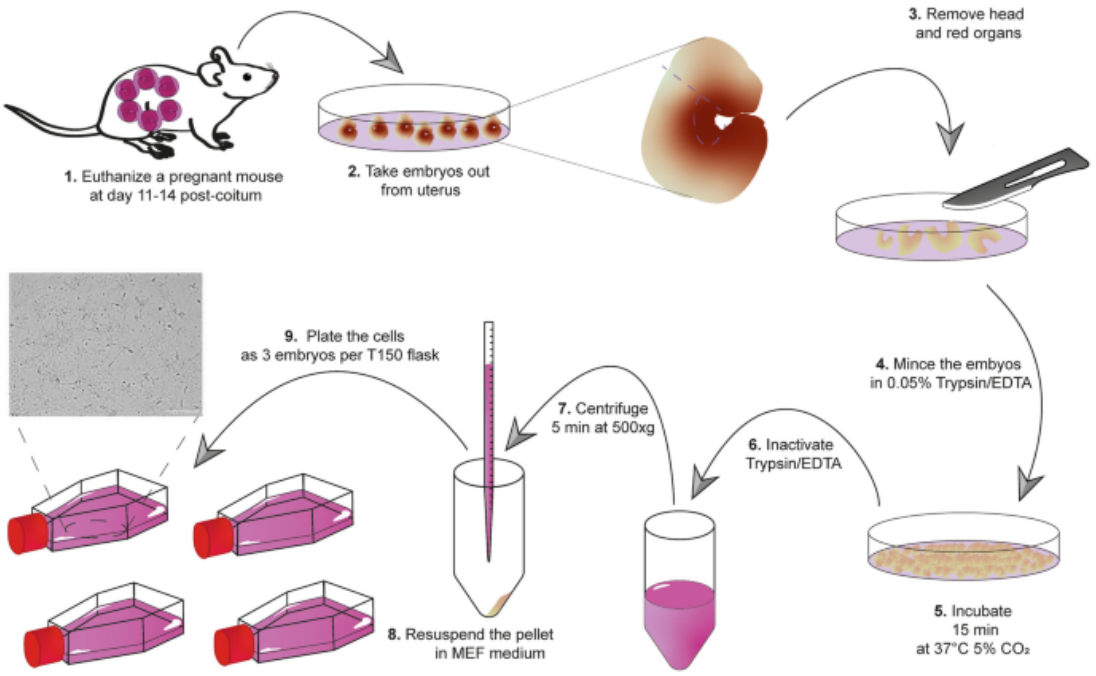

Figure 10 (Şişli et al., 2021). Visualized process of obtaining MEFs. Embryos are harvested from a euthanized pregnant mouse. Red organs and heads are removed from the embryos before mincing the embryos. cells are incubated, centrifuged and subsequently suspended in MEF media.

Their PSCs regardless of origin are cultured in a synthetic serum-free media formulation, optimized for proliferation and maintenance of an undifferentiated state (Elfenbein and Kolbeck, 2018). Next the cells are induced for differentiation into pre-adipocytes and myosatellite cells (Elfenbein and Kolbeck, 2018). These cell types are each expanded before further differentiation into myocytes and adipocytes.

For the transdifferentiation strategy they perform serial passage of the fibroblasts until they achieve an immortal fibroblasts cell line (Elfenbein and Kolbeck, 2018). These cells are then grown to the desired quantity before transdifferentiation (Elfenbein and Kolbeck, 2018). The reprogramming strategy is not described in detail, but is simply the overexpressing of specific genes, allowing for direct reprogramming from fibroblasts directly into adipocytes and myocytes (Elfenbein and Kolbeck, 2018). Whether the strategy for transdifferentiation is similar to that of the differentiation of the PSCs, by the use of episomal vectors (just expressing factors for the desired cell types instead), is not stated. This strategy would avoid the need for an intermediate pluripotent cell line, but during the process of obtaining the immortal cell line, undesired mutation might be introduced. In other cases they use MSCs instead of fibroblasts for transdifferentiation into both myocytes, and adipocytes (Elfenbein and Kolbeck, 2018).

After obtaining the adipocytes and myocytes, they are harvested, centrifuged and compacted to create a textured product. For their product of "sushi-grade" salmon the myocytes are differentiated to obtain approximately 80% fast twitch cells and 20% slow twitch cells, but how they ensure this ratio is not stated (Elfenbein and Kolbeck, 2018). But as stated by Bomkamp *et al.* (2022) there are differences in marker genes for slow and fast muscle fibers, while two different MyoD isoforms also have been found and could be differently expressed in slow and fast muscle fibers.

