The Science Behind Lab-Grown Meat

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Introduction

The idea of meat consumption without the need of animals has been around for a long time. Winston Churchill famously mentioned the concept in his 1932 compilation *Thoughts and Adventures* [1] and "carniculture" was mentioned in the old science fiction novel *Space Viking* [2]. More recently, scientists have realized that perhaps by utilizing traditional cell culture techniques, it would be possible to grow muscle cells (i.e. meat) *in vitro* for consumption. This realization was culminated in 2013 with the presentation and consumption of the world's first *in vitro* burger created by Mark Post and funded by Sergey Brin with a cool price tag of $330,000 (which was actually a bit mis-represented and included cost of setting up the lab) [3, 4]. The event was purposefully done to raise awareness for the strategy and has since spawned 4 [known] companies pursuing the idea – Dutch-based *Mosa Meats* (Mark Post's company), U.S.-based *Memphis Meats* [5], Israel-based *Supermeat* [6], and Japan-based *Shojinmeat* [7].

In this post, I will intentionally avoid going into significant detail concerning why *in vitro* meat can be beneficial and rather turn my focus on explaining the details of how it can be done, as well as dive into common questions and misconceptions surrounding the technology. I use *in vitro* ("in a dish") throughout, however other common references to this technology include "lab-grown meat", "clean meat", "cultured meat," or "cellular agriculture."

Why? Environmental and Ethical Considerations

Environmental:

The environmental impact on animal agriculture is tremendous and is a topic for its own discussion. In brief, animal agriculture uses an immense amount of the arable land on the planet, contributes to deforestation, water waste and contamination, antibiotic resistance, and greenhouse gas emissions [8]. Essentially, people who claim to be environmentalists but eat meat are hypocrites. It has been speculated that *in vitro* meat may result in up to 50% lower energy usage, 75-95% lower green house gas emissions, 99% lower land use, and 80-95% less water use depending on comparisons between products [9]. It is estimated that world population will tip 9 billion by 2050 with meat consumption increases estimated as high as 73% [10]. For these reasons, *in vitro* meat and other agricultural technologies offer the opportunity to severely mitigate the harmful effects of the current agricultural industry and should be welcomed alternatives to current practices.

Ethical:

In recent podcasts, both Sam Harris and Paul Bloom as well as Tim Ferriss and Ezra Klein come to the conclusion that factory farming is one of today's societal ills that will be looked upon in disgust by the humans of the future [11,12]. It's hard to imagine otherwise as it would be an extremely difficult claim to support saying that the animals commonly used in factory farming (chicken, cows, pigs, etc) are not sentient creatures with their own consciousness, emotions, and ability to feel pain. As such, the justification for the cruelty and suffering endured on their part for human consumption is limited given the amount of available alternatives in some of today's societies. At least in America, the realities of factory farming remain out of sight and out of mind as we are so far removed from the process when we pick up a chunk of meat wrapped in plastic at the grocery store. And the political will for this to remain can be seen through the passing of 'Ag-gag' laws that literally prosecute those who wish to reveal the realities of factory farming through images or videos [13]. Eating meat is not wrong but the mental gymnastics that most people perform to rationalize their everyday choices become less and less reasonable as new technologies and alternatives emerge.

How? The Process of Growing in vitro *Meat*

The Satellite Cell

In order to understand *in vitro* meat, one must have a quick primer on stem cell biology. A stem cell is a cell defined by its ability to self-renew or differentiate (change) into another cell type. There are many types of stem cells, defined by their potency or ability to differentiate into other cell types [14]. For instance, a totipotent stem cell is a stem cell that can differentiate into any cell type of the body, including cells of the trophoblast which make up the extra-embryonic tissues collectively known as the placenta. Totipotent stem cells only exist roughly up until the 8 or 16 cell stage of development. Embryonic stem cells and induced pluripotent stem cells (iPSCs) are cells that can differentiate into cells of all 3 germ layers (ectoderm, mesoderm, and endoderm) and are commonly used in academic research to study development and disease as well as in therapeutic regenerative medicine. Multipotent stem cells are progenitor cell types which can differentiate into a limited number of cell types, usually but not always within a single germ layer. Together, these cells are commonly referred to as "adult" stem cells and are the type most commonly used in current stem cell therapies. Much of the biology concerning *in vitro* meat will be derived from what we know based on development as well as iPSCs and adult stem cells.

The vast majority of specialized tissues (organs) in the human body contain reservoirs of adult stem cells or 'progenitors' which can become activated in response to cell death, injury, or other biological cues. We refer to these progenitor cells based on the tissue type which houses them – "neural progenitor cells" exist in the brain, for instance. In skeletal muscle, we refer to these cells as "myosatellite" or "satellite cells." Satellite cells sit along the skeletal muscle fibers [**Figure 1**] where they await activation in response to injury or stress. The ability of these satellite cells to self-renew and differentiate decreases with age, which in part may explain why people steadily lose muscle mass as they age, a condition known as sarcopenia.

While each cell in your body contains the same genetic starting material, specialized cell types are dictated by the expression of a particular set of genes. During development, the regulation of these genes changes rapidly in response to morphogen gradients and other biological signaling mechanisms as cells become specialized. In the context of skeletal muscle differentiation, the mesoderm arises from the primitive streak where it splits into the lateral plate, which forms heart muscle and parts of the circulatory system, and paraxial mesoderm, which forms skeletal muscle, smooth muscle, bone, and brown fat [**Figure 2**].

Thus, the specific cell types that precede the formation of a bonafide skeletal myotube can be marked by specific genes that are expressed in each cell type [**Figure 3**]. Based on our knowledge of development, we can then use these genetic markers in order to purify specific cell types of interest – such as the satellite cell.

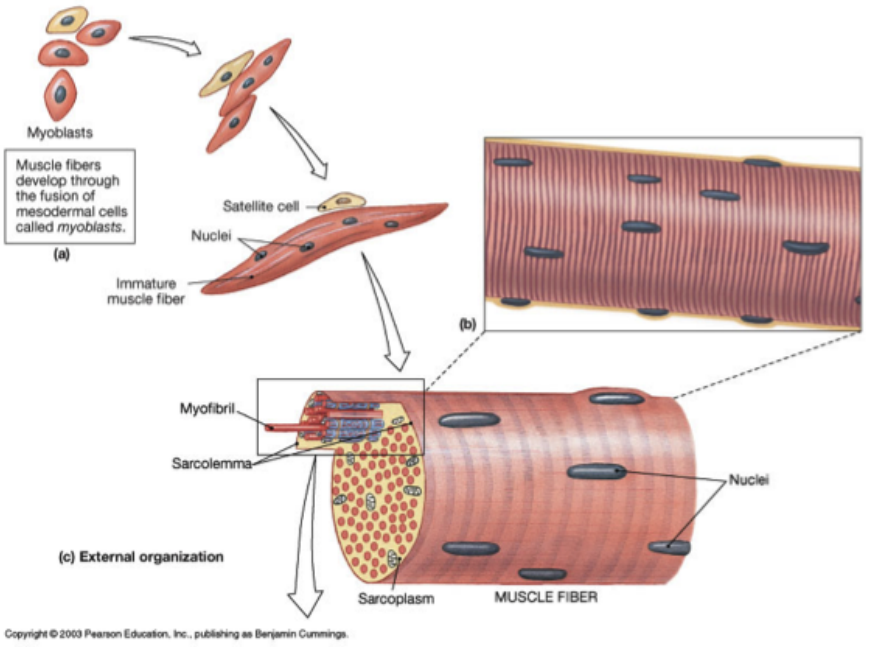


Figure 1: **Figure 1**: Schematic showing a satellite cell lying adjacent to an individual muscle cell which forms muscle fibers.a caption

