In vitro cultured meat production

P.D. Edelman, M.Sc.[1] D.C. McFarland, Ph.D.[2] V.A. Mironov, Ph.D., M.D.[3] and J.G. Matheny, M.P.H.[4*]

1. 8-9c, Rijnsteeg, 6708 PP Wageningen, The Netherlands, 0031-317-502206 (voice), p.edelman@gmx.net

2. Department of Animal and Range Sciences, 107 Animal Science Complex, South Dakota State University, Brookings, South Dakota, USA, 605-688-5431 (voice), 605-688-6170 (fax), Douglas.McFarland@sdstate.edu

3. Department of Cell Biology and Anatomy, Medical University of South Carolina, 173 Ashley Avenue, Suite 601, Charleston, South Carolina, 29425, USA, 843-792-7630 (voice), 843-792-0664 (fax), mironovv@musc.edu

4. Department of Agricultural and Resource Economics, University of Maryland, 6843 Eastern Ave, Takoma Park, Maryland, 20912, USA, 202-486-1306 (voice), 301-891-6815 (fax), jmatheny@jhsph.edu

*Corresponding author

Abstract

In this paper we discuss the concept and applications of producing meat by culturing edible animal muscle *in vitro*. The *in vitro* production of processed meats is probably feasible with existing tissue engineering techniques and may offer health and environmental advantages over existing meat production systems. The production of highly-structured, unprocessed meats faces considerably greater technical challenges. In the short term, cultured meat production is likely to remain economically impractical except, perhaps, for long-term space travel.

Introduction

One of the more infamously inaccurate predictions of the future came from Winston Churchill, who claimed in 1932, "Fifty years hence we shall escape the absurdity of growing a whole chicken in order to eat the breast or wing by growing these parts separately under a suitable medium."[1] Churchill's prediction of a method for producing "cultured meat" *in vitro* should have been realized by 1982. We're still waiting. Nevertheless, there are virtues to his futuristic suggestion.

Although meat has enjoyed sustained popularity as a foodstuff, in recent years consumers have expressed growing concern over some consequences of meat consumption and production. These include:

- Nutrition-related diseases, such as cardiovascular disease and diabetes, associated with the over-consumption of animal fats. These diseases are now responsible for a third of global mortality.[2] Over-consumption of meat may be responsible for a quarter of all ischemic heart disease, or 1.8 million deaths, annually.[3,2]
- Foodborne pathogens found in meats, such as Salmonella, Campylobacter, pathogenic E. coli, Avian influenza, and Bovine spongiform encephalopathy (BSE). In the United States, foodborne diseases – the most common causes of which are contaminated meats -- are responsible for over 76 million episodes of illness, 325,000 hospitalizations, and 5,000 deaths each year.[4]
- In the United States, alone, the annual medical costs related to over-consumption of meat are believed to be between $30 and 60 billion.[5]

- Inefficient use of resources. Obtaining nutrients from meat, rather than directly from plants, uses considerably more cropland, water, fertilizer, pesticides, and energy. Given the inputs required to house, transport, and slaughter animals; transport and process feed grains; and transport and process meat, intensive meat production is only 25 percent as energy efficient as soybean production.[6] Obtaining our food exclusively from plants would make it possible to feed substantially more people using available resources.[7]
- Pollution. Annually, 1.4 billion tons of farm animal wastes are produced in the United States.[8] Together with animal feed production, meat production is responsible for the emissions of nitrogen and phosphorus, pesticide contamination of water, heavy metal contamination of soil, and acid rain from ammonia emissions.[9] In addition, in the United States, a quarter of all the human-induced production of the greenhouse gas, methane, comes from farm animals and their waste products.[10]
- Depletion of fishing stocks. 75 percent of existing fishing stocks are either fully- or over-exploited.[11]
- Use of farm animals. 9 billion farm animals are killed each year in the United States to produce meat.[12]

While per capita meat consumption in the United States and other developed countries has plateaued in recent years, meat consumption in developing countries continues to increase, having doubled over the last 20 years.[13] It is not clear what the consequences of this increase will be, particularly when coupled with a growing global population. Conventional meat production may be capable of feeding a population of 9 billion, but may do so at a high cost. We may increasingly be pushed to reduce our consumption of meat or to adopt alternative animal farming systems.