Wildtype has a suggestion on how they will transition from 2-dimensional feeder cell dependent culturing of PSCs to 3-dimensional culture. They do this by growing iPSCs as embryoid bodies, where stem cells attach to each other instead of a surface (Elfenbein and Kolbeck, 2018). They do this via the hanging drop method, where cells grow for 72h within a droplet of media that in turn result in the formation of embryoid bodies, that can then be grown in suspension culture (Elfenbein and Kolbeck, 2018). Subsequent differentiation is done within the suspension culture.

Suspension culture adaptation is also suggested by the use of microcarriers for the multipotent cells (eg. pre-adipocytes and myosatellite cells) (Elfenbein and Kolbeck, 2018). They screened for abundant, inexpensive, partially soluble and neutral-tasting polysaccharides and evaluated glucomannan derived from konjac as a good candidate (Elfenbein and Kolbeck, 2018). They tested the use of microscaffolds against typical 2-dimensional culture and after 5 days of growth, myosatellite cells' ability to differentiate into myocytes was improved by the use of microscaffolds (Elfenbein and Kolbeck, 2018). The microscaffolds they use contain recombinant ECM proteins (to promote attachment of the cells), growth factors (to stabilize the cell type) and encapsulated lipids (Elfenbein and Kolbeck, 2018).

To overcome the pricing of media and substitute FBS, Wildtype has made an inhouse approach to develop media and strategies to minimize costs through: Media reconstruction, recombinant expression of components and a conditioned media system (Elfenbein and Kolbeck, 2018). Wildtype suggests slowly transitioning cells to plant-based formulations such as mushroom extract or soybean hydrolysate (Elfenbein and Kolbeck, 2018). Another suggestion is in-house recombinant expression of expensive components in a suitable production organism not specified in the patent (Elfenbein and Kolbeck, 2018). Lastly the possibility of conditioned media, where one cell line is grown in the media to secrete growth factors, to then be used for growth of another cell line (Elfenbein and Kolbeck, 2018). Wildtype also touched upon the expansion to bioreactors and how they have been able to produce 2 pounds (~0.9 kg) of cell biomass per week in five, 20L rocker bioreactors (Elfenbein and Kolbeck, 2018).

Wildtype also proposes different recombinant strategies. They present multiple strategies using genetic constructs that can induce differentiation. The first strategy (see

Figure 11) is a system of 2 constructs: One that contain one or more pluripotency genes (e.g. Oct4, Sox2, Klf4, 1- Myc), flanked by *LoxP* to maintain cells in an undifferentiated state, which can be excised in the presence of Cre recombinase; and a second that contain the tetracycline response element (*TRE*) one or more genes for differentiation and a Cre recombinase gene (Elfenbein and Kolbeck, 2018). The *TRE* element makes it possible to induce transcription of both; the differentiation genes and the Cre recombinase gene, by the addition of doxycycline or tetracycline. In this way working as a tetracycline-on system (Addgene, 2022). When Cre recombinase is expressed, the pluripotency and proliferation genes flanked by *LoxP* will be excised according to the principle of a deletion event according to the Cre-*Lox* system (see Figure 12).

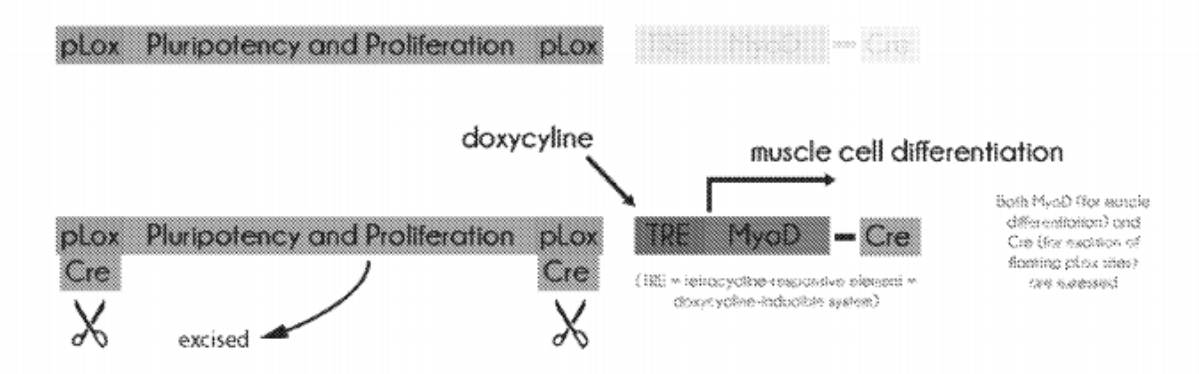

Figure 11 (Elfenbein and Kolbeck 2018). Visualization of Wildtype strategy, to switch from initial pluripotent and proliferate cell line to differentiation into myogenesis by the induced expression of MyoD and Cre. The expression of Cre excises the pluripotency and proliferation genes according to deletion by the Cre-Lox system. Induction is done by the addition of doxycycline through a tetracycline-on system.

