J Food Sci Technol (Mar–Apr 2011) 48(2):125–140
DOI 10.1007/s13197-010-0198-7

REVIEW ARTICLE

Prospectus of cultured meat—advancing meat alternatives

Zuhaib Fayaz Bhat · Hina Fayaz

Revised: 8 October 2010 / Accepted: 7 December 2010 / Published online: 30 December 2010

Abstract The in vitro production of meat is probably feasible with existing tissue engineering techniques and may offer health and environmental advantages by reducing environmental pollution and land use associated with current meat production systems. By culturing loose myosatellite cells on a substrate, it is probably possible to produce cultured meat by harvesting mature muscle cells after differentiation and processing them into various meat products. Besides reducing the animal suffering significantly, it will also ensure sustainable production of designer, chemically safe and disease free meat with favourable nutritional profile as the conditions in an in vitro meat production system are controlled and manipulatable. However, the production of highly-structured, unprocessed meat faces considerably greater technical challenges and a great deal of research is still needed to establish a sustainable in vitro meat culturing system on an industrial scale. This review discusses the requirements that need to be met to increase the feasibility of meat production in vitro, which include finding an appropriate stem cell source and being able to grow them in a three dimensional environment inside a bioreactor, providing essential cues for proliferation and differentiation.

Keywords Cultured meat · Meat substitute · Need · Challenges

Z. F. Bhat (✉)
Division of Livestock Products Technology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu,
R. S. Pura,
Jammu, Jammu and Kashmir, India 181 102
e-mail: zuhaibbhat@yahoo.co.in

H. Fayaz
Division of Biotechnology, University of Kashmir,
Hazratbal,
Srinagar, Jammu and Kashmir, India 190006
e-mail: bhat.hina@rediffmail.com

Introduction

The livestock sector is the fastest growing agricultural sub-sector globally, employing 1.3 billion people (Steinfeld et al. 2006a) and supporting about 4 billion people worldwide (Thornton et al. 2002). Besides the draught power, livestock provides us foods of animal origin including meat required to maintain the health of a human body (Nestle 1999). Humans are taxonomically omnivorous and meat provides several essential nutrients unavailable in plant sources. Meat is specifically valuable as a source of omega-3 fatty acids, vitamin B12, protein and highly bioavailable iron (Bender 1992; Verma and Banerjee 2010). The consumption of meat and other animal products can alleviate nutritional deficiency which is still widespread in developing countries and can secure a better physical and mental development of children (Delgado 2003; Speedy 2003). Population growth, urbanization, economic growth and flourishing markets all lead to the increasing demand for meat and animal products (Delgado 2003; Costales et al. 2006; Steinfeld et al. 2006a, b). Also, changing nutritional needs driven by growing incomes and demographic transitions, there is an increased need for livestock products including meat on a global scale (Rosegrant et al. 1999; Speedy 2003; Steinfeld et al. 2006a, b). Apart from the nutritional status, meat and other animal products play an important social role in the modern society. Until 2020,

meat demand is expected to increase highly in developing countries and slightly in developed counties (Rosegrant et al. 1999; Delgado 2003). To meet these increased meat demands of modern society, animals are intensively kept and production is optimized disregarding the well-being of the animals. Throughout history, domestic animals were able to adapt to the changing conditions, however since World War II the pace of change is increased to such a dramatic extent that this is no longer fully possible (Crok 2003). Because of the high number of animals being used an efficient and cheap production system is required. Herding of animals in confined spaces in unfavorable conditions is practiced. The adaptability of the animals is not high enough to cope with these unnatural conditions, and high stress levels are observed, resulting in disease, abnormal behaviour and death (Crok 2003).

Globally, 30% of the land surface is used for livestock production with 33% of arable land being used for growing livestock feed crops and 26% being used for grazing (Steinfeld et al. 2006a). About 70% of the fresh water use and 20% of the energy consumption of mankind is directly or indirectly used for food production, of which a considerable proportion is used for the production of meat. Livestock species, particularly ruminants, are responsible for greenhouse gas emissions, including methane from alimentary tract fermentation and nitrous oxide that may be emitted from decomposing manure and fertilizer. The livestock sector contributes 18% of the anthropogenic greenhouse gas emissions and 37% of the anthropogenic methane emissions to the atmosphere worldwide (Steinfeld et al. 2006a). It is anticipated that by the year 2050 global population will increase from 6 billion (in 2000) to 9 billion people which will be accompanied by a rise in annual greenhouse gas emissions from 11.2 to 19.7 gigatonne of carbondioxide, carbon equivalent and in the same period annual global meat production will rise from 228 (in 2000) to 465 million tonnes (Steinfeld et al. 2006).

