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Tissue Engineering Approaches to Develop Cultured Meat from Cells: a mini review

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Running title: Cultured meat from stem cells

Abstract

Cultured meat production is an innovative and emerging process to produce animal meat in laboratories, using tissue-engineering techniques. This novel approach to produce meat involves *in vitro* culture of the animal muscle tissues rather than rearing whole animals to obtain animal flesh for consumption. Conventional meat production results in several adverse consequences such as poor nutritional value of meat, food-borne diseases, depletion of environmental resources, pollution etc., associated with animal slaughter. Cultured meat, on the other hand, is essentially an animal-free harvest produced in controlled conditions. Cultured meat can provide healthier, safer, and disease-free meat to consumers, as well as mitigate the negative environmental effects associated with traditional meat production. Academically, this new method is considered adequately efficient to supply meat and meat products to consumers. However, *in vitro* cultured meat production is still in the early stages of development and requires in-depth research and advanced technical skills for optimized production and commercialization. This review focuses on the history and development of cultured meat production, with insights on the advantages, consequences, and potential of animal-free meat harvest.

Key words: Cultured meat, Stem cells, Bioreactors, Tissue engineering, Pollution, Disease-free meat

Introduction

Meat consumption is an essential part of the human diet. Meat for consumption is traditionally obtained from animals that are reared and slaughtered on farms. However, limited land resources and the negative perception to animal slaughter are encouraging scientists to develop innovate techniques to produce meat without rearing animals. *In vitro* meat production is the process by which muscle tissues from animals are grown in laboratories, using tissue-engineering techniques, to manufacture meat and meat products. The use of laboratory-grown animal tissue to produce meat eliminates the necessity of sacrificing the animal. Cultured meat can offer several advantages—most notably, health and environmental advantages—over traditional meat production, and benefit animal welfare and the livestock sector, essential to an agrarian economy (Haagsman *et al*., 2009). The use of livestock for food production is important to man's existence on earth, and contributes economically to the country's agricultural sector. The global population is projected to reach 9 billion people in 2050. A fast growing population will increase the annual carbon dioxide (released from greenhouse gas emissions) from 11.2 gigatons to 19.7 gigatons. Meanwhile, owing to rising income and urbanization, annual global meat production is expected to increase from 228 million tons to 465 million tons (FAO, 2006). The adverse effects of traditional meat production on the environment—such as depletion of fresh water resources, soil erosion, biodiversity loss, and destruction to habitats—have also compelled scientists to focus on cultured-meat research and development for commercialization (Asner *et al*., 2004; Savadogo *et al*., 2008).

Laboratory-grown meat must possess physical characteristics (such as appearance, texture, and flavor) similar to livestock meat, and should be affordable to consumers. To overcome these challenges, different meat culture techniques are being developed and tested for *in vitro* production of skeletal muscles, fat, fibrous tissue, bone, and cartilage, in laboratories. The technology to produce cultured meat from stem cells was explored many years ago; however, it has not yet been commercially developed (Senan *et al*., 2015). Scientists have achieved some success with these techniques in the initial phases to develop meat-based products, without requiring whole animals. Cultured meat produced from bovine stem cells was successfully used to make the world's first burger with biosynthesized meat (Post, 2012). The source material for the production of cultured meat can be taken from live animal biopsies or animal embryos, which can be inoculated in suitable media for proliferation, and grown separately from the animal. For high-quality cultured meat, the composition and source of ingredients used to produce the meat are considered important. Protein synthesis in cultured muscle cells could be enhanced by different combinations of ingredients in various conditions to improve the nutritional quality of cultured meat. A schematic representation of cultured meat production is depicted in Figure 1.

Cultured meat production can be a convenient method to develop ground-meat processed products such as sausages, burgers, nuggets, etc. (Datar and Betti, 2010; Hocquette, 2016). However, *in vitro* meat production at a commercial level still requires significant in-depth research. In the near future, cultured meat will be an essential part of human diet; nonetheless, in the short term, the extremely high cost of biosynthesized meat is the main hurdle to its feasible commercialization (Bhat and Bhat, 2011a).

