Synthetic imitation of food: What are we talking about?

Here, I think we need to set the context of what are we talking about, in particular the global research for alternative proteins via various paths, either vegetable alternatives which mimic the meat or milk products or lab grown alternatives so that we are clear, in the context of the vegan/vegetarian market segment.

The market for alternatives to traditional animal food products protein has been significantly growing on the global scale in the last decade, and market projections (Fortune Business insights) expect its turnover to reach around 12.3 billion euros by 2029, from the current 11.1-billion-euro market revenues (Pitchbook).

This market can be divided into two main streams of products: **plant-based alternatives**, and **lab-grown alternatives**. The former consists of ultra-processed vegetable-based patties whose intent is to resemble (in the color, form, taste, texture, smell, juiciness, the effect of cooking, etc.) animal-sourced products (mainly dairy or meat); the latter being animal cells collected by a living animal and artificially-induced to reproduce (replicate) in a bioreactor. The result of lab-grown imitation of meat is a multitude of identical muscle cells that need to be transformed into patties or nuggets to be consumed, given that current technology does not allow for the artificial creation of entire meat cuts.

Vegetarians, vegans, and flexitarians are the main market segment that plant-based proteins products aim to target, while meat-eaters with "health and environmental concerns" who do not want to give up meat in their diets is the group targeted by animal proteins imitated in a lab products, together with a subgroup of vegans who avoids meat for ethical reason.

Synthetic imitation of meat

'Synthetic imitation of meat', meat 'grown in a lab' meat, or, 'cellular agriculture', is the process of producing animal-based foods and other products directly from animal cells, in a laboratory. Through a sample of animal cells that are taken either from alive animals' muscles or embryos, and put in a nutrient-rich medium, the cellular tissue is grown under controlled conditions in bio-reactors to develop into muscle, fat or other tissues' cells to form animal cells conglomerates and other animal products (like leather, gelatin, collagen) (Warner, 2019).

The media where the cells are grown is synthesized from bovine fetal serum, even if currently many companies are trying to develop animal-free options serum due to costs and ethical reasons.