The water use for livestock and accompanying feed crop production also has a dramatic effect on the environment such as a decrease in the fresh water supply, erosion and subsequent habitat and biodiversity loss (Asner et al. 2004; Savadogo et al. 2007). Land use, including that for the livestock sector, has increased dramatically in the past decades, leading to loss and fragmentation of habitats. 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 (de Haan et al. 1997). In addition, there is the problem of antibiotics being used as growth promoters for animals kept in intensive farming. This use probably contributes to the emergence of multi-drug-resistant strains of pathogenic bacteria (Sanders 1999). Another problem is that of animal disease epidemics and more serious threat is posed by the chicken flu, as this can lead to possible new influenza epidemics or even pandemics, which can kill millions of people (Webster 2002). Nutrition related diseases, such as cardiovascular disease and diabetes, associated with the over-consumption of animal fats are now responsible for a third of global mortality (WHO 2001). Food-borne illnesses have become increasingly problematic, with a six fold increase in gastro-enteritis and food poisoning in industrialized countries in the last 20 years (Nicholson et al. 2000) and the most common causes of food borne diseases in EU, USA and Canada are contaminated meats and animal products (Barnard et al. 1995; Mead et al. 1999; Nataro and Kaper 1998; European Food Safety Authority 2006; Fisher and Meakens 2006). Thus, it is clear that conventional meat production has a great impact on land, water, animal welfare and energy use, as well as on the emission of greenhouse gases and pollutants (Avery 1997; Solomon and Johnston 1997; van Eelen et al. 1999; Reay 2002). Continuation of production as usual will lead to further environmental degradation and destruction of habitats. However, solutions are within reach, many of which are from the scientific sector, however, although these will not be immediate but will need investment in the form of time and money, and possibly changes in consumer's habits. Scientific innovations can and should come from all sectors involved and an important contribution can be made via the generation of meat alternatives using improvements of already existing concepts and products. An example of such a concept is to make edible products from in vitro muscle cells, cultured from stem cells, outside the animal in a bioreactor (Figs. 1 and 2). Although, in vitro meat technology is still at a very early stage, this holds great promise as a solution to reduce livestock's impact on the environment.

In vitro culturing means to grow cell types of either animal or plant origin without the organism from which it is derived. Culturing involves the extraction of cells from the organism and transferring them onto or into a suitable growth medium. This medium contains nutrients, energy sources, growth factors, etc. depending on the goal of the cell culture. Cell (or tissue) culturing may be performed for the production of edible animal muscle, better known as meat, that requires the proliferation of a small amount of muscle cells to a large muscle cell mass or tissue. The idea of cultured meat for human consumption is not new but was predicted long back by Winston Churchill in the 1920s. In essay 'Fifty Years Hence' later published in 'Thoughts and adventures' in 1932, he declared that "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. In 1912, Alexis Carrel managed to keep a piece of chick heart muscle alive and beating in a Petri dish. This experiment demonstrated that it was possible to keep muscle tissue alive outside the