The importance of cultured meat

The first efforts at culturing meat were intended to produce cultured muscle proteins for space flights and inhabitants of space stations. NASA cultured muscle tissue (obtained from the common goldfish, *Carassius auratus*), ranging 3-10 cm in length, in Petri dishes (Benjaminson *et al*., 2002). Muscle tissues cultured in crude cell extracts increased in cell mass. These cells were subsequently washed, dipped in olive oil and spices, covered with breadcrumbs and fried, and tasted by a test panel, which concluded that the product was palatable (Churchill, 1932). Different processed meat products have been prepared from *in vitro* meat cultured from muscle tissue, as opposed to the traditional method of slaughtering animals (Datar and Betti, 2010; Benjaminson *et al*., 2002; Edelmam *et al*., 2005). Consequently, different approaches for *in vitro* meat production, with competing potential, arose. One of the important *in vitro* meat production techniques involved obtaining and growing muscle tissues in a suitable medium and harvesting them (Benjaminson *et al*., 2002).

As *in vitro* meat technology advanced, tissue engineers were involved in the process to select and place adult cells on a scaffold, grow them in bioreactors, and use the cultured cells for muscle tissue production (Bhat et al., 2017; Catts and Zurr, 2002). Other initiatives aimed to use stem cell propagation by placing them on top of each other (Kelland, 2012), and using inkjet technique to spray cell material onto sheets or other structures (Bhat and Bhat, 2011b).

In 2013, scientists made the world's first *in vitro* meat burger that changed the philosophy in the domain of *in vitro* meat development. The burger contained five ounces of cultured meat (beef) patty, cooked and tasted by a panel of sensory judges in London, which concluded that it tasted similar to a conventional burger. It took three months to grow the beef using stem cells from a cow's shoulder. The monetary investment for this burger was more than $330,000. The event motivated consumers, particularly those with animal welfare concerns, to encourage the commercial introduction of such cultured meat products (Zaraska, 2013).

Although *in vitro* meat production appears to be a novel and contemporary concept, the idea of cultured meat for human consumption was conceived long back by Frederick Edwin Smith, a writer, who predicted, "It will no longer be necessary to go to 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" (Ford, 2009). Similar thoughts on cultured meat were also discussed by Winston Churchill in his essay 'Fifty Years Hence' (later published in the book 'Thoughts and Adventures' in 1932), and Rene Barjavel, a French science fiction author, in his novel 'Ravage' in 1943 (later translated as 'Ashes, Ashes' in 1967). In 1912, Alexis Carrel successfully kept a piece of embryonic chick heart muscle alive and beating in a Petri dish. A summary of the content and conclusions of recent reviews about the production of cultured meat from stem cells is shown in Table 1.

Biosynthesis of cultured meat from cells

Prokaryotic cell

The first oil crisis also ushered in the era of single cell protein (SCP) research in which unicellular organisms were studied for human consumption. Extensive research was carried out to investigate the use of SCPs for human food as well as animal feed (Haagsman *et al*., 2009). The term SCP was coined to represent microbial biomass products produced by fermentation. These proteins consisted of processed microorganisms (such as yeasts or bacteria) grown in cultures and used as food source, especially for livestock (Nasseri *et al*., 2011). A variety of microorganisms and substrates are used to produce single-cell proteins (Haung and Kinsella, 1986). However, high nucleic acid content and low cell wall digestibility are the two limiting nutritional and toxicological factors in SCPs (Alvarez and Enriquez, 1988).