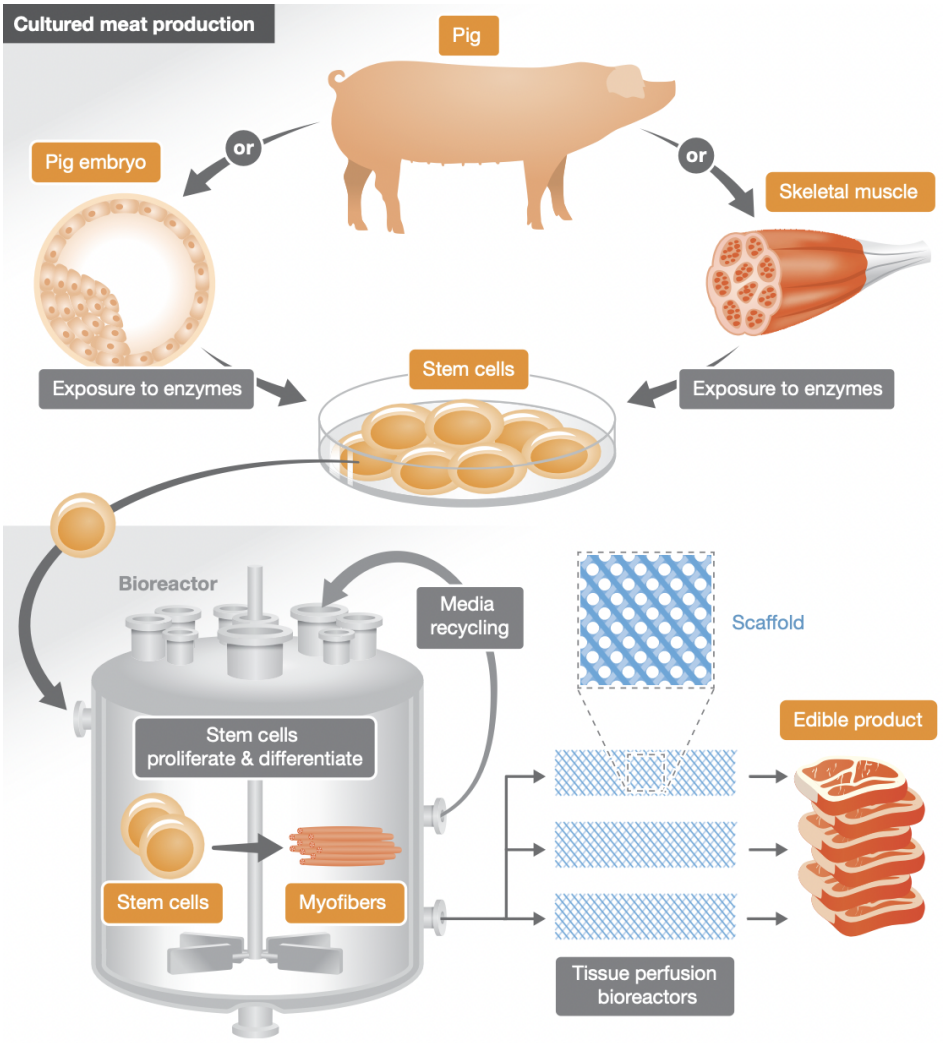

Figure 1: Production of synthetic imitation of meat. Source: Science & Society

Warner (2019) goes into detail in the production steps, describing the biology of the process.

First of all, the **source of the cell sample** has to be decided: either stem cells coming from an embryo, or from a live animal's muscle. A *stem cell* has the unique ability to develop into many different types of body cells, and all cell stems can self-renew (make copies of themselves through division) and differentiate (develop into more specialized cells). These stem cells are called 'pluripotent'. As an organism ages, the regenerative potential of the cells rapidly decreases, and stem cells found in more aged individuals can only replicate the tissue from which they have been removed. This kind of stem cell is called 'unipotent'. Thus, embryos contain stem cells that have the characteristics to replicate an infinite number of times -under controlled conditions- and grow into different body cell types (pluripotent). Stem cells that are retrieved by an autopsy of an *adult subject's tissue* (called adult stem cells) have limited replicating potential (50 to 60 times) and can only replicate the tissue from which they have been exported (unipotent). A third category of cells sample, Warner recalls, is called '*induced pluripotent stem cells*', which are artificially engineered in vitro to replicate embryonic stem cells, meaning that the adult unipotent stem cell has been artificially forced to become pluripotent. Finally, a sample can be obtained from muscle tissue derived from a living subject to derive '*myosatelite stem cells'*, which can only differentiate into muscle cells

and replicate for a maximum of 20 times. For this reason, stem embryo cells are normally preferred for cell-based meat production (Warner 2019).

The **growth medium** is the nutrient-rich serum that replicates the biochemical conditions of the environment where the cells would normally grow inside the animal's body. It consists of water, oxygen, carbohydrates, lipids, amino acids, vitamins, minerals and other biochemicals. Proper myogenic regulatory factors and different hormones (insulin, thyroid hormone, growth hormone) have to be added at different stages of growth (Warner 2019, Datar and Betti, 2009). Obviously, the growth serum has to be adapted according to the kind of animal cells that are grown (i.e., bovine cells need a different recipe to growth compared to poultry cells). Moreover, the formulation of the serum might be required to be changed over the course of the culturing process (Datar and Betti, 2009). Due to its effectiveness, the ideal growth medium for cell proliferation and growth is bovine fetal calf serum.

The next step in synthetic imitation of meat production is **scaffolding**, the process through which the starting sample of cells is put in a bioreactor with the growth serum and starts the actual growth. In this phase, cells proliferate, differentiate, mature and, eventually, fully function as skeletal muscle cells. In order to do that, stem cells need to be attached to a biological scaffold that will serve as a base on which they will grow. This might be made of collagen. In this phase, it is necessary to biomechanically stimulate the satellite cells so to allow them to transform into muscle precursors cells (called myoblasts); further, an electrical stimulus will also be needed to create neuronal activity required for the development of mature muscle cells.

Lastly, the '**harvest'** of the muscle cells and the **manufacture** of the final product conclude the production process. Current offers can only be used for processed meat products (such as hamburgers, or nuggets) since the cultured copycat product does not have the same structure as real skeletal muscle tissue.

