

# AlcheMeat—how the future of animal production rests with biochemistry

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Innovations in regenerative medicine, stem cell biology, tissue engineering, and biopharmaceutical production are responsible for saving countless lives and completely transforming healthcare. But what if this research could be applied to more than individual medical treatments? New applications within these fields could further address public safety concerns, global malnourishment, environmental issues, and animal abuse. In fact, these types of studies could lead to prevention of many of the diseases they initially set out to treat! Foci within these areas, such as telomerase expression, cell differentiation, induced pluripotency, three-dimensional tissue formation, recombinant protein production and CRISPR, are now being used for something entirely different – making meat.

The majority of the human population regularly eat meat. Meat is high in protein, convenient, affordable and, for most people, delicious. But once you scratch the surface of the process behind eating meat, you will come to find that it's not all it's cracked up to be. Given that several types of meat are classified as carcinogens and packed with cholesterol, sodium and saturated fats, it is easy to correlate consumption with the top two killers in America: cancer and heart disease. Many foodborne illnesses stem from contaminated meat products, 80% of our antibiotics are fed to farm animals, and zoonotic diseases are easily spread within the concentrated animal feeding operations that source the majority of our meat products. Animal production is responsible for more greenhouse gas emissions than all planes, trains and automobiles combined, leading the way in man-made climate change. Desertification, deforestation, erosion, acidification, eutrophication and species extinction are all extremely damaging results of intensive farming practices. The 9 billion pigs, cows, chickens, turkeys, and other land animals farmed and slaughtered for food each year in the US alone might appreciate a change within the food system.

Despite the growing body of evidence relating meat production and consumption to these health, environmental and welfare concerns, we still can't get enough of it. Seeing as the world probably won't be going vegan anytime soon, we need a solution, and fast. Luckily, biotechnology is offering new approaches.

Clean meat research is a focus within the field of 'cellular agriculture', a movement made up of scientists, entrepreneurs and activists hoping to change the food system for the better by growing animal products without animals.

## Clean meat in the making

Clean meat has been in the making for over a century, starting with 1900s Nobel laureate Alexis Carrel, who grew chicken heart muscle in a dish for months on end. William Churchill predicted we would be growing chicken-free chicken breasts by the 1980s, and artist Oron Catts cooked up some clean frog meat for an exhibition in 2003. It wasn't until 2013, however, that the field really got going, when Dutch physiologist Mark Post served up some Google-funded clean burgers during a broadcast in London. This led to a burst of research and startups in the field. In addition to beef, companies and academic institutions have now produced clean chicken, duck, turkey, fish and pork, with several products expected to hit the shelves within the next 3 years. So how does one grow clean meat, what are some of the challenges the field must overcome, and how can interested scientists apply their expertise to help raise the steaks?

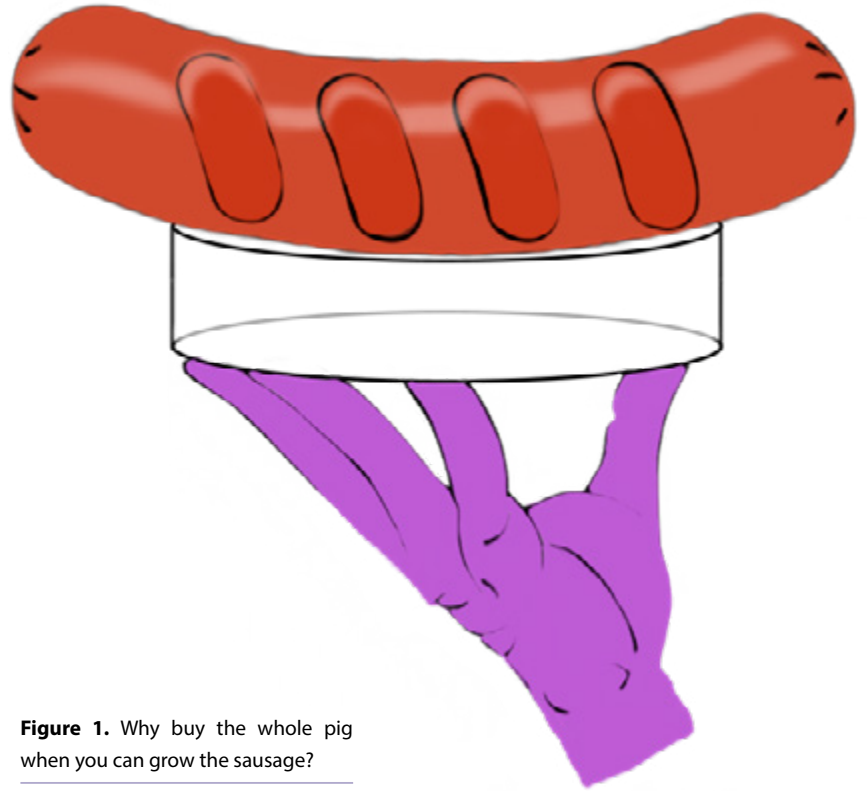
In theory, the process is quite simple. Get your hands on some animal cells, seed them into a bioreactor containing the proper nutrients and environmental