Stem cells

In the last two decades, stem cell selection, identification, and modification have been significantly promoted owing to their potential use in various fields of study. Stem cells have characteristic abilities to retain themselves in the undifferentiated form for a specific number of population doublings (Roelen and Lopes, 2008). Different types of stem cells are required to develop *in vitro* meat and meat-based products. Among these, myoblast or satellite cells are the most important (Mauro, 1961). In living animals, adult stem cells are generally responsible for muscle regeneration. Satellite cells easily differentiate into myotubes and mature myofibrils when cultured cells reach maximum numbers, and therefore, it is a preferred cell source for skeletal muscle tissue engineering. Satellite cell subsets also have better regeneration capacity (Collins *et al*., 2005).

Adult stem cells

Some cells in living organisms have the ability of self-renewal, which is required for repair and regeneration of damaged or diseased tissues. Stem cells also possesses self-renewal characteristics, owing to which they can be transformed and differentiated to different cell types. Pluripotent adult stem cells, like embryonic stem cells, can be used for the production of *in vitro* culture (Slack, 2008). Adult stem cells or progenitor cells are preferred sources for cultured meat generation, independent of their original *in vivo* source. Adult cells are obtained from animal species such as pig (Kues *et al*., 2005; Zeng *et al*., 2006) and cattle (Kook *et al*., 2006; Colleoni *et al*., 2005). However, these cells may have limited differentiation capacity.

Adipose tissue-derived adult stem cells

Adipose tissue-derived adult stem cells are unique multipotent cells, which can be used for cultured meat production (Gimble *et al*., 2007). They are derived from subcutaneous fat in the adipose tissues, and get transdifferentiated to myogenic, osteogenic, chondrogenic, or adipogenic cell lineages (Kim *et al*., 2006). Adipose tissue-derived adult stem cells have been observed to immortalize at high frequency and undergo rapid transformation in long-term culturing (Rubio *et al*., 2005). Mature adipocytes could dedifferentiate *in vitro* into a multipotent pre-adipocyte cell line known as dedifferentiated fat (DFAT) cells, which have the ability to transdifferentiate into skeletal myocytes (Kazama *et al*., 2008). The characteristics of different types of mammalian stem cells are summarized in Table 2.

Culture medium

Cyanobacteria can be used as a potential food source for cell growth in meat culture. Cyanobacteria are fast-growing photosynthetic bacteria with protein content of up to 70% dry weight, and can be easily cultured for biomass in a culture medium (Ford, 2011). Mammalian cell cultures also require complex medium as compared to prokaryotic cells that require simple conditions for growth. The availability of vitamins, lipids, and amino acids are essential factors required for replication and maintenance of cells. Additionally, mammalian cells mostly prefer a solid surface for attachment to consume the food materials (Haagsman *et al.,* 2009).

The provision of essential growth factors is important for proper nutrition, growth, and development of cells in culture. Some cells have developed systems to release and synthesize these growth factors. For instance, liver cells can provide growth factors for themselves in the medium (Edelman *et al.,* 2005). Serum and plasma beneficial for mammalian cell proliferation can be provided in liquid media. Fetal calf serum is generally added at 5-20% final concentration in the medium. Serum-free media can delay culture development; hence, serum provision is mandatory to obtain good results (Jochems *et al*., 2002).

Bioengineering or bioreactors for tissue culture

Bioreactors are generally used for growing prokaryotic and eukaryotic cells (yeast, bacteria, or animal cells) under controlled conditions. They are used to produce pharmaceuticals, vaccines, or antibodies at industrial scales. Bioreactors provide a controlled environment for cells by maintaining the temperature, pH, and oxygen level in the culture chamber (Carrier *et al*., 2002). Mammalian cell cultures are cultivated in synthetic medium under controlled conditions, with an oxygen gradient to ensure proper availability of oxygen (Radisic *et al*., 2008). The provision of oxygen in the bioreactors increases the mass transport rate between culture medium and cells, and it is regulated in bioreactors to maintain high oxygen concentration (Bhat and Bhat, 2011b).