It is worth reporting that "the generation of a whole muscle, complete with blood supply, connective tissue cells, muscle cell and associated structure, has neither been achieved in human biology and medicine nor in cellular meat production. […] The cell-based production of a whole steak is technologically far more complex and requires technological break-throughs in providing a vascular supply through a thick layer of muscle tissue as well as determining the right growth media for co-culture of muscle, fat, connective tissue and endothelial cells" (Warner, 2019). Additionally, Warner (2019) points out that the flavor of natural meat flash is derived from more than 750 components. Its texture, mouthfeel, and tenderness derive from several specific biochemical conditions (happening in the *post-mortem* stage) that, eventually, make meat what it is, questioning if the synthetic copycat will ever manage to replicate the complexity of the natural model: "hence the manufacture of a product from purely muscle cells, such as cell-based meat, will have a different texture and mouthfeel to a product made from muscle derived from the post-mortem carcass of a living animal". In fact, "muscles are transformed to meat through a complex biochemical process" that happens in muscles after death, determining taste and texture of meat as well (Fraeye et al., 2020).

Regardless of the millions of financial resources raised in funding, the industry reports some bottlenecks in its development to scale up, notably due to the high production, and research and development costs. In fact:

- **Cell-line development** -the funding block of the lab-grown business, being the actual genetic sequence from which cell replication starts from- are time-intensive activities, knowledge-specific, and many are protected by intellectual property rights making it difficult to access.
- **Growth media** represents the most expensive asset in the production line, specifically due to the use of the component of Fetal Bovine Serum -that is sourced from bovine embryos and to which, for now, no actual replacement is available- which reaches up to more than €2 000 per liter, representing, on average, 95% of the production costs.
- **Scaffolding** requires large surfaces of space to perform at its best, allowing cells to access nutrients. Actual scaffolding tools limit the potential to scale for the industry.
- **Bioprocess** is currently done in small batches and it is highly demanding on energy, posing a limit to scaling up the processes in terms of energy costs and efficiency in production.

Additional challenges that the lab-grown imitation of meat industry faces concern the products itself, in the sense that current technology allows growing cells one at a time and constantly requires access to nutrients and oxygen, which is why it is grown in strips, limiting a lot of their after-production use. Due to these barriers, muscle and fat that can naturally be found in a steak have to be grown separately and prevent the creation of meat cuts rather than ground beef that can be used for patties. 3D printing is an explored solution that could be used to fix this issue.

Precision fermentation

Precision fermentation is a parallel industry that is growing in the alt-meat industry as a technique to replicate milk and dairy products in labs as well. The process of precision fermentation involves some organisms as a 'base' such as bacteria, yeast, or algae (microflora) that interact within a nutrient-rich medium serum to create an eatable outcome such as milk proteins, animal and vegetal fats, collagen, honey, egg whites, enzymes, flavoring agents, vitamins, and natural pigments. In order to do so, the living material base is often composed of genetically engineered microorganisms that have been inserted into the whey protein or casein DNA sequence and cultivated in fermentation tanks.

The organisms (the microflora, i.e., yeast, bacteria or algae) are programmed through genetic modification so to allow them to excrete a particular sellable material, usually edible fats or proteins that are biologically similar to animal products that can be further processed to create a similar-animal-looking product. In particular, the **precision fermentation industry uses the DNA sequence of the whey gene protein (the protein that is found in milk) to insert it in the gene sequence of the microflora, using then Genetically Modified Organisms (GMOs) to produce whey**.

Once this process is done, the microflora that incorporated the gene encoding for whey protein is encouraged to grow and reproduce in a nutrient-rich serum. As a result, through fermentation, the flora converts sugar into whey. The protein is then separated from the

microflora, purified, and dried. The end product is a protein powder that can be used by food makers to replicate milk and dairy products.
Most of the start-ups in this industry use microflora from all the six biological kingdoms, targeted for the specific outcome (being fats, protein, carbohydrates, etc.).

Remarks:

Through these processes, the industry that imitates food in the lab currently offers several products that are proposed as replacement of animal-based traditional food both in the food industry, as well as in the fashion and beauty industries. In fact, besides meat and dairy, several other animal-based products are targeted by the synthetic replica industry (cell-cultured or fermentation). Amongst others: **seafood** (Schiok meats, Blue Nalu, Bluu Seafood, Fisheroo, Umami meats, Blue Fin Foods); **egg's white** (Onego); **foie gras** (Gourmey, Foieture); **oils** (Zerocare); **human breast milk** (Biomolq, Wilk, Me&Ma); **leather** (Bolt Threads, Vitro Labs inc, Modern Meadow); **gelatin** and **collagen** (Jellatech, Geltor); **chocolate** (California cultured), **coffee**[1] (Stem); **pet food** (bond tech food, Because Animals, Wild Earth, Pristine Pet foods), **caviar** (Caviar Biotec), **skin care ingredients** (IntergiCulture, Avant Meats), **fur** (Geneus Biotech).