conditions for proliferation, and when you have enough cells, combine them with a meat-like scaffold and grill up some burgers. In practice, however, there is still a bit of work to be done before you'll find any clean meat options at your local drive-thru. Until then, it is up to us scientists to perfect the process of clean meat production.

## What type of cell to sell?

Like our current meat production methods, clean meat begins with a cell. Clean meat scientists are experimenting with several different cell types, including tissue-specific cells, embryonic stem cells, mesenchymal stem cells and induced pluripotent stem cells. While there may be several optimal cell types for clean meat production, there are certainly some traits that would be extremely beneficial for each cell type of interest to possess. These include fast proliferation rates, unlimited growth potential and differentiation flexibility. Tissue-specific cells, such as the muscle-based satellite cells and myoblasts, fat-producing adipocytes and plentiful fibroblasts, can be harvested from a simple biopsy the size of a sesame seed. They proliferate well in culture, easily differentiate into their tissue-specific cell type, and with a little guidance, can differentiate into other cell types as well. However, tissue-specific cell types may not be able to proliferate for more than a few months before undergoing senescence – at least, not without a bit of genetic editing, spontaneous immortalization or experimental media additives.

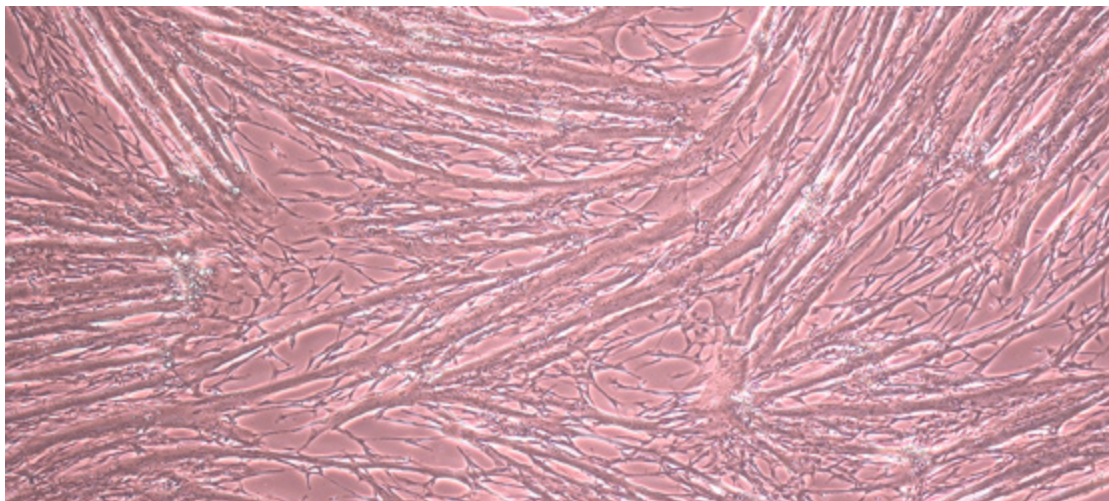
We continue to study the exciting process of controllable immortality within tissue-specific cell types for clean meat applications, but scientists have also turned to stem cells. Embryonic, mesenchymal and induced pluripotent stem cells are able to continuously



**Figure 1.** Why buy the whole pig when you can grow the sausage?

evade cell ageing, possibly due to their ubiquitous expression of a very special protein: telomerase.

Telomerase is a longevity-promoting protein that acts by adding telomeres to the end of our chromosomes, thus allowing cells to continuously divide indefinitely without the threat of chromosome shortening or fusion. While the exact role of telomerase in immortality varies across species, many farm animal cell lines are able to grow indefinitely when telomerase expression is



**Figure 2.** Myoblasts easily differentiate into multinucleated muscle fibres when crowded and exposed to low nutrients

upregulated. Another benefit of stem cells is their ability to theoretically differentiate into any cell type.