One unexplored alternative is to produce edible animal muscle (i.e., meat) *in vitro* using tissue engineering techniques. Such cultured meat would enjoy some advantages over conventional meat, and the techniques required to produce it are not beyond imagination. In this paper we discuss these techniques, their rationale, and the contributions to their development likely to be made by skeletal muscle tissue engineering. To tissue engineers this subject is of interest as cultured meat production is an application of tissue engineering principles whose technical challenges may be less formidable than those facing many clinical applications.

Cultured meat production

Most edible animal meat is made of skeletal muscle tissue. Thus the production of cultured meat *in vitro* must draw upon techniques developed for skeletal muscle tissue engineering. These techniques have been discussed in depth elsewhere.[14] The idea that they could be applied to produce edible meat appears to have been seriously pursued by only three groups of researchers. Their efforts can be divided roughly into scaffold-based and self-organizing techniques.

In scaffold-based techniques, embryonic myoblasts or adult skeletal muscle satellite cells are proliferated, attached to a scaffold or carrier, such as a collagen meshwork or microcarrier beads, and then perfused with a culture medium in a stationary or rotating bioreactor. By introducing a variety of environmental cues, these cells fuse into myotubes, which can then differentiate into myofibers.[14] The resulting myofibers may then be harvested, cooked, and consumed as meat (Figure 1). van Eelen, van Kooten, and Westerhof hold a Dutch patent for this general approach to producing cultured meat.[15] However, Catts and Zurr appear to have been the first to have actually produced meat using the method.[16]

A scaffold-based technique may be appropriate for producing processed meats, such as hamburger or sausage. But it is not suitable for producing highly structured meats, such as steaks. To produce these, one would need a more ambitious approach, creating structured muscle tissue as self-organizing constructs[17] or proliferating existing muscle tissue *in vitro*.

The latter technique was employed by Benjaminson, Gilchriest and Lorenz.[18] They placed skeletal muscle explants from goldfish (*Carassius auratus*) in diverse culture media for seven days and observed an increase in surface area between 5.2 and 13.8 percent. When the explants were placed in a culture containing dissociated *Carassius* skeletal muscle cells, explant surface area grew by 79 percent.

Explants have the advantage of containing all the cells that make up muscle in their corresponding proportions, thus closely mimicking an *in vivo* structure. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if separated for long periods by more than 0.5 mm from a nutrient supply.[17] Thus, without vascularization, the production of large, highly structured meats will not be possible.

Future efforts in culturing meat will have to address the limitations of current techniques through advances that make cultured cells, scaffolds, culture media, and growth factors both edible and affordable.

Cells

Skeletal muscle is a tissue consisting of several cell types. Skeletal muscle fibers are formed by the proliferation, differentiation and fusion of embryonic myoblasts and, in the postnatal animal, satellite cells, to form large multinucleated syncytia.[19] Attempts to force skeletal muscle fibers to proliferate are typically counterproductive, as most myonuclei remain postmitotic.[14] Although embryonic stem cells are characterized by high proliferation and differentiation potential, considerable effort must be applied to force them to differentiate and cell yields from harvests are low. Moreover, it is not clear whether embryonic stem cells forced to commit to a skeletal muscle lineage will have the proliferative characteristics of embryonic stem cells, or become indistinguishable from myoblasts. Thus the most practical cell source for cultured meat is probably embryonic myoblasts or postnatal/posthatch skeletal muscle cells called satellite cells.

Satellite cells with high proliferative potential have been isolated and characterized from the skeletal muscle of chickens, turkeys, pigs, lambs and cattle.[20-24] In each case media conditions have been established by these investigators to support the proliferation and differentiation of cells to form immature muscle fibers called myotubes in culture (Figure 2).