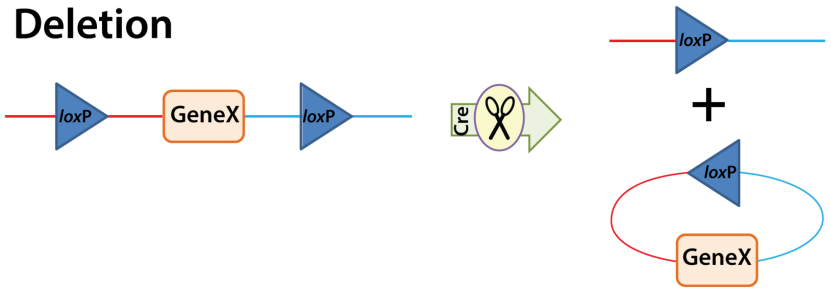

Figure 12 (Juchheim, 2015). Deletion event by the presence of Cre recombinase and 2 identical Lox regions (in this case LoxP) having the same direction.

How they introduce the reverse tetracycline-controlled transactivator (rtTA), a transcription factor for the tetracycline-on system to work, is unclear. It is probably necessary to express rtTA recombinantly in the cell as well, but this part of their strategy is not specified. The strategy illustrated in Figure 11 uses *MyoD* as an example for muscle differentiation, but the expression could also be different combinations of *MyoD, Myf5, MRF4* and *MyoG* to induce myogenesis (Elfenbein and Kolbeck, 2018). For their induction of adipogenesis *LPL, LEP, AGPAT2*, *ADIPOQ, PLIN1*, *GLUT4* and *FABP4* are expressed in different combinations (Elfenbein and Kolbeck, 2018). In this way they can maintain pluripotency and proliferation in an independent manner, until they decide to induce myogenic or adipogenic differentiation by addition of doxycycline. Simultaneously, the expression of the Cre recombinase, then excises the genes related to pluripotency and proliferation.

Another one of their strategies also utilize the Cre-*Lox* system, where a gene of interest, together with the Cre recombinase gene with an upstream *TRE*, are flanked by *LoxP*. This system allows for the initial expression of a desired gene until the addition of doxycycline induces the transcription of the Cre recombinase gene followed by excision of the entire flanked region, resulting in termination of both the expression of the genes of interest and the Cre recombinase gene.

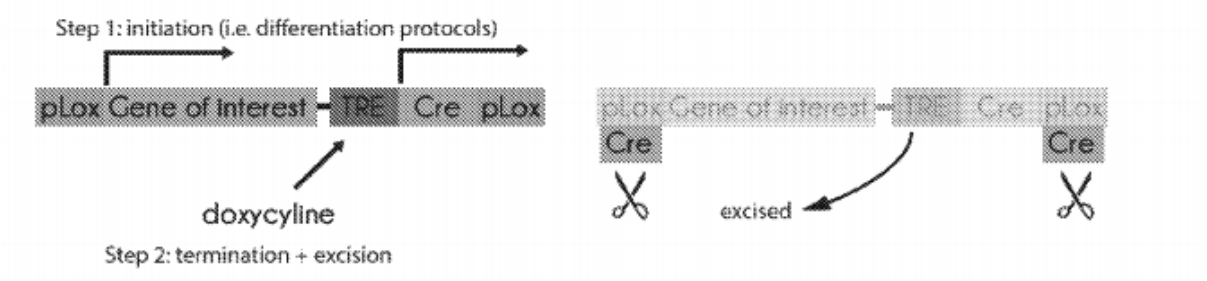

Figure 13 (Elfenbein and Kolbeck 2018). Visualization of Wildtype strategy, to transdifferentiate a cell line, then subsequently excise recombinant gene. The induced expression of Cre excises the recombinant gene. Induction is done by the addition of doxycycline through a tetracycline-on system.

They state in their patent that this will be a footprint-free excision, but this does not align with the actual mechanism of the Cre-LoxP system as one *LoxP* region will remain after the excision by Cre recombinase, see figure 12, depicting the Cre-*Lox* system for deletion. They suggest this system for the transdifferentiation approach, in this way only needing to activate differentiation genes into the desired phenotype.