Customized bioreactors that can maintain low shear force and provide uniform perfusion for large volumes have been designed for cultured meat production. NASA has developed rotating bioreactors for the production of skeletal muscle tissue (Van der Weele and Tramper, 2014).

Myoblasts are substrate-dependent cells required for proliferation and differentiation of cultured meat. Atrophy can result in diminished muscle tissue size due to muscle wastage caused by denervation or uncontrolled conditions (Ohira *et al*., 2002; Charge *et al*., 2002) during development phases and can pose a major problem to cultured meat production (Fox, 1966). Atrophy can be prevented by the imitation of myofibrils differentiation and proper contraction of skeletal muscles. Differentiation and proliferation in *in vitro* culture systems is induced by mechanical, electromagnetic, gravitational, and fluid-flow methods (De Deyne, 2000; Kosnik *et al*., 2003). The repetitive contraction and relaxation can increase the length of skeletal muscles by 10% (Powell *et al*., 2002). Therefore, in the absence of growth factors and medium, myoblasts are seeded with magnetic particles to induce differentiation of the cells (Yuge and Kataoka, 2000).

Electrical stimulation

Application of electrical stimuli during *in vitro* meat culture is important for the development of mature muscle fibers (Bach *et al*., 2004; Wilson and Harris, 1993). The contraction activity enhances myotube differentiation to different isoforms of the myosin chain for sarcomere development (Fujita *et al*., 2007; Naumann and Pette, 1994). Electrical stimulation is useful in accurately checking the engineered muscle function (Dennis *et al*., 2009). Due to the active contraction of muscles, muscle constructs exert a force by generating an electric field in bioreactors; however, the force generated by muscle constructs is only 2-8% of the force generated by mature rodent's skeletal muscles (Dennis *et al.,* 2001).

Mechanical stimulation

Mechanical transduction is a complex mechanism that can provide mechanical stimuli to cells (Hinz, 2006; Burkholder, 2007) with the help of integrin receptors that attach to the extracellular matrix protein of cells to develop an insoluble meshwork (Juliano and Haskill, 1993). These events induce differentiation and proliferation in the cells (50). Furthermore, quiescent cells are activated by cyclic strain (Tatsumi *et al*., 2001), thereby increasing myoblast proliferation (Kook *et al*., 2008). The mechanical stimulation can effect cell differentiation and proliferation in muscles and the applied stretch, timing and frequency of stimulus are important factors that affect the application of mechanical stimuli.

Advantages of cultured meat

Cultured meat offers several benefits over traditional meat. Among these, the foremost benefit is that it can greatly reduce the suffering of animals, as it does not involve animal slaughter to fulfill the requirements of meat eaters, while satisfying all their nutritional and hedonistic needs (Holmes and Decay, 2008).

Cultured meat production systems also provide control over meat composition and quality by modifying flavor, fatty acid composition, fat content, and especially, the ratio of saturated to unsaturated fatty acids (Bhat and Bhat, 2011c). Additionally, several health boosting and functional ingredients can be added to the meat during its formulation, by manipulating its ingredients (Van Eelen, 2007). Cultured meat techniques can also potentially develop new exotic meat varieties using near-extinct or endangered species. Furthermore, it may be used to develop novel meat variants for vegetarians.

In vitro meat production systems are energy- as well as time-efficient, as they conserve energy that would have been wasted during metabolism and development of extra organs, to be utilized for the development of skeletal muscles alone. Additionally, by using *in vitro* meat production techniques, rearing animals like chicken and cows for months and years will be unnecessary, as tissue engineering will ensure quicker meat production (Madrigal, 2008). The first '*In vitro* Meat Symposium' in 2008, held in Norway, indicated that the first commercial *in vitro* meat products would be commercially available in the next 5 to 10 years at prices competitive with European beef (~$5,200-$5,500 per ton or 3,300 to 3,500; Alexander, 2011).