Maintaining stem cells in an undifferentiated state, however, can be troublesome. Many researchers are working on methods that establish better control over stem-cell fate. Additionally, stem cells can be costly and difficult to grow in culture when compared with tissue-specific cell types. Other issues may arise from stem-cell harvests. Multiple harvests from animals for clean meat are not required, as a single cell could potentially lead to the production of 30,000 times the global annual meat demand if allowed to double exponentially over a 3-month period. However, initial isolations are needed to establish cell lines, and many may not like the idea of harvesting cells from animal embryos. Like tissue-specific cell types, however, mesenchymal stem cells are found throughout adult tissues, rendering the need for embryonic harvests unnecessary.

Perhaps even more appealing is the option of induced pluripotency. Researchers are now able to take isolates from several different sources, such as blood samples, skin scrapings, cheek swabs, hair follicles, or feathers, and turn these tissue-specific cells into pluripotent stem cells. These cell harvests are much less invasive than embryonic collections or tissue biopsies, and produce cells that share the same characteristics as a stem cell. It is worth noting that the easiest way to induce pluripotency currently involves genetic modifications, and while more natural methods are possible, additional research is needed to boost efficiency.

## Not all media is good media

Once you have your hands on some animal cells, you need to provide them with the nutrients, chemical signals and environmental conditions required to promote fast and controllable growth. This is accomplished by using a suitable cell culture medium. In the past, researchers have supplied cell cultures with all the necessary factors normally found within the body by going straight to the source of cell nutrients – blood; fetal cow blood, to be exact. We can't very well claim to produce animal-free animal products if we are relying on feeding our clean meat cells with fetal cow blood. Biomedical researchers have, however, begun 'steering' away from animal-based media additives for several reasons. Variations in batches, unknown pathogens, and undefined additives found in fetal bovine serum lead to unanticipated and hard-to-replicate results within cell culture experiments. These drawbacks have led several research supply companies to produce

chemically defined, serum-free media formulations for specific cell types.

While we are moving further and further away from harvesting blood from fetal cows in the name of science, large-scale cell culture media formulations still face some challenges in the form of cost. Serum-free media is made of several different growth factors that must be produced recombinantly, in a similar fashion to that of biopharmaceutical production. This process can be pretty pricy, but as production scales up, the costs will drop dramatically. The push for scaled growth factor production means more affordable clean meat AND cell culture media for biomedical research.

Other options for serum-free media additives involve co-culturing with cells like liver, which naturally produce growth factors within the body. A plethora of growth factor analogues and functional domains could be hiding within the unexplored proteomes of several different plant species, and it may be possible to synthesize growth factors from plant proteins as well. We can also potentially remove growth factors from the media entirely by creating cells that can make their own growth factors. Furthermore, we could edit cells to bypass the initial growth signalling pathways and grow independently of growth factors, assuming they are given appropriate nutrients and maintained under the proper pH, temperature and osmotic pressure. While there is still much to learn about clean meat media, we are getting closer every day.

## Bioreactors: where bio and farm meet

Now that you have your cells and know what to feed them, it's time to find them a home. However, the perfect bioreactor is still up for debate. Adhesion-based setups are intuitive options, as the majority of cell types found in meat are anchored to other cells when housed in the body. But adhesion-based reactors may involve more complicated and time-intensive harvests, extra detachment enzymes and unwanted premature differentiation and senescence when compared with alternative production methods. Given the difficulties associated with anchorage-dependent bioreactor systems, suspension-based culture may be more promising in the long run.

In fact, stir-tank bioreactors are the go-to setup for many large biopharmaceutical companies. Using yeast, bacteria and even mammalian cells such as Chinese hamster ovary cells, we are currently growing cells to produce recombinant proteins on a very large scale. This process, however, yields only a small amount



of the intended product – the protein isolate. Clean meat production, on the other hand, is not focused on producing recombinant proteins; we want the entire cell. So while biopharma is constantly seeking to increase protein production yield, clean meat systems will be able to harvest much more product, and undergo much less downstream processing.

The cost of suspension-based recombinant protein production may be high at the moment, but considering the anticipated higher yields and simple processing of clean meat cell proliferation, it is safe to assume that this system will be much more affordable. As we continue to scale production, clean meat proliferation methods could lead to decreased costs associated with biopharmaceutical production as well, creating more accessible medicine.

## Scaffolds: the matter of the meat

When it comes to clean meat scaffolding, we have several different options. Scaffolding can be used as a means of growth, in addition to serving as textural and structural support. We are still a few years away from a rack of ribs, a turkey leg or a T-bone steak, but the texture and appearance of processed meats such as burgers, meatballs, sausages, cold-cuts and nuggets can be easily reproduced thanks to the growing plant-based meat field.