Wildtype also describes how Cre recombinase could potentially be stored as reservoirs at the cell surface (see figure 14), but they do not specify what they are bound to or what is needed to induce their release. What they gain from this compared to induction of Cre by their other strategies is not made clear.

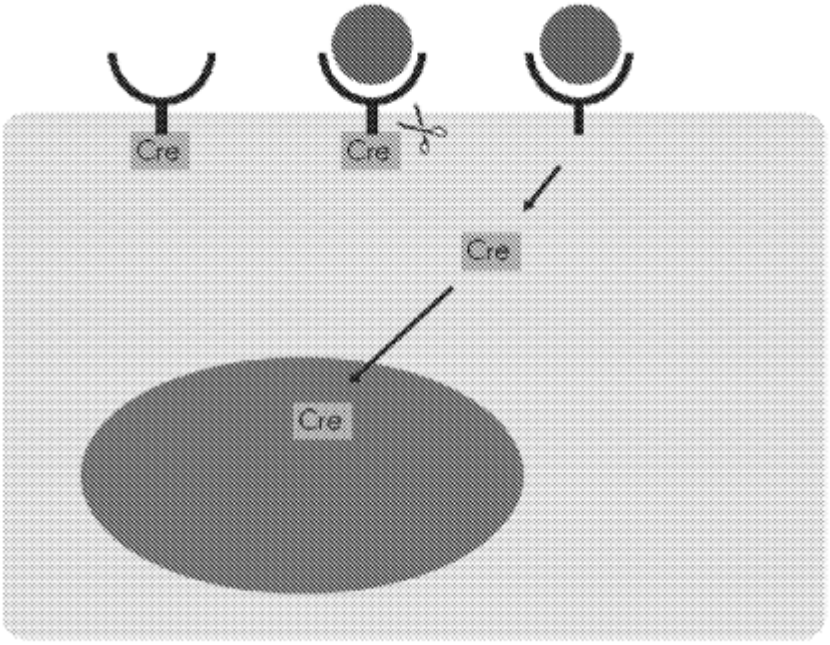

Figure 14 (Elfenbein and Kolbeck 2018). Wild type strategy to store Cre at the cell surface of cells.

Wildtype also suggests other genetic strategies, where a genetic switch is used to switch for proliferation to differentiation. These strategies utilize the Cre-*Lox* system where 2 different *Lox* pairs (*LoxP* and *Lox5171*) are inserted in such a way that the Cre recombinase can perform 2 subsequent steps: 1. an inversion (reversible) and 2. a deletion (irreversible), details are shown in Figure 15.

One detail that Wildtype has overseen is that the deletion will not remove both *Lox* of each pair, as shown previously in Figure 12 one *Lox* from each pair will remain after a deletion event. The remaining *Lox,* will not influence the functionality of this strategy, as the remaining *LoxP* and *Lox5171* are different from each other, the Cre recombinase will not be able to perform a deletion event with them. The pair would need to be identical, for this to be possible.