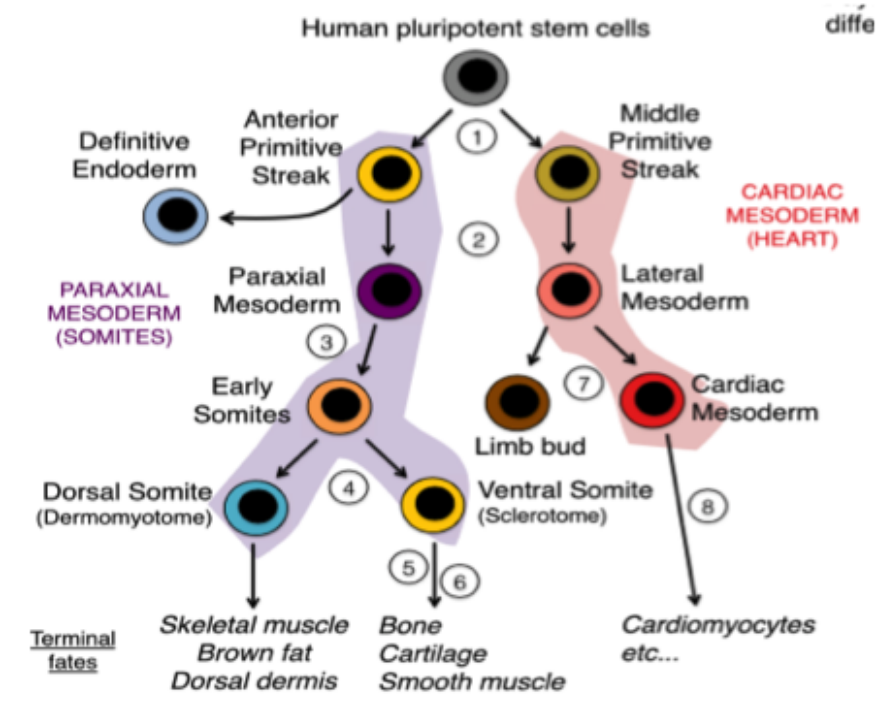


Figure 2: Figure 2: Stepwise development of mesodermal tissue and the adult cell types produced. From Loh et al, 2016 (http://dx.doi.org/10.1016/j.cell.2016.06.011).

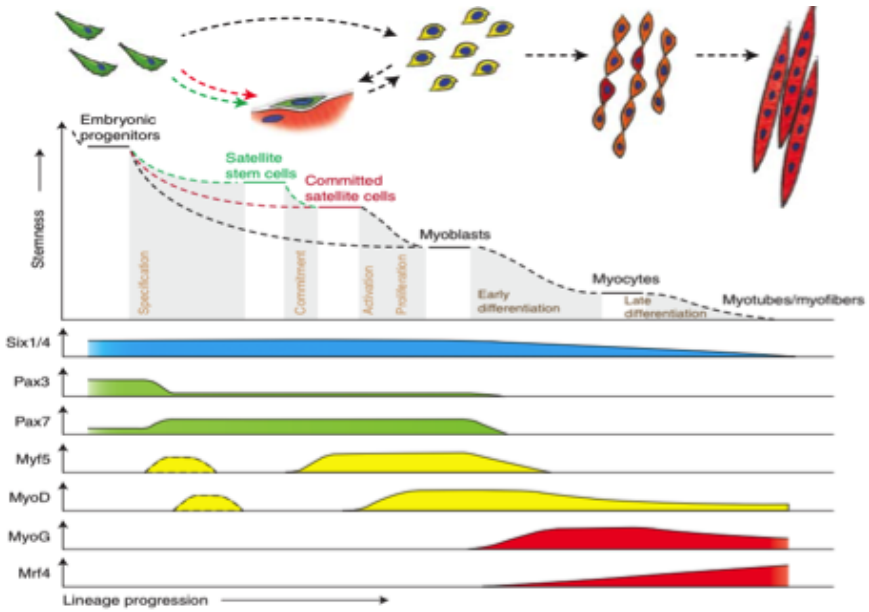


Figure 3: Figure 3: An overview of transcription factor changes that delineate skeletal muscle differentiation. From Bentzinger at al., 2012. (doi: 10.1101/cshperspect.a008342).

Obtaining Satellite Cells From Muscle

By taking a muscle biopsy roughly the size of an eraser head under local anesthesia, researchers can then isolate satellite cells following enzymatic digestion of the tissue to separate individual cells. Although I am unsure *exactly* how bovine satellite cells are isolated, it is likely through a fluorescent- or magnetic- activated cell sorting protocol (FACS or MACS). Essentially, these processes allow for the separation of cell types based [usually] on the proteins present on their surface or other features (size, shape, etc). In this case, both of these methods utilize antibody-based sorting via specific proteins expressed on the surface of the satellite cell, typically a population positive for proteins such as Integrin α7, CD34,CD56, CXCR4, Vcam1, CD82 and negative for Sca1, CD45, CD31, and CD11b [15, 16, 17, 18, 19]. Once sorted, the satellite cell population can be confirmed with immunocytochemical staining for markers such as PAX7 and expanded *in vitro*.

As for the animal, it can go on living its life as normal. In the future, it may therefore only be necessary to have a few animals that have been selected for their cells' characteristics to choose for biopsies. However, the possibility exists to eliminate the farming (in the classical sense, for food) altogether (discussed later).

Creating Muscle

As shown in Figure 3, activated satellite cells will turn into proliferative myoblasts through a gene regulation shift which involves down regulation of PAX7 and up-regulation of MYF5 and MYOD, both important transcription factors for muscle identity. During this transition, these transcription factors alter gene expression to transform the cell from a myoblast into a myocyte which will align and fuse with other neighboring myocytes to form multinucleated myotubes, which when performed in vitro is usually upon serum starvation (discussed later). The cells here can be identified based on staining of important molecular markers, where up-regulation of Myogenin, Mrf4, and MHC mark terminally differentiated myotubes (**Figure 4**). Concurrent with these genetic changes, internally the cell undergoes structural changes to form characteristics of sarcomeric organization to achieve contractile functionality through the coordinated use of actin, myosin,

calcium, ATP hydrolysis, and other proteins (**Figure 5**). Indeed, iPSC-derived human skeletal myotubes will spontaneously contract in culture which may be mediated by gap junctions which are present on skeletal muscle during development [Supplementary Video 1 & 20]. This is in contrast to many primary muscle cell lines, where spontaneous contractions are not as frequent and therefore contractions must be elicited exogenously. Additionally, skeletal muscle contraction promotes protein synthesis and myotube hypertrophy and is a vital component of the in vitro growing process [21, 22]. In an animal, single myotubes will align and form myofibrils, muscle fibers, fiber bundles, and finally muscle. However, re-creating this in a dish is not as trivial.