Cultured meat production will also reduce dependency on natural resources and land resources, which will provide the opportunity to use that land for other recreational or beneficial purposes (Datar and Betti, 2010). Cultured meat production is a relatively humane way of producing meat with comparatively low adverse effects on environment; thus, it will be encouraged by the scientific, environmental, and animal rights communities (Schneider, 2013). Cultured meat production can also decrease the incidence of diseases spread mostly by animals. As cultured meat can be produced locally, it reduces the transportation costs incurred to deliver the product to the consumer. This, in turn, reduces carbon dioxide emissions and volume of methane released by rumen of cows into the atmosphere. As a greenhouse gas, methane far less abundant in the atmosphere and 20 times more efficient than carbon dioxide. Cultured meat would also reduce the level of waste nitrate released by cattle farms. With a fast-growing global population, cultured meat would offer safe, nutritious, and affordable meat for future populations. It would reduce food shortages, decrease food-borne diseases, reduce pollution, and increase food production (Ford, 2011). Cultured meat also has some moral advantages, which are summarized in Table 3.

Challenges and future directions

The technology for cultured meat is still in its infancy. Cultured meat may have to overcome challenges such as generation of appropriate stem cells, availability of pure and healthy media for stem cell culturing, suitable differentiation media for production of muscle cells, technical challenges in tissue engineering of muscle fiber, developing industrial-scale bioreactors, and consumer acceptability (Haagsman *et al.,* 2009). According to predictions, commercial production of cultured meat will replace the conventional livestock meat in future, and slow-grown red meat will vanish from the market. It is also believed that as cultured meat only include muscle, it will not contain artery clogging saturated fat, hormones, life-threatening microorganisms (such as *Salmonella*), dioxins, antibiotics, etc. which are found in conventional meat. Besides, polyunsaturated fatty acids and functional ingredients beneficial to health could be added during production (Hyena, 2009).

Conclusion

Conventional meat production systems are associated with several problems such as animal welfare issues, sources of infectious diseases, nutritional disparities, limited resource availability, biodiversity loss due to destruction of habitats, and environmental degradation due to pollution and global warming. In this scenario, alternatives to conventional meat systems should be investigated. *In vitro* meat holds great potential to replace slaughtered animal meat and meat-based products. Moreover, with the escalating demand for meat, bridging the demand-supply gap with conventional meat production is difficult. Cultured meat production should be promoted to provide eco-friendly and disease-free meat to consumers. However, it is also important to conduct in-depth research and develop strong technical knowledge to further refine the technology and make it economically feasible and commercially viable to develop healthy and safe meat for consumers.

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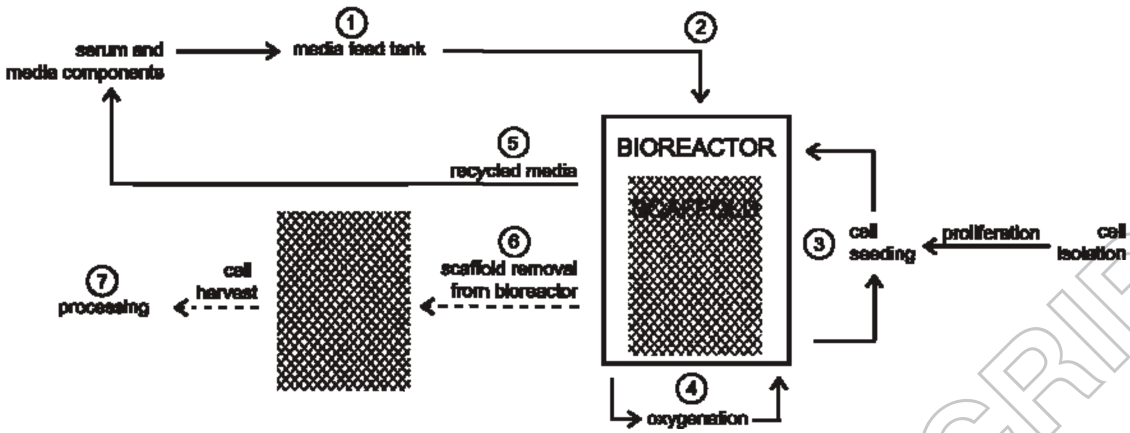