Ketelings et al (2021) are not the only ones who argue that further research is needed in order to improve some aspects of this technology, especially on risk assessments, hazards & risks, and safety perception (Figure 2).

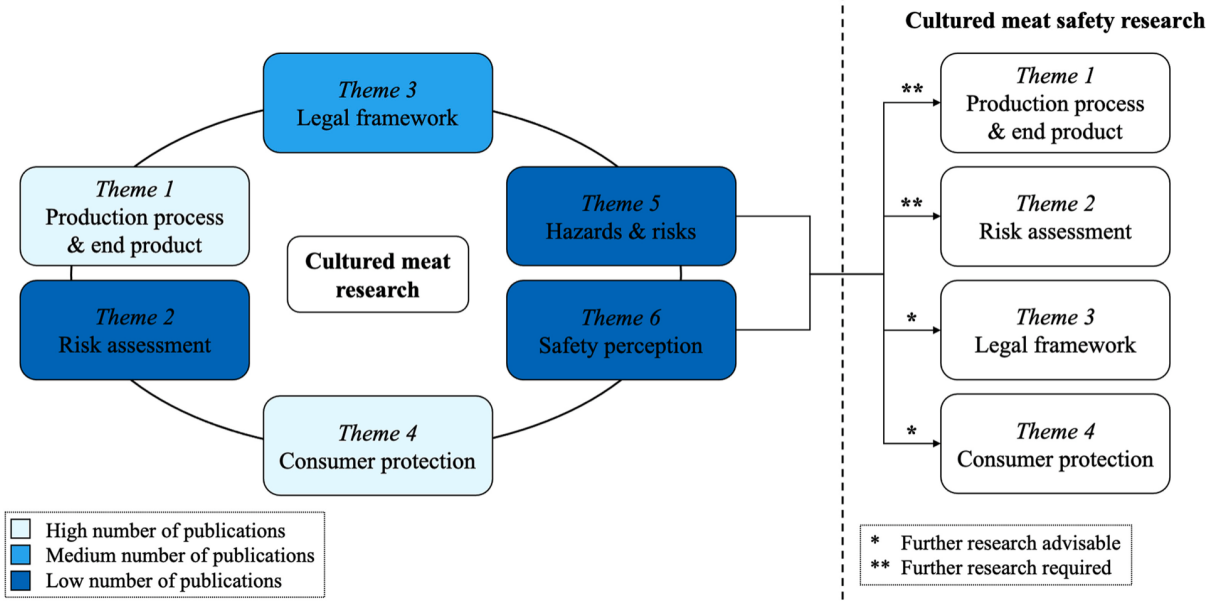

Figure 2: Scientific publications in the areas of synthetic imitation of animal protein production. Source: Ketelings et al. (2021)

[1] Both for coffee and chocolate, the technology used is the same that is use to grow cultivated meat: by taking a DNA sample of coffee/cocoa, the cells are then grown in bioreactors in a nutrient-rich media to deliver coffee/cocoa (in the form of powder) to be used by food manufacturers and processors as ingredients for the final product.

Sources:

- Datar, Betti, “Possibilities for an in vitro eat production system”. Innovative Food Science and emerging technologies, Vol 11, 13-22, 2010. https://files.givewell.org/files/labs/animal-product-replacements/Datar_and_Betti_2010.pdf

- Fortune Business Insight data, found in The Washington Post. Marina Lopes, ‘In Asia, meat substitutes have investors biting’. 2022.

- Fraeye, Kratka, Vandenburgh, Thorrez, “Sensorial and Nutritional aspects of cultured meat in comparison to traditional meat: much to be inferred”. Frontiers in nutrition, 2020. https://doi.org/10.3389/fnut.2020.00035

- Ketelings, Kremers, de Boer, “The barriers and drivers of a safe market introduction of cultured meat: A qualitative study”, Food Control, Volume 130, 2021, 108299, ISSN 0956-7135, https://doi.org/10.1016/j.foodcont.2021.108299.

- Pitchbook

- Warner, “Review: Analysis of the process and drivers for cellular meat production. *Animal, 13*(12), 3041-3058, 2019. doi:10.1017/S1751731119001897