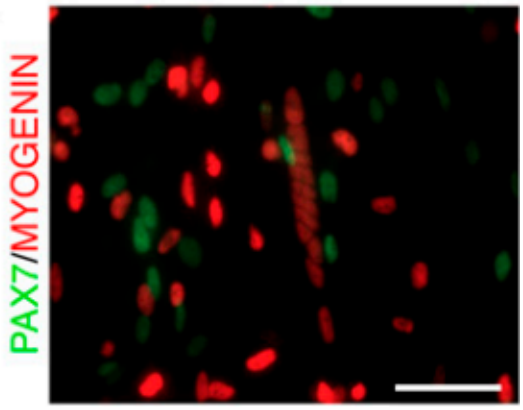


Figure 4: **Figure 4:** Pax7 and Myogenin represent mutually exclusive cell populations in iPSC-derived skeletal muscle. A multinucleated myotube can be seen forming which contains 11 individual nuclei. From Swartz et al., ref [23].

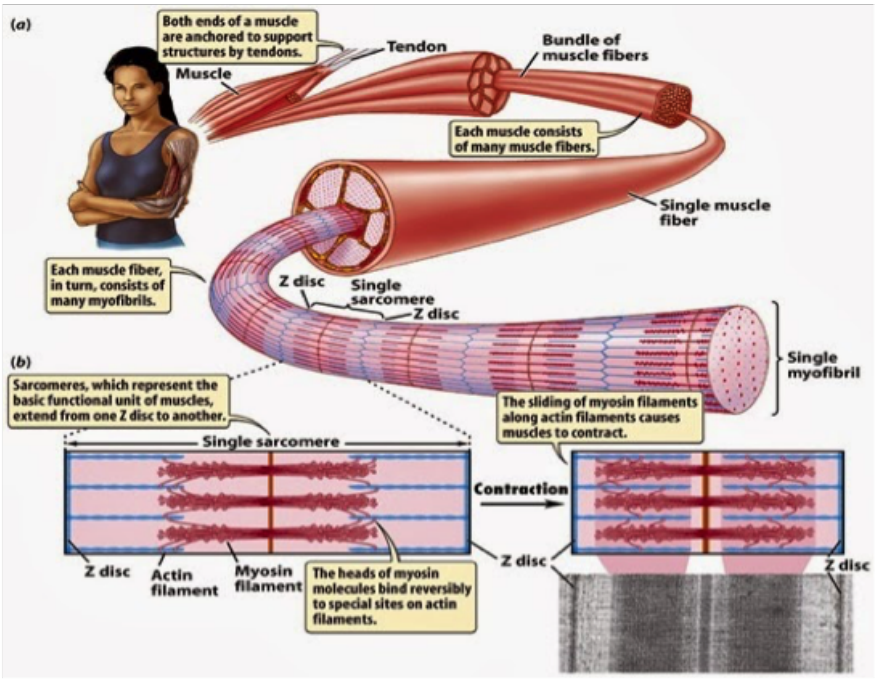


Figure 5: Figure 5: A breakdown of sarcomeric organization involved in muscle contraction.

In order to form skeletal myotubes at scale and in a way that allows them to be packed together to form "meat," researchers have used alternative strategies. For instance, in this video [24], one strategy is to seed cells around a gelatin ring which promotes muscle fusion, contraction, and hypertrophy. By utilizing this method, individual myotube rings can be harvested and compacted together forming a shape similar to ground meat. More recent strategies focus on the use of plastic microcarriers which increase the surface area for cells to bind to and thus enable the culturing of larger numbers of cells in a smaller space [25]. This technique has been used in the past for growth of mesenchymal stem cells [26] and allows cells to grow in suspension for use in bioreactors which are more suitable for production at industrial scales.

In either case, as far as I can tell, the process occurs in three main phases: proliferation, fusion, and polishing. The proliferation phase aims to produce as many dividing satellite cells as possible, using the methods previously described and likely in a specialized medium containing morphogens that will allow for self-renewal of the satellite cells but not lineage commitment, possibly through the use of Wnt proteins such as Wnt7a [76]. Once enough cells are produced, the cells will be transferred to a separate apparatus in a new medium that will promote the production of myocytes and myocyte fusion into myotubes. Classically, this is done via serum starvation, however as I discuss below the use of serum-free components will be essential and thus alternative yet similar methods likely exist. The process of fusion may additionally be aided by nano-patterned plastics or other scaffolding materials, substrates, or biomaterials which will promote myotube maturation as well as provide tensile points of attachment. Once mature myotubes are produced, they can be harvested and "polished" into a final product, likely through some method of compaction of the cells into a final shape and form. This entire process could likely take place within a month with a final product consisting of up to dozens of billions of cells starting from a few thousand.

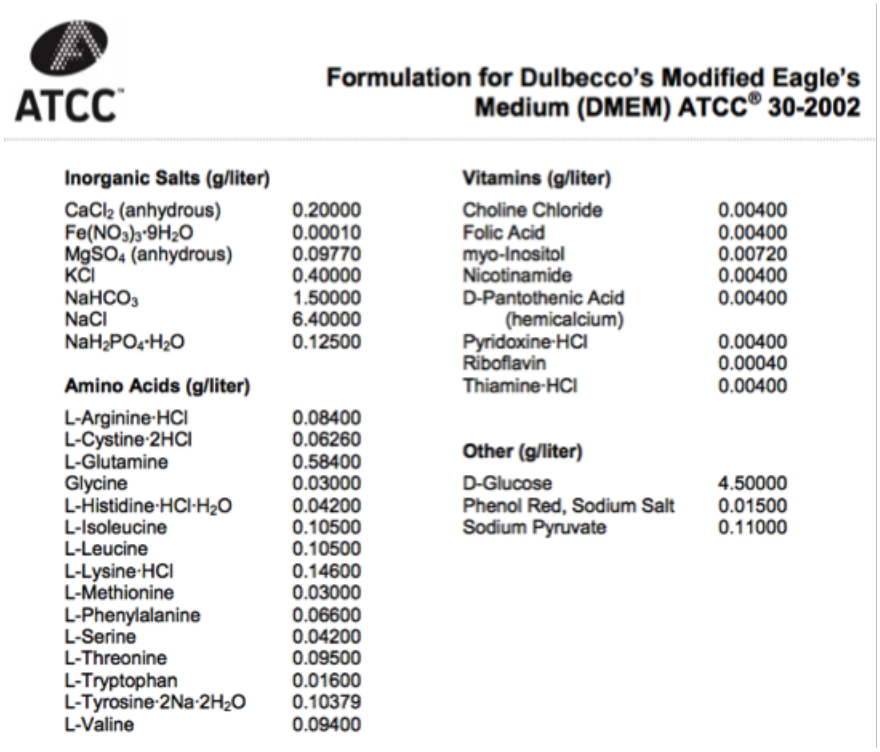
*I could not find exact details on this process as it's likely proprietary, so a lot of this portion can be considered educated speculation.

