LAB-BASED MEAT PRODUCTION: SCIENCE FICTION OR REALITY?

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INTRODUCTION - WHY CULTURED BEEF?

Producing meat through cell culture, currently known as cultured –or clean- meat is being developed as an alternative to livestock produced meat. Starting with the authoritative report by the FAO in 2006, it is increasingly clear that livestock meat production, beef in particular, is unsustainable (FAO, 2006). The 2011 update of that report shows that it is physically impossible to match the 70% rise in demand for meat by 2050 given the constraints of available resources (FAO, 2011). Accepting these constraints and production limits while demand for meat rises, will make meat an increasingly scarce and expensive commodity. Most likely, meat producers will try to serve the demand by increasing production, leading to higher volumes of feed production. Since in factory farming, feed consists mainly of corn and soy, increasing meat production will lead to reduced availability of these staple foods for direct human consumption and thus pose a threat to global food security.

In addition to the threat to food security, it is estimated that between 15 and 20% of all greenhouse gas emission is attributable to livestock farming (FAO, 2006). The globally accepted urgent need to curb climate change further challenges livestock meat. It is also noted that the growing global appetite for meat drives deforestation. Although these environmental effects depend on regional conditions for keeping livestock and on the intensity of livestock farming, their global significance is scientifically accepted.

The third reason for looking at meat alternatives is the growing care of consumers for animal welfare (Dawkins, 2006; De Backer and Hudders, 2015). This is exemplified by the recent ban on caged eggs in the EU following a massive change in consumer behavior favoring free range eggs, despite their marginally higher price. Most, if not all, of the animal welfare issues in the bio-industry are related to the high intensity of farming and the need for cost-effective production. Thus, meat alternatives from partially animal-free production will lead to improved animal welfare, simply by reducing the number of animals in the bioindustry.

While culturing meat might theoretically be a solution for all of these challenges, the technology is still in its infancy and requires appreciable research effort and investment to become a reality. Originally derived from medical technology and still exercised mainly by biomedical investigators, for the technology to be fully developed and integrated, multidisciplinary networks, including cell biologists, biochemists, bioprocess engineers, animal scientists, meat scientists and social scientists, are required.

TECHNOLOGY

The technology to culture meat is derived from medical tissue engineering and is based on large scale cell culture of satellite cells that can subsequently be differentiated into skeletal muscle (Seale et al., 2000) and fat tissue (fig 1).

The process starts with harvesting satellite cells from a small piece of muscle obtained by a transcutaneous needle biopsy of a cow (fig 1). The stem cells are retrieved by mechanical and enzymatic disruption of the muscle fibers, allowing satellite cells to grow out. This routinely results in a more than 95% purity of satellite cells. The satellite cells start to divide and produce myoblasts. Further multiplication of these myoblasts can be stimulated by standard cell culture techniques. This replication stage is the most resource intense stage, where the cells need to be fed a nutrient-rich fluid a.k.a. “medium”. Medium contains all necessary nutrients for cells to grow. It typically is supplemented with a 10-20% serum, derived from calf blood. Successful efforts have been made to replace the serum by serum-free or chemically defined medium. Myoblasts have sufficient but limited replicative capacity with a maximum of 45 doublings being reported in human cells (Hughes et al., 2015), although this can probably be extended (Magalhaes, 2014). Without extension, this would theoretically allow the production of several hundred kg of meat from one biopsy; more, when the number of doublings is increased. Myoblasts are, like most mammalian cells, “anchor dependent” meaning that they grow when attached to a surface such as bottom of a culture flask or Petri dish.

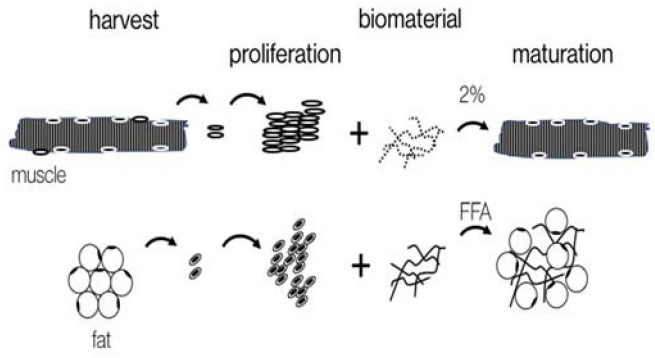

Figure 1. Schematic representation of meat culturing process. It starts with harvesting the satellite cells from muscle and the adipose tissue derived stem cells from fat. The proliferation results in the multiplication factor. Combined with a suitable biomaterial, the tissue matures in either a full-fledged muscle fiber with 2% serum or fat tissue under the influence of free fatty acids (FFA).