Figure 1: A potential scheme of production of cultured meat: (Derived from Datar and Betti, 2010)

Table 1 A summary of the content and conclusions of recent reviews about the production of cultured meat from stem cells[1]

reference	Title of article	Topics covered & conclusions
(Langelaan et al., 2010)	"Meet the new meat: Tissue engineered skeletal muscle"	• A review for the manufacturing of cultured meat economically which is feasible from engineering point of view • Main hurdles to find the better source of stem cell, and developed the commercially feasible methods of 3-dimensional structure inside the bio-reactor
(Stephens, 2010)	"*In vitro* meat: zombies on the menu?"	• Possibility the production of *in vitro* meat with reference to emerging regulative, moral and social matters • The author did not use the terminology conducive to others, in vitro meat is "zombie meat".
(Bhat and Bhat , 2011c)	"Prospectus of cultured meat- Advancing meat alternatives"	• The authors noted that the manufacturing of meat-products is good nutritional value, disease free as well as its chemically-safe and they said that it will be easily to achieve as compare to the raw-meat by-products production along with all organoleptic and physical properties • List the strong points of products of cultured-meat. • For the future, six basic demands along with comments on the list have concluded that great potential *in-vitro* meat
(Tuomisto and Mattos, 2011)	"Environmental impacts of cultured meat production"	• Modeling method used by authors and they assume to differentiate the production of cultured meat with different conventional methods such as chicken, beef, sheep and pork related to the land usage, outflow of greenhouse gas, energy usage and H_2O used/kilogram of eatable meat. • Result proved that all of these factors was superior for the production of cultured meat except that the production of chicken was better with energy use factor. • They deduce that in spite of uncertainty "cultured meat production, environmental impact as a whole is much lower than the production of meat conventionally"
(Dodson et al., 2012)	"Cell supermarket: Adipose tissue as a source of stem cells"	• Cultured meat is not directly concerned in this review, but the success of cultured meat would be desirable to involve adipocytes in order to assure good palatability • It can be induced from the description of the type of cultured cells derived from adipose tissue
(Post, 2012)	"Cultured meat from stem cells"	• A need of this review to overcome the problems of cultured meat • Three main motives were identified for the production of commercial cultured meat such as: 1) to meet the projected demand for meat increases; 2) concern about the environmental impact of production of meat from cattle; and 3) concern about ethics • Emphasizes the product needs to mimic the meat which produced conventionally as close as possible • Noted that the manufacturing of cultured meat product, but remaining challenges comprise: 1) Satellite cells harvested fine-tune, (2) to improve the efficiency of culture media (biological and economical) and its efficacy, (3) the development of "tissue engineering" features, and (4) Ensure the product is accepted by consumer
(Welin et al., 2012)	"*In vitro* meat: What are the moral issues?"	• A review of cultured meat manufacturing with respect to ethical issues involved • Their conclusion is that, going in the direction of *in-vitro* meat acceptance but: "It will need a bit 'of time to obtain there, it will take people a long time to adapt" • They noted that the culture meat development is an important aspect of medical concern in "tissue engineering"