Cell Culture Medium Components

Many articles describing the creation of *in vitro* meat describe the growth process as "in a bath of nutrients" as if "nutrients" here means anything. So let's break down what is actually used. Some of the most common mammalian cell culture basal medium formulations consist of what you might expect a cell needs to survive, namely amino acids, salts, vitamins, and sugar (**Figure 6**). The cells are grown in an incubator warmed to 37 degrees Celsius (body temperature) and typically 5% CO2, which mimics blood CO2 levels of ˜40 mmHg and predominantly acts as a pH buffer in the form of bicarbonate (HCO3-). The cell culture medium is replaced every 24-72 hours, depending on the amount and metabolic rate of the cells being worked with. This removes cellular waste products, dead cells, and restores pH balance so that the cells can keep living. All told, it mimics the environment found in the body fairly well but can be improved upon by furthering our understanding of biology and having future robotic monitoring and control of cell culture conditions.

Serum

Biology is complex, and there are lots of additional factors required for cell metabolism and growth. Classically, researchers have used animal serum as an easy work-around to delivering these factors to cells. The animal serum of choice is usually fetal bovine serum (FBS), although horse serum, chick embryo extract, or other sera types are sometimes used. FBS [27] is, as the name might suggest, derived from fetal calves which are by-products of the dairy industry. The process involves sterile collection of the blood, coagulation, and centrifugation to remove clotting factors and blood cells, followed by filtration before being available for sale. FBS contains a variety of growth factors, hormones, and necessary components for cell survival (**Figure 7**). In sum, it's not the most "animal friendly" product imaginable. In skeletal muscle, it helps



Formulation for Dulbecco's Modified Eagle's Medium (DMEM) ATCC® 30-2002

Inorganic Salts (g/liter)	
$CaCl_2$ (anhydrous)	0.20000
$Fe(NO_3)_3 \cdot 9H_2O$	0.00010
$MgSO_4$ (anhydrous)	0.09770
KCl	0.40000
$NaHCO_3$	1.50000
NaCl	6.40000
$NaH_2PO_4 \cdot H_2O$	0.12500

Amino Acids (g/liter)	
L-Arginine·HCl	0.08400
L-Cystine·2HCl	0.06260
L-Glutamine	0.58400
Glycine	0.03000
L-Histidine·HCl·H_2O	0.04200
L-Isoleucine	0.10500
L-Leucine	0.10500
L-Lysine·HCl	0.14600
L-Methionine	0.03000
L-Phenylalanine	0.06600
L-Serine	0.04200
L-Threonine	0.09500
L-Tryptophan	0.01600
L-Tyrosine·2Na·2H_2O	0.10379
L-Valine	0.09400

Vitamins (g/liter)	
Choline Chloride	0.00400
Folic Acid	0.00400
myo-Inositol	0.00720
Nicotinamide	0.00400
D-Pantothenic Acid (hemicalcium)	0.00400
Pyridoxine·HCl	0.00400
Riboflavin	0.00040
Thiamine·HCl	0.00400

Other (g/liter)	
D-Glucose	4.50000
Phenol Red, Sodium Salt	0.01500
Sodium Pyruvate	0.11000

Figure 6: Figure 6: Formulation of a standard basal medium.

cells to proliferate and survive and the reduction of serum from 10% to 2% in medium formulations promotes the fusion of skeletal myotubes. However, if the purpose of *in vitro* meat is to eliminate animals from the process altogether while catering to moral imperatives, vegetarians, and vegans alike, then serum-free medium formulations need to be used.

According to Mark Post of Mosa Meats, the first meat burger produced in 2013 utilized serum, however moving forward this will not be the case [28]. This is further supported by Uma Valeti of Memphis Meats when I asked them on Twitter last year (**Figure 8**) and in a discussion with Sam Harris [29].

So how difficult will it be to re-create a medium formulation without serum? Are all of those hormones really indispensable? As it turns out, researchers have been working for a long time in creating serum-free medium formulations. The reason for this is because serum is notoriously variable from lot to lot, expensive, and can lead to mycoplasma or other contaminants. As a result, nearly all embryonic and induced pluripotent stem cell culture is performed serum-free. In the lab, I can differentiate pluripotent stem cells to terminal neuronal lineages in completely serum-free medium formulations. Other formulations have been made or are in the pipeline for other cell types. For skeletal muscle, several groups have published on the derivation or expansion of skeletal myotubes from murine or human pluripotent or myosatellite stem cells using completely serum-free conditions [30, 31, 32]. This trend has continued with the recent publication using a serum-free method for derivation of muscle from porcine pluripotent stem cells, where the first author here is a co-founder of Memphis Meats [33]. Indeed, while I use serum in my own published protocol for human skeletal muscle derivation [23], expansion of skeletal muscle progenitors can be performed in serum-free conditions, specifically DMEM F12, 1% N-2 Supplement, and 1% Insulin-Transferrin-Selenium supplement. The trick seems to be via the addition of supplements or knockout serum replacements [34] which contain only some of the essential factors found in serum, such as insulin, transferrin, selenium, putrescine, and progesterone

Composition of FBS

Component	Average	Range
Endotxins (ng/ml)	0.35	0.01 - 10.0
Glucose (mg/ml)	1.25	0.85 - 1.81
Protein (mg/ml)	38	32 - 70
Albumin (mg/ml)	23	20 - 36
Hemoglobine (µg/ml)	113	24 - 181
Bilirubin, total (µg/ml)	4	3 - 11
Bilirubin, direct (µg/ml)	2	0 - 5
Urea (µg/ml)	160	140 - 200
Urate (µg/ml)	29	13 - 41
Creatinin (µg/ml)	31	16 - 43
Insulin (µU/ml)	10	6 - 14
Cortisol (ng/ml)	0.5	0.1 - 23
Growth hormone (ng/ml)	39.0	18.7 - 51.6
Parathormone, PTH (ng/ml)	1.72	0.085 - 6.18
Triiodothyronine, T3 (ng/ml)	1.2	0.56 - 2.23
Thyroxine, T4 (ng/ml)	0.12	0.08 - 0.16
Thyroid-stimulating hormone, TSH (ng/ml)	1.22	0.2 - 4.5
Follicle-stimulating hormone, FSH (pg/ml)	95	20 - 338
Testosterone (pg/ml)	400	210 - 990
Progesterone, P4 (pg/ml)	80	3 - 360
Prolactin = Luteotropic hormone, LTH (pg/ml)	176	20 - 500
Luteinizing hormone, LH ?? (pg/ml)	8	1,2 - 18
Prostaglandin E (ng/ml)	5.9	0.5 - 30.5
Prostaglandin F (ng/ml)	12.3	3.8 - 42.0
Vitamine A (ng/ml)	90	10 - 350
Vitamine E (ng/ml)	1.1	1 - 4.2
Cholesterol (µg/ml)	310	120 - 630
Lactate-dehydrogenase, LDH (mU/ml)	864	260 - 1,215
Alkaline Phosphatase (mU/ml)	255	110 - 352
Aspartate-Aminotransferase, ASAT (mU/ml)	130	20 - 200

Figure 7: Figure 7: Typical composition of fetal bovine serum used in cell culture.

(found in N-2 supplement). The hormones typically shamed in bovine farming include things such as recombinant bovine growth hormone and synthetic hormones such as Zeranol, Trenbolone, and Melengestrol [35], although I divert to someone who has a better understanding as to the possible ill effects of these on human health. Thus, it's likely clean meat will be advertised as "hormone-free" but contain indispensable hormones such as insulin. Additionally, other efforts are underway in using plant-based protein extracts known as vegetal serum or peptones [36, 37]. In sum, while I cannot say exactly what serum-free formulations are being used by these start-ups, it is clear that it *is* currently possible.