The simplest cultured meat system would likely use a single myogenic cell line from one of these animals, or a co-culture with fat cells. Following culture and harvest, cells might then be prepared for consumption as a processed meat. To replicate the taste and texture of unprocessed meats is a more ambitious goal, as vascular cells would be needed, as would fibroblasts for the production of connective tissue, and these would have to be properly organized in a three-dimensional structure. A proper growth factor milieu would be essential to direct the construction of a structured skeletal muscle tissue.

It is unclear how much cultured meat a single cell could yield. Cells in culture are believed to undergo a fixed number of doublings, called the Hayflick limit. The Hayflick limits of farm animal muscle progenitor cells have not been well-established. It has been shown that satellite cells cloned from turkey breast muscle express telomerase.[25] This finding suggests that some domestic animal satellite cells may generate enough daughter cells to produce huge quantities of cultured meat. (For instance, back-of-the-envelope calculations suggest that a single parent cell with a Hayflick limit of 75 could theoretically satisfy the current annual global demand for meat.) For other species, it may be necessary to proliferate a sufficient number of stem cells in culture before differentiation into myoblasts -- or to use cells transfected with the telomerase gene, that have higher Hayflick limits.

Fields

Mechanical, electromagnetic, gravitational, and fluid flow fields have been found to affect the proliferation and differentiation of myoblasts.[14, 26] Powell and others found that repetitive stretch and relaxation equal to 10 percent of length, 6 times per hour, increased differentiation into myotubes.[27] Yuge and Kataoka seeded myoblasts with magnetic microparticles and induced differentiation by placing them in a magnetic field, without adding special growth factors or any conditioned medium.[28] Electrical stimulation also contributes to differentiation, as well as sarcomere formation within established myotubes.[14,26]

Scaffolds

Myoblasts are attachment-dependent, meaning that a substratum or scaffold must be provided for proliferation and differentiation to occur.[29] For cultured meat, a scaffold and its by-products must be edible and may be derived from non-animal sources. A further challenge is to develop a scaffold that can mechanically stretch attached cells to stimulate differentiation. A flexible substratum is also necessary to prevent detachment of developing myotubes that will normally undergo spontaneous contraction.

Cytodex-3 microcarrier beads have been used as scaffolds in rotary bioreactors (Figure 3). However, these beads have no stretching potential. One elegant approach to mechanically stretch myoblasts would be to use edible, stimuli-sensitive porous microspheres made from cellulose, alginate, chitosan, or collagen that undergo, at minimum, a 10 percent change in surface area following small changes in temperature or pH (Figure 4). Once myoblasts attach to the spheres, they could be stretched periodically. Preliminary studies will need to be conducted to establish that such variation in the pH or temperature would not negatively affect cell proliferation, adhesion, and growth.

Developing a scaffold for large, highly structured meats presents greater technical challenges, due to the need for vascularization. It may be possible to build a branching network from an edible, elastic, and porous material, through which nutrients are perfused. Myoblasts and other cell types can then attach to this network. Approaches to creating such a network for the purpose of tissue-engineering have been proposed by creating a cast onto which a collagen solution or a biocompatible polymer is spread. After solidification, the original material is dissolved, leaving a branched network of microchannels behind, which can be stacked onto each other to form a three-dimensional network.[30] However, this approach does not lend itself to mass production.

Alternatively, one could attempt to create a highly structured meat without a scaffold. Benjaminson, Gilchriest, and Lorenz proposed solving the vascularization problem through controlled angiogenesis of explants.[18]

Culture Media and Growth Factors

To enjoy many of the potential advantages over conventional meat production, cultured meat would need to employ an affordable medium system. Such a medium must contain the necessary nutritional components and be presented in a form freely available to myoblasts and accompanying cells, as no digestive system is involved. Improvements in the composition of commercially-available cell culture media have enhanced our ability to successfully culture many types of animal cells.