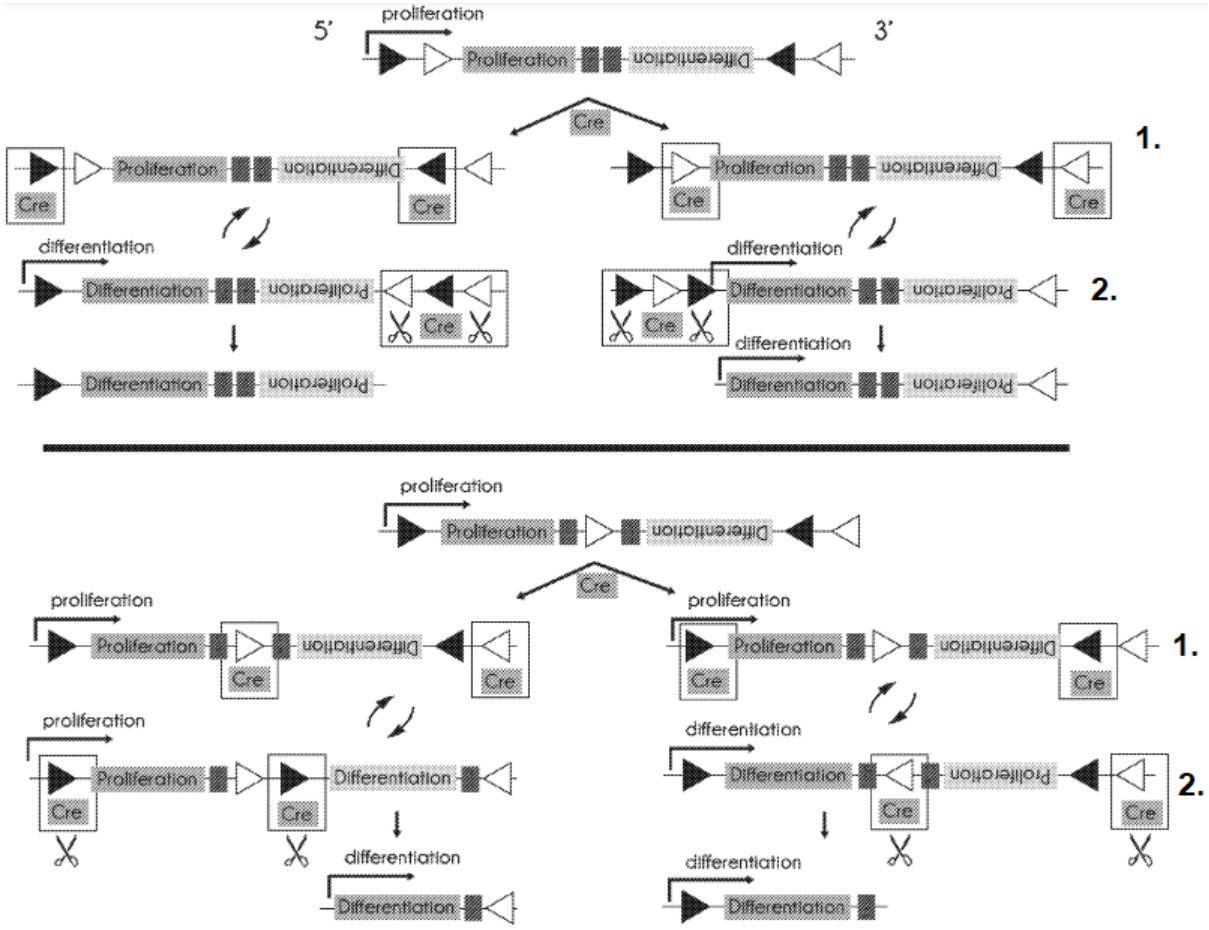

Figure 15 (Elfenbein and Kolbeck 2018). Shows two strategies utilizing the Cre-LoxP system for switching from proliferation to differentiation by 1. inversion and 2. deletion. Black triangles are LoxP and white triangles are Lox5171. Lox pairs pointing towards each other leads to inversion. Lox pairs that are aligned leads to deletion.

Further perspectives

There are challenges in regards to consumer acceptance when it comes to cultivated meat, even more so if the product is GMO. whether plant based products wil be preferred, despite nutritional values differing from the typical animal derived products could be a challenge to overcome.

Hybrid products might be a way to ease consumers into accepting cultivated products, but could also be perceived as less appealing by consumers. As cultivated products are more expensive than plant based products, it might only be available for the more wealthy populations. But if this could be made into an advantage where cultivated meat is a luxury product available in gourmet restaurants. Combined with an approach of scaling out, this might be a way to get cultured meat to the consumers much faster. This

approach would be suitable for the requirement of eg. restaurants, rather than big scale slaughter houses (The Good Food Institute, 2022a).

From a sustainable point of view, a future where conventional farming of animals is excluded would contribute significantly to the environmental impacts of humans on earth. Political intervention to make industrialized meat production illegal or at least heavily taxed due to its footprint, the ethical aspects of animal exploitation, its role in spread of disease and development of antibiotic resistant strains, would create possibilities for cultivated meat to enter the market.

Conclusion

Throughout this report, the general workflow for cultivated meat has been described. Challenges are still seen in this novel technology including development of cell lines suitable for production. Cell lines both need to be able to initially proliferate and maintain pluripotency followed by subsequent differentiation into desired ASC types. Media formulations for cultivated meat have been expensive due to especially the cost of necessary GFs. Substitution of FBS is essential if the argument for producing cultivated meat is to avoid animal production as a whole. More research is needed within the use and potentials of cheaper FBS substitutes, such as mushroom extracts. For bioreactor design, the main challenges are the need for vascularization, reduction of shear stress damage and design of reactors suitable for more textures products. When it comes to the textured products, scaffolding materials should be considered in regards to their ability to support attachment of cells and whether these materials have to be dissolved or be part of the final product. Wildtype is an example of a company that has taken some of these considerations into account, and actively are seeking solutions to many of these challenges. They have managed to establish various cell lines, established genetic strategies, with a minimal recombinant footprint. Furthermore have they been able to produce specific muscle fibers (fast- and slow twitch).

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