Cellular Substrates

Cells typically grow in a dense protein jungle known as the extracellular matrix (ECM), which provides structural and biochemical support [38]. Additionally, the ECM allows cells to sense elasticity and stiffness in order to get a [pun-intended] "feel" for the environments they are in. Work from Helen Blau and others has shown a vital importance for the stiffness and elasticity of the ECM substrate on skeletal muscle self-renewal and differentiation [39, 40]. The same is true for other cell types, which is an often over-looked fact that cell biologists of the future will ask, "what were they doing?" So why is this relevant?

As it turns out, the most commonly used substrate for primary muscle cell growth is a low percentage

Figure 8: Figure 8: One of many public statements by Memphis Meats declaring serum-free cell culture.

(0.1 – 0.2%) of porcine gelatin, derived from pigs. Alternatively, Matrigel, which is derived from mouse sarcoma cells, has a similar Young's Modulus (measure of stiffness) as the human satellite cell niche (0.45 kPa versus 0.5 kPa, which I include in my manuscript's discussion section here [23]) and can also be used as a differentiation substrate. So we are again left wondering if *in vitro* meat is really devoid of animal products. Luckily, efforts have already been made in this area as well through the use of synthetic hydrogels to mimic desired stiffness and elasticity features of certain cell types such as muscle [41]. Alternatively, recombinant proteins such as laminin-521 have fared well in promotion of skeletal muscle growth [42]. Again I speculate on the use of hydrogels as a cell substrate here, but mainly want to make the point that this area can be free of animal products as well. Additionally, it's quite possible that in order to properly scale production, substrate-free bioreactors will be the primary choice for growing *in vitro meat.*

Taste

One of the most immediately obvious differences, based on what's been publicly shown, between clean meat and the packaged product you buy at the store is taste and texture. And make no mistake – those two features likely outweigh considerations of cost, ethics, and environmental considerations that would play into a consumer making the switch. This is evident in the slow, yet coming-of-age progress in the plant-based meat industry, where Beyond Meat's "bloody" beet-juice burger has been met with rave reviews. So what's being done to address these issues?

In this recent podcast [43, start 41:30], Mark Post discusses how efforts are already underway to incorporate adipose- (fat-) derived stem cells and using fatty acids to drive differentiation of fat cells. It should be assumed that similar efforts are underway at other labs in order to find the ratios of fat cells necessary to yield the best taste, flavor, and texture. To be clear, one shouldn't assume that the intention is to produce a perfectly marbled ribeye steak off the bat. Rather, the intention is for different ratios of fat cells and muscle cells to be able to be molded into different shaped meats that mimic the taste and textures of '*real*' meats. Incorporation of 3D bioprinting may aid in the formation of repeatable shape and patterning of skeletal myotubes with intertwined fat cells and scaffolds for myotube alignment have also been used for these purposes [44]. For tenderness, the length of time in culture and amount of electrical stimulation can be used to mimic animal muscle. For instance, veal is tender because the animal has not yet had time to develop strengthened, mature muscles. In the same way, preventing or reducing contractions *in vitro* may mirror this process [45]. It remains unclear to me whether different cell types will be differentiated and

expanded independently of the skeletal muscle before combining the two, or whether they will be grown as a co-culture from the start.

The last consideration for taste is the location of the original muscle biopsy. Intuitively, the taste of the muscle may change based on what type of body part (or animal) from which it originates, however this has yet to be truly tested. Indeed, from a developmental perspective, the skeletal muscle of different body areas is inherently different in terms of certain gene expression patterns as well as fiber-type composition [46, 47]. It's also entirely possible that muscle tissue from different animals will taste distinct and that multiple animal types can be combined to create unique meat combinations. Additionally, efforts to improve the shelf-life as well as give the meat color are also being explored [48]. Finally, much work in the plant-based "meat" industry has gone towards isolating compounds such as heme that are critical to re-creating a meaty aroma. Therefore, the addition of synthetic compounds may be able to make up for lack of a true "meaty" taste or flavor.

Immortalized lines

How many cells can you obtain from a single muscle biopsy sample after expansion? Cells are inherently constrained in their capacity to replicate indefinitely due to telomere shortening – a phenomenon known as the Hayflick Limit [49, 50]. Thus, a population of purified satellite cells is confined up to around a maximum of 40 times *in vitro* although 20-30 in practice is more realistic. Although this results in a large amount of cells by number, the volume of cells needed to make a single patty is likely in the tens of billions and alternative ways need to be explored to increase efficiencies and lower costs.

One alternative way would be starting from iPSCs, which were discussed earlier. When a cell is reprogrammed into a pluripotent state, it activates endogenous telomerase expression thus bypassing the Hayflick Limit through restoration of telomere length. This makes the cell essentially immortal, although increased cell divisions lead to higher likelihood of DNA replication errors such as chromosome duplications, deletions, inversions, etc, which can lead to apoptosis. Starting with iPSCs would allow for easy and large scaling of the initial starting population. These cells could then be guided through a differentiation protocol that mimics development, such as those previously discussed, in order to finally arrive at skeletal myotubes which can be formed into a meat product.

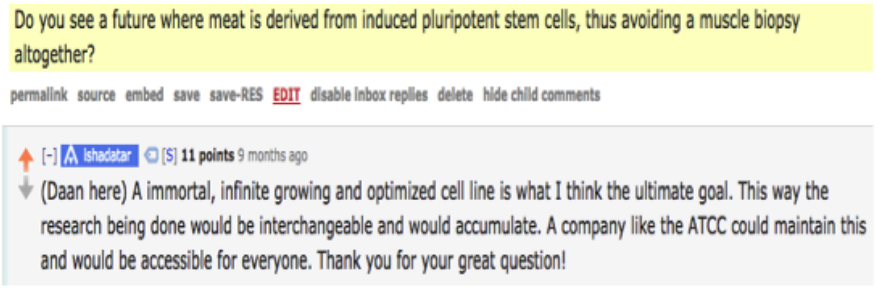


Figure 9: Figure 9: A representative from New Harvest Org. envisions the use of immortalized cell lines for *in vitro* meat during a Reddit AMA.

An even better alternative would perhaps just be creating an immortalized satellite cell line (**Figure 9**). Scientists have figured out ways to artificially immortalize cell lines through the addition of viral elements that alter cell cycle checkpoints such as SV40 or over-expression of telomerase [51]. Thus, addition of these factors to an isolated myosatellite cell line can lead to an "off-the-shelf" immortalized line which can be banked and shared among researchers or companies. Moreover, researchers can create multiple cell lines and select the ones with the best characteristics in terms of cell division, protein expression, taste, etc, to further optimize the production pipeline and final product. This is being done at North Carolina State

University, where scientists have immortalized turkey cell lines that led to the creation of a small turkey nugget in just 2 weeks after thawing [52], indicative of a huge decrease in the already sped-up time that it takes to grow a full sized chicken {**Figure 10**). In the future, meat production companies will therefore likely have stocks of optimized immortalized cell lines for the quick production of "off-the-shelf" meat of a specific animal, taste, or texture, which will lead to the limiting or complete elimination of repetitive biopsies.