McFarland and others developed a serum-free medium that supported the proliferation of turkey satellite cells in culture.[20] Kosnik, Dennis, and Vandenburgh refer to serum-free media developed by Allen et al., Dollenmeier et al., and Ham et al.[14] Benjaminson and others succeeded in using a serum-free medium made from maitake mushroom extract that achieved higher rates of growth than fetal bovine serum.[18] And it has recently been shown that lipids such as sphingosine-1-phosphate can replace serum in supporting the growth and differentiation of embryonic tissue explants (W. Scott Argraves, Medical University of South Carolina, personal communication, 22 May 2004).

In addition to supplying proper nutrition to growing muscle cells in culture, it is necessary to provide an appropriate array of growth factors. Growth factors are synthesized and released by muscle cells themselves and, in tissues, are also provided by other cell types locally (paracrine effects) and non-locally (endocrine effects). The liver is the primary source of circulating insulin-like growth factor-I. Appropriate co-culture systems may be developed such that liver cells (hepatocytes) provide growth factors necessary for cultured muscle (meat) production. Typically, investigators initiate differentiation and fusion of myoblasts by lowering the levels of mitogenic growth factors. The proliferating cells then commence synthesis of insulin-like growth factor-II, which leads to differentiation and formation of multinucleated myotubes.[31] So, the successful system must be capable of changing the growth factor composition of the media.

Bioreactors

The importance of bioreactor design to tissue engineering has been discussed elsewhere.[32,33] Cultured meat production is likely to require the development of new bioreactors that maintain low shear and uniform perfusion at large volumes. Much recent skeletal muscle tissue engineering research has employed NASA rotating bioreactors. Their chief advantages are that cells are in near-continuous suspension, fluid shear is minimal, and suspension is possible for tissue assemblies up to 1 centimeter. These bioreactors can sustain biomass concentrations up to 10^8 cells per mL. Research size rotating bioreactors (10 to 250ml) have been scaled up to three liters and, theoretically, scale up to industrial sizes should not affect the physics of the system. Industrial scales are already available for low-shear particle-based biofilm reactors, allowing biomass concentrations as high as 30 kg per m^3.[34]

Potential advantages of cultured meat

Relative to conventional meat, cultured meat could offer a number of benefits:

- Composition of the meat. Fat content can be controlled -- either by supplementation of fats after production, or by co-culturing with fat-producing adipocytes. Most meats are high in saturated fatty acids and low in poly-unsaturated fatty acids. The former have been implicated in the development of heart disease, while the latter have a beneficial effect on blood cholesterol.[35] With cultured meat, the ratio of saturated to poly-unsaturated fatty acids could be better controlled.
- Disease control. The incidence of foodborne disease could be significantly reduced. The chance of meat contamination would be lower due to strict quality control rules, such as Good Manufacturing Practice, that are impossible to introduce in modern animal farms, slaughterhouses, or meat packing plants. In addition, the risks of exposure to pesticides, arsenic, dioxins, and hormones associated with conventional meat could be significantly reduced.
- Efficiency. An animal kept for conventional meat production supports, in addition to muscle tissue, biological structures required for locomotion and reproduction. These include bones, respiratory system, digestive system, skin, and the nervous system. In cultured meat production, these structures do not have to be grown or supported. Cultured meat could also be produced in less time -- weeks instead of months to years before meat can be harvested. Likewise, cultured meat production should be more efficient than conventional meat production in its use of nutrients and energy, land, water, and human labor; and it should produce less waste. These efficiencies seem to depend most on the development of nutrient-rich, non-serum media.

- Replacement of exotic meats. The global trade of meats from rare and endangered animals has reduced wild populations of many species. In theory, cells from captive rare or endangered animals (or even cells from samples of extinct animals) could be used to produce exotic meats *in vitro*.
- Reduced animal use. Theoretically, a single farm animal may be used to produce the world's meat supply.