Once enough myoblasts are obtained (trillions), the cells are separated in batches of 1.5 million cells that are packed together in a temporary supporting gel, usually a bio-based material such as bovine collagen or fibrin. Reduction of serum in the medium will trigger the myoblasts to merge and form myotubes. By interaction with the gel, the

myotubes start to align and compact to form a tissue in a process that is commonly referred to as (collagen) gel contraction. Currently of animal origin, the gel is gradually replaced by alginate that is functionalized by small peptides such as RGD. Slowly, the myotubes start to contract and if the tissue is constructed in a way that anchors the ends of the myotubes, they develop tension. The tension is the biggest trigger for protein synthesis (Vandenburgh et al., 1999). The easiest scalable format to anchor the muscle fibers is by letting them grow in a ring around a central column of elastic material, thus "self-anchoring" the muscle fiber. Full maturation of the muscle fiber takes about 3 weeks, after which they are ready to be harvested. When ready, the muscle fibers are 2-3 cm long but less than 1 mm in diameter. Ten thousand of these fibers make up a hamburger patty of approximately 100 grams.

Meat also has fat tissue and fibrous tissue, which needs to be created. Fibrous tissue may not be important for a minced meat product, but will be important to recreate full thickness pieces of meat. Fat however is important for taste and texture and arguably, nutritional value. Fat tissue can be generated from a variety of stem cells, including adipose tissue derived stem cells (ADSCs). Alternatively, and more practical, the satellite cell can be used as the source for adipogenesis (Teboul et al., 1995; Asakura et al., 2001). The differentiation of satellite cells into fat cells requires stimulation transcription factor PPARγ. Naturally occurring fatty acids (FFA) are PPARγ and can be used to differentiate stem cells into fat cells. A large number of FFAs were tested and especially pristanic and phytanic acid are in our hands very effective in stimulating adipogenesis. Like muscle cells, fat cells require a temporary support gel to create a tissue, but they do not align and they do not interact appreciably, suggesting that the composition of the gel is less critical than for myoblasts.

Minced meat is currently the focus of research and development, simply because existing technology suffices to create such a product. To reform livestock meat production into a sustainable and environmentally and animal friendly manner it is essential that other types of meat are replaced as well. Creating full-thickness cuts of meat requires a more complex tissue engineering approach. Most importantly, a high-density channel system needs to be incorporated that allows perfusion of medium to every corner of the tissue, so that oxygen and nutrient delivery as well as waste removal are assured. Second, a more complex and larger 3D structure needs to be created with morphological, biochemical and mechanical features tailored for the specific cuts, but also to the needs of the cells. This can be done through 3D printing or any other type of 3D free form fabrication. Third, myoblasts, fat cells and fibroblasts (creating fibrous tissue) need to be co-cultured and differentiated along their respective lineages. These challenges are shared with tissue engineering for medical purposes. Therefore, scientific advances from both areas will lead to an eventual successful strategy, but it will take more time and effort to realize than the minced meat product (Post, 2014).

OPTIMIZING THE PRODUCT

Culturing meat aims to create the same tissue that consumers appreciate as meat. This is specified not only in its eventual sensory qualities but also in nutrition and health characteristics. Cell and tissue culture will lead to a similar product but with different feed and external mechanical and dynamic environment, it is not immediately obvious that the tissue will the same. Fortunately, all these conditions are controlled and can therefore be optimized. For a minced meat product, the two components that need to be optimized are the protein and fat composition.

Protein composition

Muscle tissue is protein-rich. Quantitative analysis of the exact protein composition of muscle turns out to be quite challenging, because it contains up to 6500 proteins spanning several orders of magnitude in expression levels (Ohlendieck, 2011). A large fraction of the weight, contain membrane bound and not soluble proteins, further complicating the extraction of proteins prior to analysis by for instance mass spectrometry. Not only is the range of expression wide, the distribution of protein sizes is also extremely spread out, with muscle tissue containing some of the largest proteins such as titin (12,00 Kd) and nebulin (600-800 kD). The most abundant proteins are myosin, actin and titin, together making up 75% of the cytoskeletal proteins of the muscle cell (Robson, 1995), which by themselves constitute between 40 and 60% of the total amount of protein (Murgia et al., 2015). Whether it is 40 or 60% depends on the type of muscle fiber, i.e. type 1, 2A, 2X or 2B. Cytoskeletal proteins in muscle cells are highly organized in contractile sarcomeres giving the cells their distinguishing cross striation pattern on light and electron microscopy. Highly aligned and tightly co-expressed myosin and actin molecules likely contribute to the texture of meat. Nutritional value, taste and mouthfeel are co-determined by the amount and composition of protein in the muscle cells, so a thorough understanding of the muscle proteome is essential for the development of a product that is aimed to substitute meat. Likely, the most abundant proteins contribute more to taste and texture of meat than the scarcer proteins, although it is possible that some very distinct proteins have specific components contributing disproportionally to taste and appearance. One such class of components is the group of heme containing proteins, with hemoglobin and myoglobin as their prototypic exponents. Hemoglobin and myoglobin are partly responsible for the red color of beef and also for its browning upon oxidation (Pearson and Dutson, 1994; Pearson and Gillett, 2012). Hemoglobin and particularly myoglobin has been associated with a serum-like taste and metallic mouthfeel of beef, so their presence may be important for sensory quality (Miller, 2012).