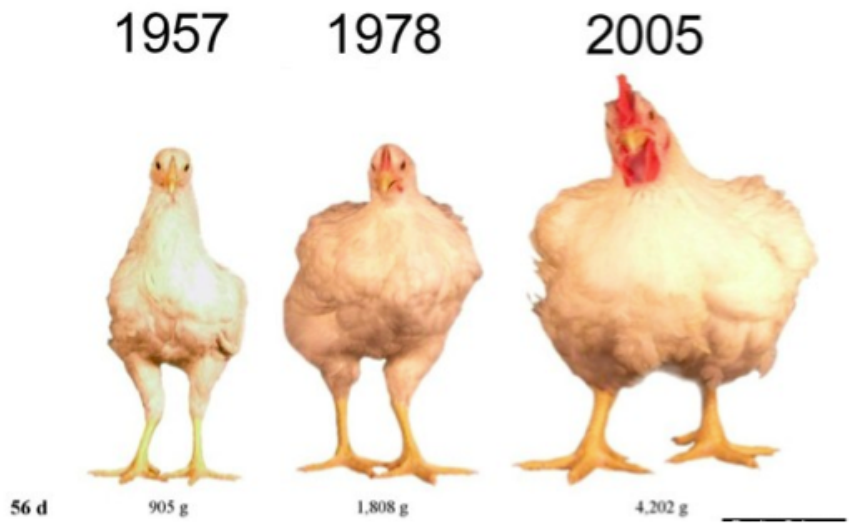


Figure 10: Figure 10: Selective breeding and feeding techniques have led to a massive increase in the broiler chicken's body and growth rate throughout time. *in vitro* methods may offer even larger yields in a shorter timeframe while using less resources.

Wait – I know a lot of immortalized cell lines are derived from tumors. Does this mean I'm eating cancer? Well, not exactly. Cancer cells have many mechanisms to avoid normal cellular checkpoints, only some of which would be similar to the methods in creating an immortalized cell line. Additionally, the cells will likely contain additionally engineered methods to "turn off" the factors involved in immortalization to allow a smooth transition into a post-mitotic, mature skeletal myotube (or other cell type) [one example here,53]. For this, many options are available (drug inducible, CRISPR-mediated, Cre recombinase, etc) although I am unsure exactly how the system would be set-up. Regardless, the FDA and clean meat companies are going to have their work cut out for them in convincing the public that engineered or immortalized cells would be safe to eat.

What happens to the livestock?

There is a staggering amount of livestock not only in the United States but other highly populated countries as well (**Figure 11**). As mentioned previously, maintaining these animal populations takes a massive toll on the environment, arable land, and water availability. If the technology described in this post becomes a reality, it is inevitable that these populations will shrink dramatically, starting in 'westernized' societies and eventually spreading throughout the world. The concept of farming animals for food will likely always exist and markets for high quality meats seem likely to remain for the foreseeable future. However, *in vitro* meat does essentially offer the opportunity to eliminate the need for animals altogether (via immortalized cells, for instance) and thus the possibility exists in the future for technological maturity to hit a point where traditional animal farming becomes practically non-existent.

Figure 11: Figure 11. Livestock populations by country. From The Economist.

Engineering opportunities for improved nutrition and optimization

Our understanding of genetics and biology now enable us to modify the genetic makeup of organisms in a controlled and predictable fashion. In doing so, the creation of genetically modified organisms (GMOs)

offers an unprecedented ability for humans to tackle real world problems related to feeding an ever-growing population in a manner which produces the least ethical, economic, and environmental burdens. **This is a good thing**. It is inescapable that GMOs do and will continue to play a vital role in human progress.

With that said, recent genetic engineering technological advances such as CRISPR/Cas9 and other CRISPR systems further hone the ability to make these changes. As far as I am aware, the lab grown meat that has been publicly shown has not been genetically modified. But I am almost certain this will not hold true over time. The first type of modification that may be implemented may be the creation of genetically modified animals where myostatin is knocked out. Myostatin is a protein encoded by the MSTN gene which essentially functions to inhibit myogenesis. In doing so, it keeps muscle growth in check. Randomly occurring mutations in the myostatin gene have led to the identification of animals with abnormally larger muscles, including humans [**Figure 12**, 54]. Researchers are now exploring engineering strategies to make these animals commercially, as they provide more lean muscle than standard farming strategies [55, 56]. Human athletes are also exploring this, through use of the myostatin inhibitor follistatin for performance enhancement or even anti-aging strategies [57, 58]. Thus, harvesting satellite cells from MSTN knockout animals or through the creation of *in vitro* MSTN knockout cell lines will likely lead to higher yields of skeletal muscle tissue.

Figure 12: Figure 12: A bull without myostatin.

For nutritional purposes, there are a variety of things that can be done. We are getting pretty good at modifying biological pathways to produce specific molecular outputs given specific molecular inputs. For instance, through enzymatic engineering, scientists were able to create a complete biosynthetic pathway of opioids from sugar in yeast [59]. Similar strategies could be undertaken to optimize nutritional output in *in vitro* meat. The low-hanging fruit here would be for the creation of meat which has high concentrations of omega-3 fatty acids such as DHA and EPA, typically found in fish, which have been studied extensively for their health benefits. Humans and other mammals do have the capacity to naturally synthesize these types of omega-3 fatty acids following ingestion of alpha-linolenic acid (ALA), but they do so in low quantities. By engineering these biosynthetic pathways (**Figure 13**) or altering traditional cellular medium formulations, we would aim to bias our meat's nutritional profiles to our liking. I imagine entire companies in the future focused specifically on these goals, however I divert to an expert in biochemistry for the feasibility and range of options possible.

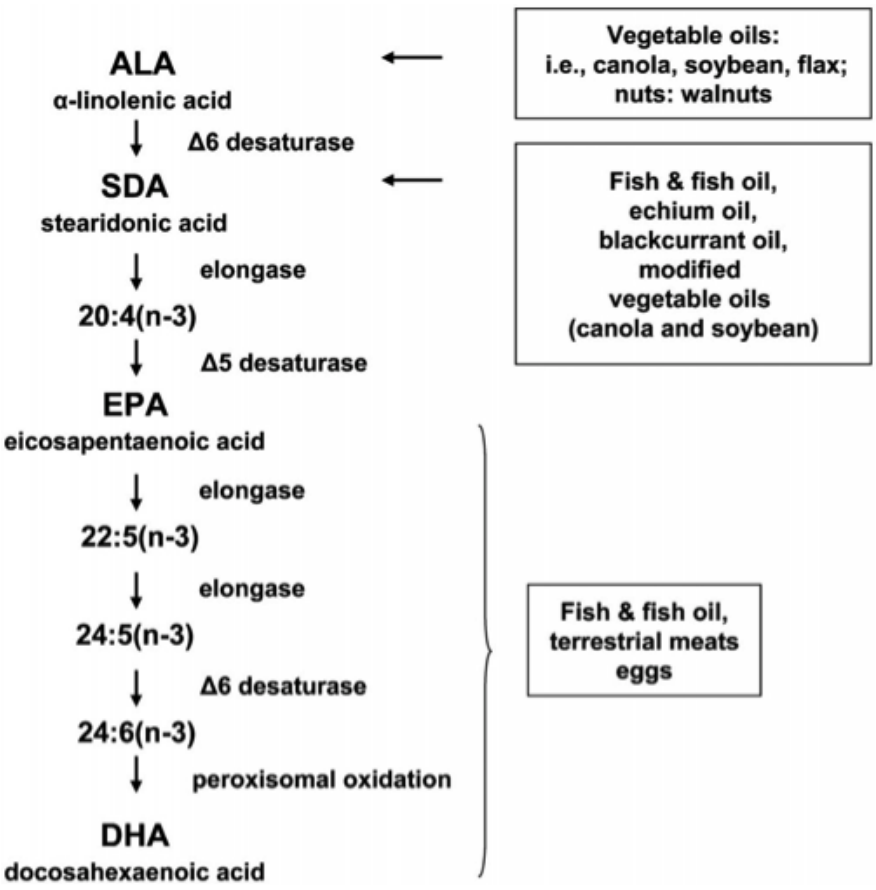


Figure 13: Figure 13: The biochemical synthesis of beneficial fatty acids. Modifications of naturally occurring enzymes may be able to bias fatty acid synthesis to higher levels of these fatty acids in future *in vitro* meats.