Consumer acceptance

It is not clear how the nutritional or aesthetic characteristics of cultured meat would compare to those of conventional meat. If scaffolds are included in the edible product, then cultured meat will have a lower protein density, since skeletal muscle is composed of only 2 percent ECM and contains more mature fibers than engineered counterparts.[36] The fat content and texture of meat contribute to their taste, and these would likewise need to be controlled to make a compelling substitute, even for processed meats.

Assuming cultured meat were aesthetically acceptable, it could appeal to the growing number of consumers concerned about food safety, the environmental effects of agriculture, and the welfare of farm animals. Cultured meat could also appeal to consumers interested in tailoring the aesthetic and nutritional characteristics of food to their individual tastes. A cultured meat production system could theoretically be sufficiently compact and automated for every household to produce its own meat -- a "meatmaker" could sit next to every "breadmaker," using ingredients purchased at a store.

At the same time, there are a number of barriers to cultured meat's acceptance by consumers. That the form or structure of cultured meat will not resemble actual muscles should pose little problem for consumers, as there is a market for boneless and skinless meat products, as well as further processed meats, such as sausage or hamburger. However, most of us have an intuitive aversion to unnatural foods. This is not a consistent aversion, since most of us consume bread, probably the first product of biotechnology, as well as cheese, butter, yogurt, and wine -- all "unnatural" foods one cannot find growing "in nature." Still, the artificiality of these products is more familiar to us at this time than cultured meat.

Probably the biggest obstacle to the adoption of cultured meat would be its initial cost. Although the nutritional and energy costs of cultured meat are theoretically lower than conventional meat, it is unclear how these would translate into economic savings.

The costs of cultured meat

The cost of conventional meat production fluctuates due to feed prices, disease and veterinary needs, weather, and the lags in production response due to changing demands. As a result, profit margins for conventional meat production are highly volatile, although producers and consumers are shielded to some extent by public subsidies.

The costs of cultured meat are unknown. Vandenburgh has estimated that to produce cultured meat using present technology would cost approximately $5 million per kilogram (Brown University, personal communication, 20 February 2004). This estimate is based on the costs of producing functional skeletal muscle tissue in laboratories on a small scale. Presumably the costs would be orders of magnitude lower if one used a suspended cell culture technique, and mass-produced the cultured meat in industrial bioreactors. Advances in the technical aspects of cultured meat production will greatly improve efficiency and lower costs significantly. Theoretically, cultured meat could afford higher resource and labor efficiencies, which could translate into lower costs, if cultured meat were produced at scale with an affordable medium.

In any case, it is unlikely that cultured meat will soon compete with conventional meat in ordinary markets. However, there are technologies now found in virtually every household -- computers, the internet, Velcro, freeze-dried foods -- that originally cost too much for mass acceptance. They found their first applications in space and defense projects. Only after reductions in cost by several orders of magnitude were they mass-produced. This could be true of cultured meat, as well.

Spaceships and bunkers

There are situations in which it is costly to re-supply people with food, and in which it is more economical to produce food *in situ*. These include scientific stations in polar regions, troop encampments in isolated theaters of war, bunkers designed for long-term survival of personnel following a nuclear or biological attack, and long-term manned space missions.

Napoleon said “an army travels on its stomach” and the same could be said of astronauts. Much of the difficulty in moving people over great distances comes from having to provide them with food. Conventional foods are not amenable to transport for long-term space missions, due to the enormous expense of transporting weight from Earth into space. The cost of launching payload to a geostationary Earth orbit, for instance, is around $50,000 per kg.[37] Thus long-term space missions, such as a settlement on the Moon or a flight to Mars, will likely involve some food production *in situ* within a settlement or spacecraft, to reduce lift-off weight and its associated costs. NASA has led research on a number of "bioregenerative" food systems in which waste products are converted into nutrients for plants or animals, which are then used as food.[38] Such bioregenerative systems both reduce the launch costs for long-term missions and extend their duration.