(Young et al., 2013)	"Novel aspects of health-promoting compounds in meat"	• As shown in the title, it focuses on health supporting "functional" or "biologically-active" composite present in meat, also briefly studied the cultured meat • Authors believe that 4 primary challenges, *in vitro* or cultured meat manufacturing such as: 1) identification of the better seed cells sources and an appropriate growth medium cost-efficient; 2) Suitable framework for the development of cell growth and differentiation; 3) The program scaling-up to industrial levels; 4) To ensure that the consumer acceptance, nutritional value, and health-promoting attributes, at least equal to conventional meat
(Goodwin and Shoulders, 2013)	"The future of meat: A qualitative analysis of cultured meat media coverage"	• Discussion and summary, in many countries the media have been running stories about cultured meat, its potential and problems • It is observed that the probable time earlier the products of cultured meat on the market-place is not as much of processed-meat and ground meat compared to produces alike to existing meat products intact as roasts and steaks
(Post, 2014)	"Cultured beef: Medical technology to produce food"	• After greatly-advertised on television this review was written, sampling the 85-grams of meat-pie which is made from muscles fibers of *in vitro* grown cultured bovine from satellite cells • In this demonstration, stages of proof-of-concept overview • Survey the attitudes of consumer in this way usually positive • It's deduced that there are still many challenges, but the meat demands increase in the coming decades according to the research point of view
(Van der Weele and Tramper, 2014)	"Cultured meat: Every village its own factory"	• The authors suggested that in the future, culture meat production is probable to be technologically achievable, and that cultured meat production has some certain benefits as compared to the production system of conventional meat • Summarize some procedures and stages • They suggested that the cultured meat production at small-scale may prove effective when it will consume • It is concluded that, economically, competition with 'normal' meat will be a challenge unless the price of conventional meat increases greatly

[1]Derived from (Kadim et al., 2015)

Table 2 Characteristics of several types of mammalian stem cells[2]

Type of stem cell	Tissues and stem cell can differentiate into	Persistency in terms of number of replications *in vitro*
Embryonic stem	Most of the animal body tissue cells	the long term persistence and can be unlimited (Zeng and Rao, 2007)
Totipotent stem	All body cells and the development of the fetus. Good example is newly fertilized egg cells	Theoretically high
Pluripotent stem	Body most tissues are more limited than the cells of totipotent stem. Example: embryonic stem cell	Variable
Induced pluripotent stem cell	Body most tissues, completely differentiated cells can become pluripotent by proper treatment (Holden and Vogel, 2008)	Variable and unknown in many cases
Adult stem cells	Classification of all tissues, with the more be specific for the tissue which originate from	50–60 divisions are limited for mostly stem cells of adult. This replication is called (Hayflick-limit) (Roobtouck et al., 2008)
Multipotent stem cells	Various-tissues dependent on the tissue's-origin. e.g., from the mesoderm of mesenchymal stem cells can be differentiated into chondrocytes, myocytes, adipocytes, fibroblasts, or osteoblasts "muscle-cells"	Variable, depending on animal age
Myo-satellite stem cell	Muscle tissues, example: committed or uni-potent stem cells it can only support one type of tissue	Decreases with age (Roobtouck et al., 2008), and *in-vitro* for adult it possibly less than 20-divisions (Mouly et al., 2005)

[2]Derived from (Kadim et al., 2015)

Table 3. Moral profiles for cultured meat[3]

	Vegetarian	**Sustainable**	**Pig in the yard**
Meat is…	Unfit for animal; so “bad”	Preferably, but not sustainable	There is contradictory
Goal	Vegetarian (or vegan) in the world.	Viable world	less/better “alienating world”
Human animal relation	Animals not suffering or no use at all	Not related *per se*	The popularity of individual animals and personal relationship to humanity
How cultured meat helps	No animals suffering, so no animal	Larger sustainability gains	New combinations and directions
Mode of reasoning	Ethics	Data quantified	Relational/ Tinkering

[3]Derived from (Van der Weele and Driessen, 2013)

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Public Interest Statement

Cultured meat production can be a convenient method to develop ground-meat processed products such as sausages, burgers, nuggets, etc. However, in vitro meat production at a commercial level still requires significant in-depth research. In the near future, cultured meat will be an essential part of human diet; nonetheless, in the short term, the extremely high cost of biosynthesized meat is the main hurdle to its feasible commercialization