Antibiotics, Sterility, and cGMP

A big question that comes to mind for a would-be consumer is the process and sterility. How are they going to ensure that the meat is not contaminated – that must require tons of antibiotics and those things are bad, right? Indeed, approximately 70% of all antibiotics in the United States goes into animals [60]. As it turns out, however, you don't really need any antibiotics if the process is sterile. In standard cell culture, we obtain a near-sterile environment through the use of culturing cells and tissues in plastic dishes which keeps bacteria or spores in the air from getting in. When we need to replace the medium for the cells, we do so in a biosafety cabinet which controls the environment through air regulation and filtration. Through this process and other standard cell culture practices, you largely eliminate the possibility of outside contamination. Indeed, virtually all of the cell culture I personally perform is done so free of antibiotics. Thus, contrary to current factory farming practices of overuse of antibiotics, all evidence points to *in vitro* meat being completely antibiotic free [61, 62, 63]. This means that a switch from factory-farmed meat to *in vitro* meat will nicely coincide with efforts to reduce antibiotic consumption that has been leading to antibiotic resistance and fear of development of new pathogenic bacterial strains. Additionally, this means that your meat will be free of foodborne pathogens (think *Salmonella* or *Listeria*), which would like decrease the incidence of foodborne

illnesses or deaths.

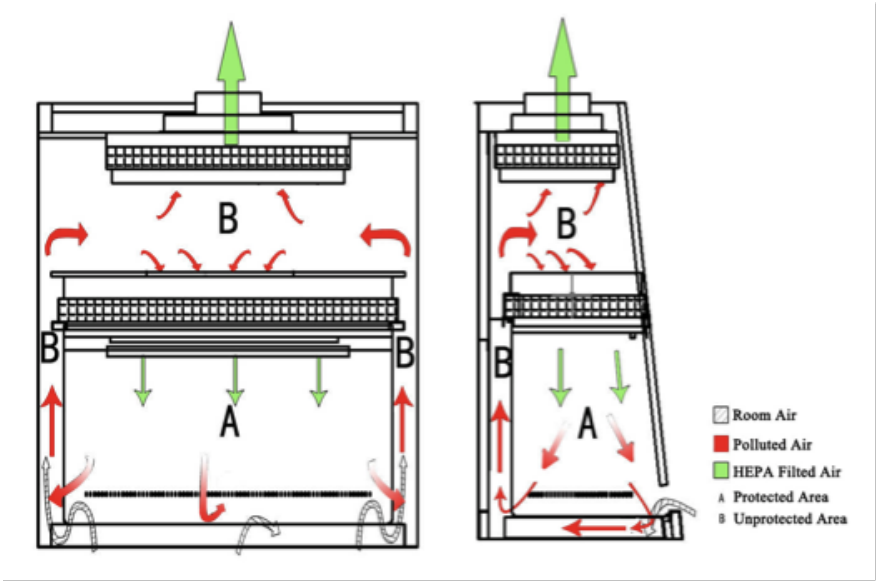


Figure 14: Figure 14: A schematic of a standard biosafety cabinet used in cell culture.

But the meat won't just be produced in some standard biosafety cabinet. Rather, the FDA has specific and stringent requirements for facilities that produce chemicals or [cellular] products that will eventually make their way into humans. And despite what some people will tell you, regulatory agencies are essential for carrying out these requirements. We refer to these requirements as *current Good Manufacturing Practice* or *cGMP*. Under cGMP regulations, the facilities, equipment, materials, and quality assurance are held to the highest standard to ensure a product is safe and identical batch-to-batch. Being cGMP compliant requires a very high investment of time and resources and as far as I know, taken very seriously. It's likely that many cGMP requirements for the production of cellular therapy products could be adapted for *in vitro* meat production, however it's also likely that a new framework specifically for *in vitro* meat would need to be created. You can get a better sense on the details of cGMP here [64, 65].

Cannibalism

Another question that comes up is the possibility of growing human meat. Of course, it would be completely possible for human cells to be taken through the same process of *in vitro* meat production. It's possible that one could even eat oneself. . . in some sort of closed-loop fantasy of the uber vegan. Maybe future space-farers will grow meat in a semi-sustainable fashion. Obviously, this idea won't be entertained by any commercial entity, but it's worth stating that it is possible and that human meat has been consumed by other humans in the past. So the possibility exists. Am I on a list yet?

Cost, scale, and investments

One of the largest current barriers to making this into reality is the cost. As previously mentioned, the cost of the first *in vitro* hamburger was roughly $325,00 in 2013. Thus, creation of technologies that enable scaling are just as important as those involved in the successful creation of the product itself. It should be assumed that a large amount of work in this industry is currently going into the scaling process, although the specifics are currently uncertain. The best data I could find on this comes from interviews with Mark Post in May 2016, who says that they plan to use 25,000 liter bioreactors that would be able to produce 882,000 pounds of meat per year, which is enough for more than 10,000 people who eat an average amount

of beef [66]. Uma Valeti of Memphis Meats has already stated that for the production of beef, the ratio of calories in to calories out has already been reduced from 23:1 to 3:1 [67], however the price of a pound of chicken was recently reported at $9,000/lb [68]. There is a funded project at Kent State University to build a bioreactor for the production of *in vitro* pork and lobster [69]. And more recently, a report in February 2017 suggested that the price had dropped to $11.36 for a five ounce burger [70], making the cost roughly 9-10x more expensive per pound than standard ground beef. Thus, we already getting closer to a price point that would be attractive to consumers.

Even with scaling, however, there are additional hidden costs that come between growing meat in a dish and getting it into a consumer's mouth. The agriculture industry has a complex supply chain that includes farming, slaughtering, packaging, shipping, etc, which adds additional costs to an end product. In a recent podcast, Uma Valeti discusses how the goal of the *in vitro* meat industry is to hijack and cut-out existing infrastructure rather than innovate their own processes [71]. Thus, production of *in vitro* meat aims to team up with distributors directly to save on costs and environmental impacts.