There are three reasons why a bioregenerative system might include cultured meat. First, the most efficient digesters of human waste products in these systems – algae and fungi -- are not ideal for human consumption but may be suitable for producing culture media. Second, a pure plant-based system will not make most efficient use of plant matter. Two-thirds of most food plant mass (leaves, stems, roots) is inedible by humans but may be used as nutrients for fungi-based culture media. Plant residues could also be cycled through live animals, such as fish or goats. But this is inefficient, both because not all plant mass can be converted into animal feed, and because not all animal mass can be converted into human food. Moreover, the rearing of live animals introduces new disease vectors and logistical problems. Third and last, apart from the nutritional adequacy of food, familiarity is also important. It is doubtful a crew accustomed to eating meat on Earth would be satisfied with a strict vegan diet. For these reasons, NASA has been the first to fund research on cultured meat.[18]

Conclusions

The prospect of cultured meat has excited the public imagination since even before Churchill. The Earl of Birkenhead shared Churchill's overly-optimistic prediction, writing that in the future, "It will no longer be necessary to go the extravagant length of rearing a bullock in order to eat its steak. From one 'parent' steak of choice tenderness it will be possible to grow as large and as juicy a steak as can be desired."[39] Later, in the 1952 science fiction story, The Space Merchants, Frederik Pohl and C.M. Kornbluth imagined the world's meat grown in one huge lump, hundreds of feet in diameter, called "Chicken Little."[40] Claude Zidi's 1976 comedy, L'aile ou la cuisse, features a scientist who competes with gourmet chefs by producing meat *in vitro*. And most recently, a number of tissue engineers placed their bets on the prospects for cultured meat.[41]

While its proliferation in the imagination is little evidence of its proliferative potential *in vitro*, cultured meat, at least of the scaffold-based variety, appears technically feasible. However, significant challenges remain before it could be produced economically. Most of these challenges are common to skeletal muscle tissue engineering, generally. The environmental cues needed to promote myofiber development are not well-understood. And it is not clear which, among them, are essential to cultured meat production. Still, it is safe to assume that the level of functionality needed for most clinical applications of muscle tissue engineering exceeds that needed to produce cultured meat with nutritional and aesthetic properties sufficiently similar to those of conventional meat. Thus, cultured meat should present fewer technical challenges than functional engineered muscle. Future research is likely to be most fruitful if focused on developing scaffold-based techniques appropriate for processed meat products, and affordable, non-serum media needed to support them.

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Figure captions

1. Scaffold-based cultured meat production: 1. Myoblasts in petri dish; 2. Porous collagen microspheres; 3. Myoblasts form myotubes on collagen microspheres; 4. Bioreactor; 5. Microwave; 6. Hamburger

2. Satellite cell-derived turkey myotubes stained with Giemsa. Cells were grown until cultures were near confluence. Cultures were then administered low serum containing medium to initiate differentiation and fusion.

3. Turkey satellite cells grown on Cytodex 3 beads in a Synthecon Rotary Cell Culture System. Note the clumping of beads that occurs as proliferation progresses.