Although no detailed proteomic analysis has been performed on cultured beef yet, muscle fibers from cultured meat had the typical cross striation indicating sarcomere development (Boonen et al., 2009; Boonen et al., 2011). The muscle fibers also show spontaneous contraction, which is enhanced upon electrical stimulation (Langelaan et al., 2010). The overall protein content is 20% like native muscle fibers (unpublished). The very limited sensory experience of tasters at a pubic launch of a hamburger of cultured beef in 2013 in London, ascribed a meat taste to the product, with no off flavors, further

suggesting that protein composition is sufficiently replicated. The tasters also described the structure being like ground meat. The muscle fibers however were still yellowish in color because of low myoglobin expression and complete lack of blood and therefore hemoglobin, in the production process. By reducing the oxygen concentration during cell culture however, the myoblasts start to express myoglobin to 5-fold higher levels, in accordance with observations in many other muscle cells of vertebrates (Kanatous and Mammen, 2010; Helbo et al., 2013).

Fat composition

Fat tissue can be cultured from satellite cells (Lepper and Fan, 2010) by stimulating them with FFA and thereby activating PPAR-γ. Making tissue of these fat cells is both mandatory and less challenging than making muscle fibers. It is mandatory as mature fat cells are difficult to maintain in an adherent state when cultured under a fluid layer: they surface due to their lower specific gravity than the watery medium. In a matrix of biomaterial, the cells stay nicely bound and form fat tissue. Unlike the formation of muscle fibers, where the interaction between differentiating myocytes with their matrix is crucial to form the structure, this interaction does not seem to be necessary to produce proper fat tissue, although the level and speed of adipogenesis depends on the matrix characteristics. The fatty acid composition of the cultured fat tissue has not been analyzed yet. For hamburgers, the separate culture of muscle tissue and fat tissue, that is later combined when patties are made, it is very easy to precisely titrate the amount of fat that will be present. Optimizing the production of fat tissue for a hamburger application is still in early development and will require additional work.

Whole cuts of meat

Processed meat is a sizable part of the entire meat market, but to achieve the goal or producing meat without threatening food security and with minimal environmental burden, it is necessary to create whole cuts of meat, such as a filet mignon or a ribeye steak. The challenges of engineering large tissues are shared with medical tissue engineering and are threefold:

1. Creating a large 3D structure of biomaterials, also known as freeform fabrication.
2. Co-culturing various cells with different culture condition requirements.
3. Formation of a channel and perfusion system for mass transport of oxygen and nutrients and waste removal.

In fact, many technologies for fabrication of 3D structures have already been developed from a variety of human cells. These include but are not limited to 3D printing, lithography, casting and moulding (Houben et al., 2016). Many biocompatible and usually biodegradable materials that are usually synthetic polymers have been investigated and developed for these functions. For food applications, the focus should be on bio-based materials such as alginates, gelatins, cellulose and chitins. In addition to the classic tissue engineering requirements for biomaterials such as mechanical properties, timely degradation and interactivity with cells, the materials or their degradation need to be safe for consumption and produce no off-taste. The freeform fabrication method of choice

needs to be scalable, extremely cheap (compared to medical applications), resource-efficient and sustainable, i.e. seemingly unlimited supply. The condition of unlimited supply can be achieved by using abundant natural materials or by recyclable materials.