Today, agriculture (both plant and animal based) receives large amounts of government subsidies [72]. From land on which to graze on, to feed and drought relief, the agriculture industry is propped up by American taxpayers. And while the public may be okay with the benefits of having a supply of cheap meats from which to choose, the external costs on the environment which are then passed on to the public are often ignored. This leads to downstream increases in healthcare costs and other public health burdens. In this sense, the detrimental impact of animal agriculture on the environment parallels that of the fossil fuel industry where quick gains for companies are chosen over external concerns. Thus, wielding of political power must play a role in accelerating the adoption of *in vitro* meat consumption in the same way that many experts feel a carbon tax will accelerate renewable energy production. Subsidizing or other efforts to support *in vitro* meat technology can go a long way in achieving faster price parity.

The most impressive part about the $11.36 burger is not the dramatic cost reduction in only 3 years, but rather that the cost reduction was performed with so few people involved. There are likely around 100 people in the entire world inside of academia and industry that are seriously involved in *in vitro* meat production, which is insane considering the potential earnings that would come from disrupting the food industry. Increasing interest in this field can be achieved in academia through new programs in cellular agriculture or by advertising job opportunities for scientists in related fields (stem cell biology, tissue engineering, industrial engineering, etc). Indeed, assistance is just starting to emerge to accomplish these goals.

New Harvest is a 501c3 research institute that funds and advocates for all types of projects involved in general cellular agriculture including lab-made milk and eggs. Indeed, a good way to view the landscape of current projects can be seen by visiting their portfolio or grant opportunity pages [73]. A group called the Good Food Institute similarly aids in providing legal, educational, and other support to companies or scientists within the space [74]. Together, these organizations aim to get new companies in cellular agriculture off the ground. While plant-based companies such as Beyond Meat have attracted large funding rounds led by the Gates Foundation and others, new venture capital may be aimed at *in vitro* meat projects. For instance, a $150 million dollar venture capital fund from Tyson Foods may help kick start some of these alternative meat projects [75]. Nevertheless, it is likely that as proof-of-principle results continue to be positive that a real influx of funding will arrive and this will usher in a larger amount of scientists and engineers who aim to solve these scaling problems.

Reception

The public reception to *in vitro* meat seems to be mixed, with some very excited, some disgusted, and some confused. One of the main motivations for making this blog post was due to the wild amount of misinformation I read in comment sections on Reddit and Facebook whenever an article on this topic is discussed (**Figure 15**). It's depressing. It's clear that distrust of science in America irrationally proceeds to grow with President Trump's cabinet serving as exhibit A. The long battle with the public's perception of genetically modified food products doesn't help either. Many people already have their minds made up

on any form of modified foods as they seek out unprofessional opinions to validate their own confirmation biases while disregarding the opinions of those who have dedicated their lives to becoming experts in a field. The urge to appeal to naturehas proven to be hard to overcome in the past.

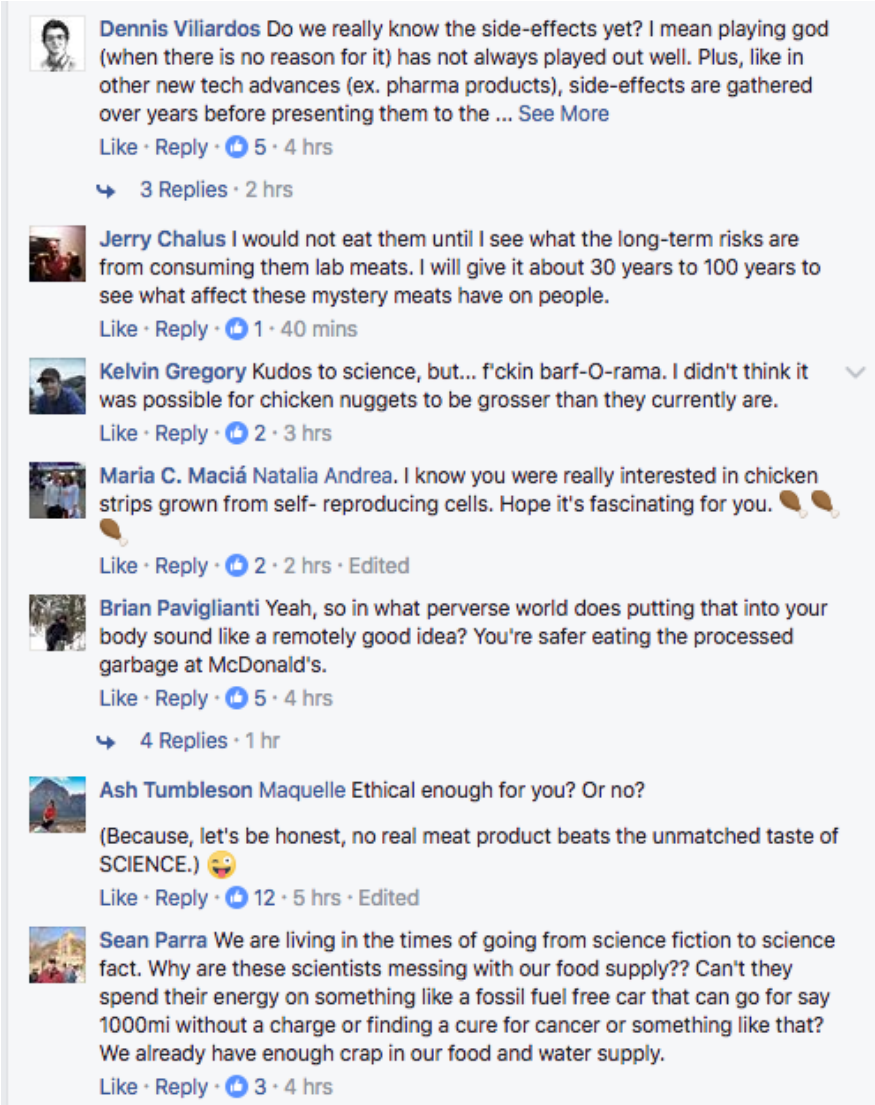


Figure 15: Figure 15. Your typical comments under a recent Wall Street Journal post.

In the end, however, I believe that the true test of the public will be for these companies to put out a product that is nearly indistinguishable in looks, taste, and texture as 'real' meat and comes with the added benefits previously discussed. One advantage *in vitro* meat does have in convincing the public of its safety or reducing fears of the "gross factor" is transparency. It will be possible for future *in vitro* meat facilities to be visited in the same fashion as microbreweries are today. This will allow people to see the process and the product that they're getting up close and personal, which is radically different from current practices. Additionally, *in vitro* meat provides a realistic approach to facing the fact that humans are omnivores and enjoy eating meat. I don't think the majority of the world could commit to a vegetarian or vegan lifestyle but they may be able to adjust to slightly different form of meat.

Conclusions

In sum, recent advances in our understanding of muscle cell biology, cell culture techniques, and tissue engineering now offer an unprecedented opportunity to disrupt traditional animal agriculture. In the face of population growth, environmental, and ethical concerns, there seems to be few options aside from the pursuit of new technologies. Over the next decade, I foresee rapid growth in *in vitro* meats and alternative agriculture as the first products are expected to hit the market in ˜5 years. Hopefully I've outlined the feasibility and implications of this soon-to-be familiar technology.

Disclosure: It's worth mentioning that I am not a vegetarian or vegan, although I do consciously consume less meat than average largely due to environmental reasons. Roughly 90% of my meals do not contain any meat. I do not receive funding from nor am I associated with any companies mentioned in this post.

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