4. Porous collagen microsphere

Figure 1

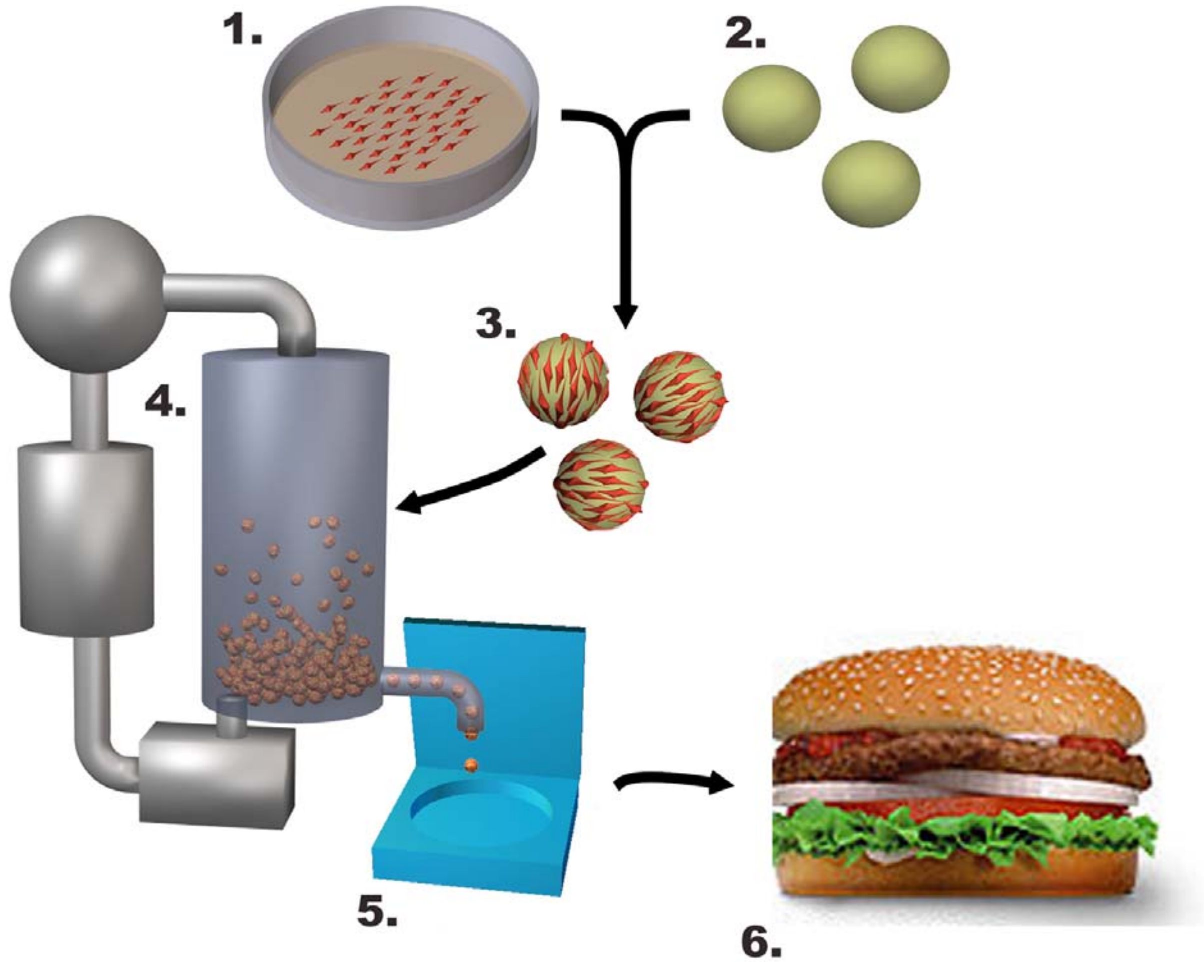

Figure 2