Co-culturing and co-differentiating cells of different origin with distinct differentiation protocols, such as muscle cells, fibrocytes (forming fibrous tissue) and fat cells, can be challenging. Our lab has experience with co-culture of fibrocytes and endothelial cells (blood vessels) (Pullens et al., 2009) and several co-culture protocols that include muscle cells have been developed elsewhere (Lesman et al., 2014; Cerino et al., 2016). Obvious co-cultures such as adipocytes and muscle cells have led to novel insights into the reciprocity of these cells, perhaps even leading to new ways to tenderize cultured meat (Choi and Myung, 2014). Thus, this research has challenges as well as opportunities that stretch beyond the cultured meat application

One of the goals in medical tissue engineering of large structures is to create a blood vessels system (Post et al., 2013) that not only facilitates oxygen and nutrient transport to and waste removal from the tissue during culture, but also serves to connect the tissue to the recipient blood supply upon implantation. For the development of whole cut cultured meat, the latter is obviously not required, so it may not be necessary to faithfully recreate a blood vessel system. A much simpler channel and perfusion system might suffice. Much will depend on whether the vascular cells that make up the blood vessels exert an influence on muscle other than just a transport conduit. It is presently unknown if blood vessels contribute to sensory qualities of meat, but it is possible that the vascular cells directly affect muscle cells in a positive or negative manner. We have observed minor effects of cultured endothelial cells on muscle differentiation, when co-cultured in direct contact with each other or with a distance between the cells requiring diffusible substances to drive the interaction (unpublished observations).

Together, the necessary developments to realize whole cut cultured meat are numerous but they will likely be successful and lead to an appropriate gamut of cultured meat products.

ROAD TO PRODUCT DEVELOPMENT

The development of cultured meat is no longer restricted to academic research. Currently, six companies are busy getting cultured pork, beef and chicken to the market (Brown, 2017). Market introduction will likely still take a couple of years, because the production process is entirely new and not regulated yet. For these products to enter the market, production needs to be scaled and cost-effective and the products must be regulated by governmental agencies.

Large scale cell culture methods have been developed for mammalian cells, but have not been applied yet to their full extent. As most mammalian cells including myoblasts, are anchor dependent, they need to grow on a surface. To allow cell growth in large fermenters that are already available for bacterial and yeast culture, myoblasts will be grown on microcarriers (Moritz et al., 2015). Many microcarriers are commercially available but only few have been tested with myoblasts. Issues such as seeding density,

efficiency of attachment of cells to the microcarriers, bead-to-bead transfer and the efficiency of cell harvest from the beads require extensive research and optimization (Rafiq et al., 2013; Merten, 2015). Not only scalability, but also efficiency and cost-effectiveness of cell culture critically depend on the success of this process as they are intimately related to the maximum achievable cell density. An ideal alternative to microcarrier based myoblast culture would be single cell suspension culture.

The use of fermenters for cell growth and the subsequent use of tissue engineering technology makes producing cultured meat in effect a biotechnology, with more examples in the pharmaceutical industry than in food technology. It is therefore likely that the early companies aiming to bring their products to the market will either retrieve know-how from or form alliances with biotech companies, rather than with traditional food manufacturers.

Safety of novel foods such as cultured meat is of utmost importance to build consumer trust. Governmental regulatory bodies judge if cultured meat is a novel food and therefore requires an assessment of its safety before consumers are exposed. The regulatory process will likely differ from country to country, and will therefore be a factor in determining where market introduction will start. As the final composition of cultured meat is very similar to livestock meat, it is expected that safety tests will be passed.

CONSUMER ACCEPTANCE AND SOCIETAL CHALLENGES

Cultured meat would need to be widely accepted by consumers in order to be an effective alternative to livestock meat. Surprisingly, a relatively large number of studies has already been focusing on consumer acceptance of cultured –or clean- meat. Different methodologies were used, ranging from focus groups to web-based or cohort-selective surveys. Cohorts from many difference countries and continents were surveyed, with somewhat similar outcomes. A sizable minority, in some cases even a majority, of participants expressed willingness to try and/or by cultured meat when it becomes available. The latest study performed at Maastricht University tested 200 subjects from a cross section of the local population for consumer acceptance, but in the setting of a tasting experience. All 200 subjects sampled a piece of meat that was described as being cultured. There is still notable reservations around cultured or clean meat, but it seems that the obstacles are surmountable given time and thoughtful communication.

SUMMARY

Culturing meat as an alternative to livestock meat is an exciting technology that could be a solution to the food security and environmental issues associated with the increasing appetite for meat of the global population. The exact protein composition of cultured meat is still unknown although from morphologic observations it can be inferred that the bulk of cytoskeletal proteins will be in the same range as livestock meat. Complementing the cultured meat tissue with cultured fat is in development but still at an early stage. Culturing meat is a challenging technology with still some hurdles ahead before it can be introduced in the market in an appreciable quantity and at a reasonable

price. It is likely that consumers can be enthused for this technology on the basis of its perceived benefits.

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