The main hurdles left within plant-based meat involve the replication of taste and animal-based protein content, both of which can be addressed with cell-based meat production. Plant-based scaffolds include meaty fruits, veggies and fungi such as coconut, jackfruit, artichoke and mushroom. We can also create textured wheat, bean and pea isolates that can be produced to form a meat-like product with the help of extrusion methods.

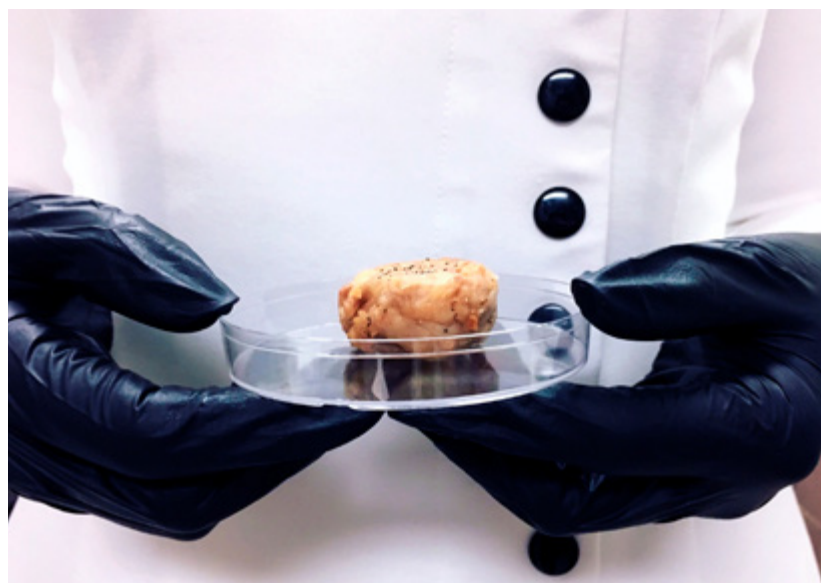
Synthetic scaffolds utilized within regenerative medicine are also promising. While these types of scaffolds may initially be harder to produce when compared with the ease of harvesting crops or operating an extruder, they offer more control in terms of texture, shape, nutrient distribution and even cell-type differentiation. As we learn more about the intercellular interactions involved in cell fate, it may be possible to design scaffolds that can promote different types of differentiation within different areas of the scaffold. With this insight, we could grow marbled, meat-on-the-bone products, all from a single cell source seeded on to an edible or degradable scaffold.



**Figure 3.** Future meat production facilities may look more like a brewery than a barn.

As we expand on our clean meat options, borrowing research from the field of whole organ synthesis may prove successful when attempting to replicate the organoleptic properties of current meat products. Here, the clean meat field holds an advantage over growing entire organs. While organ developers must create tissues that are able to perfectly function without being rejected by the body, clean meat just has to taste good and not kill anyone.

## Food for thought



**Figure 4.** The world's first clean turkey meat, created by seeding turkey muscle cells on to de-cellularized jackfruit fibres

We are constantly gaining a better understanding of the biochemistry involved in cellular metabolism and proliferation. As we make new discoveries about novel cell signalling pathways and molecular interactions, and as computational biology, machine learning and data-scraping technologies advance, so too will our knowledge of and applications for biochemistry. There are still many hurdles within clean meat science – we must determine the most efficient cell types, affordable media formulations, scalable bioreactor processes and satisfying scaffold designs. But as we continue to optimize the clean meat system, we add to the discoveries within regenerative medicine, stem cell biology, tissue engineering and biopharmaceutical production.

Advances in the field could lead to meat without trans fats, do-it-yourself meat-growing kits and meat options during space travel, all while addressing the many issues associated with current meat production. What's more, clean meat research could help find cures for muscular diseases, uncover the mysteries of aging, explore tools for organ synthesis and supply our growing population with more accessible biopharmaceuticals. More and more industrial leaders and academic institutions are getting involved with the research, and the release of clean meat to the market is so close we can taste it. Just as we are no longer draining whales for oil or whipping horses for transport, soon we won't need to kill a pig in order to bring home the bacon. ■



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*Marie is using her passion for animal welfare and interest in biological science to explore and promote the cellular behaviours needed for large-scale muscle cell production using serum-free media. Email: [marie\\_gibbons@hms.harvard.edu](mailto:marie_gibbons@hms.harvard.edu)*

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