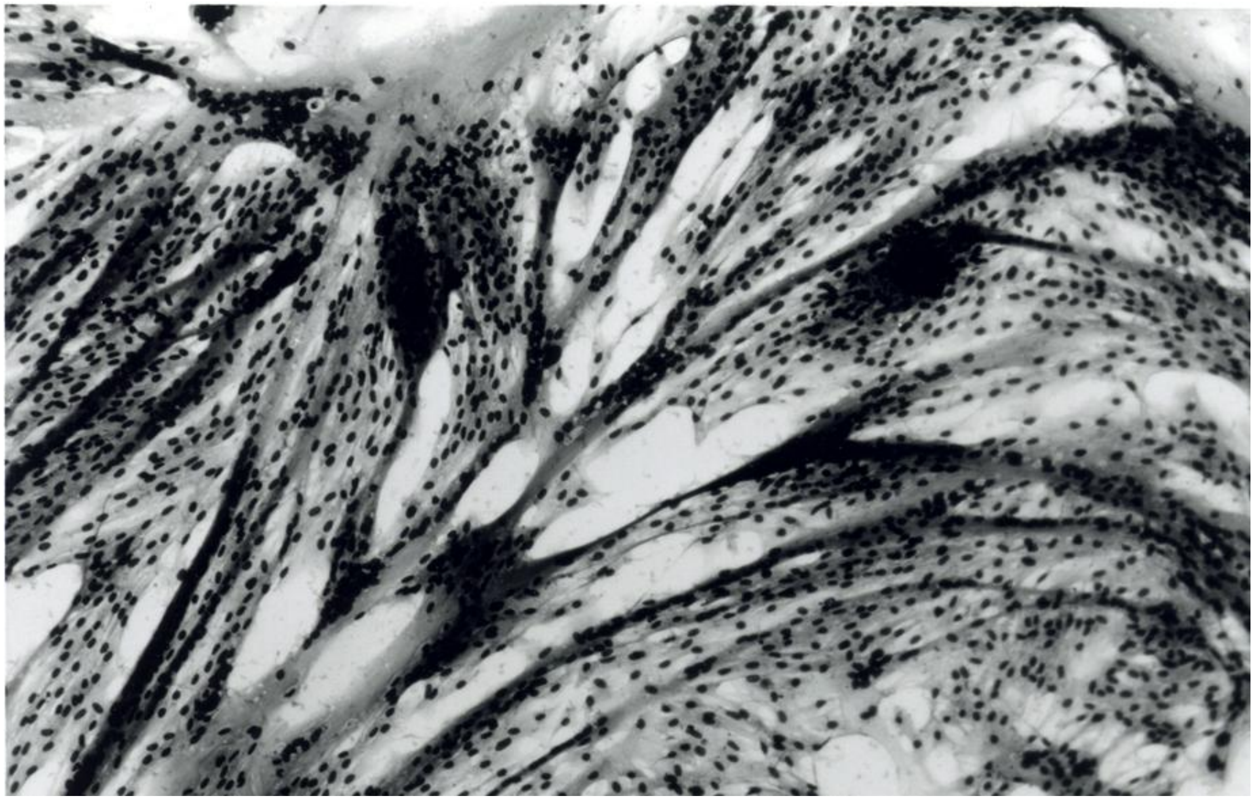

Figure 3

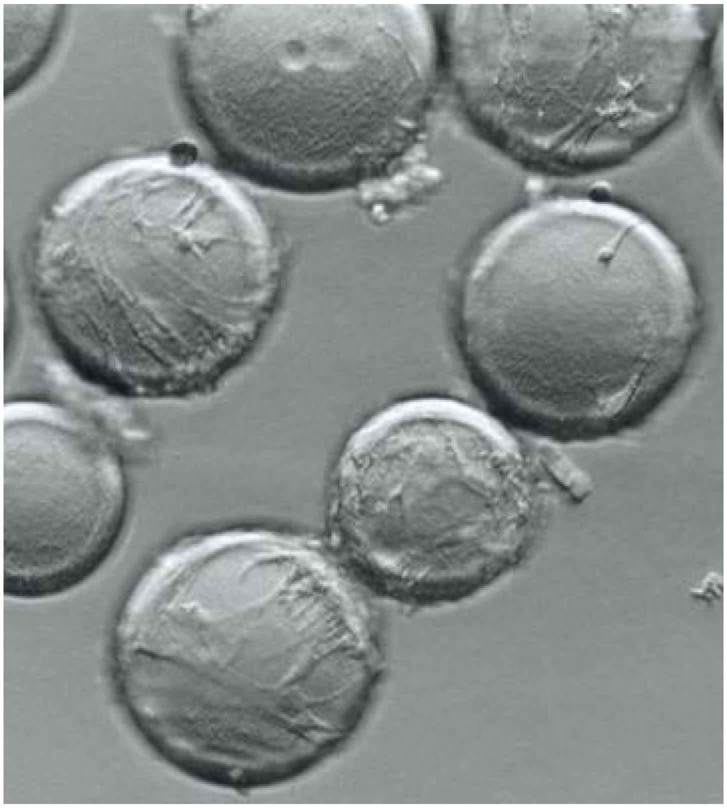

Figure 4