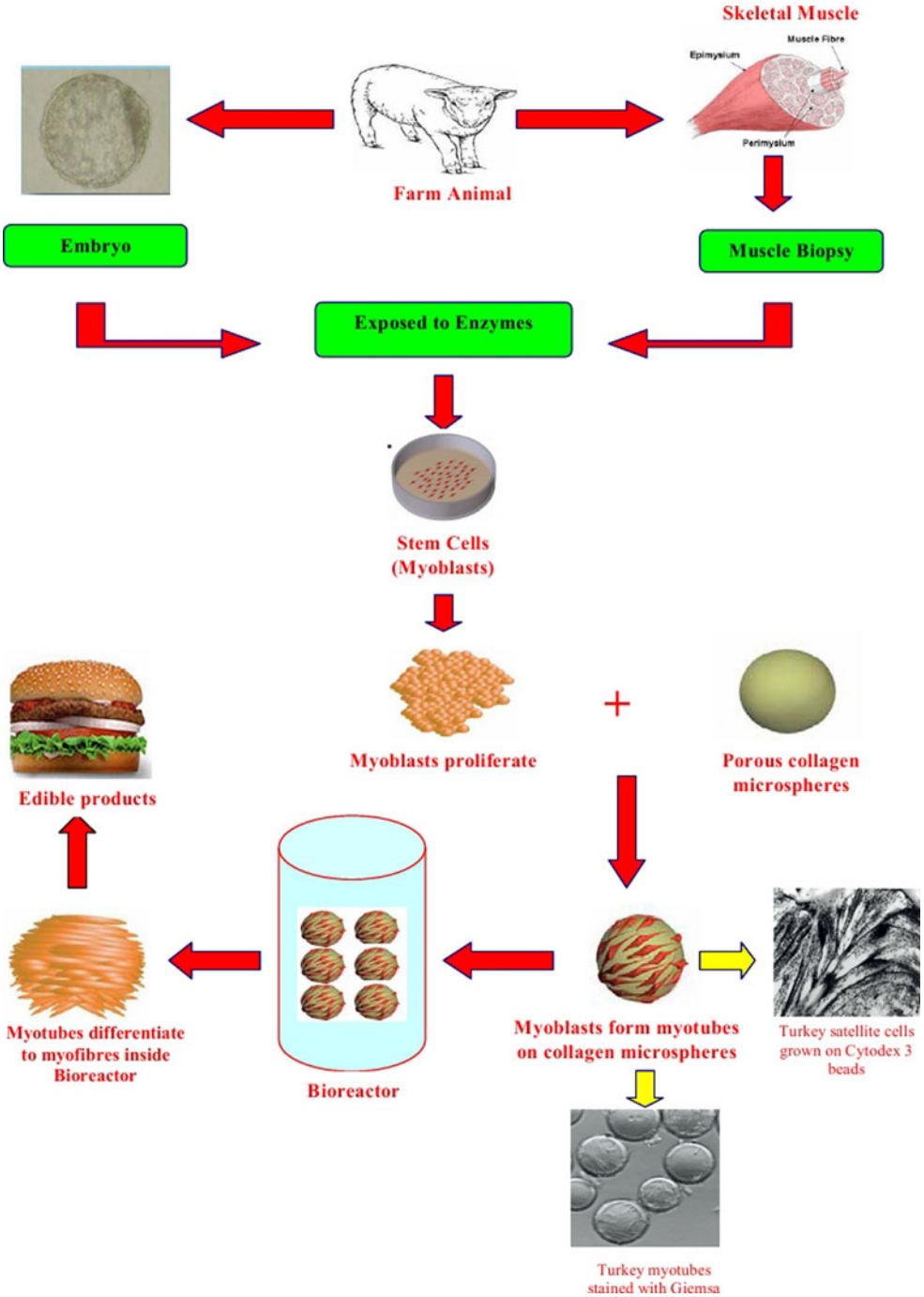

Fig. 1 Scaffold-based cultured meat production

body, provided that it was nourished with suitable nutrients. It was much later in the early 1950s when Willem van Eelen of Netherlands independently had the idea of using tissue culture for the generation of meat products. Since at that time the concept of stem cells and the in vitro culture of cells still had to emerge, it took until 1999 before van Eelen's theoretical idea was patented. Some efforts have already been put into culturing artificial meat. SymbioticA harvested muscle biopsies from frogs and kept these tissues alive and growing in culture dishes (Catts and Zurr 2002). Other research initiatives have also achieved keeping muscle tissue alive in a fungal medium, anticipating on the infection risk associated with serum–based media. In 2002, a study involving the use of muscle tissue from the common goldfish (Carassius auratus) cultured in Petri dishes was published in which the possibilities of culturing animal muscle protein for long term space flights or habituation of space stations were explored. In this study muscle tissue cultured with crude cell extracts showed a limited increase in cell mass and the cultured muscle

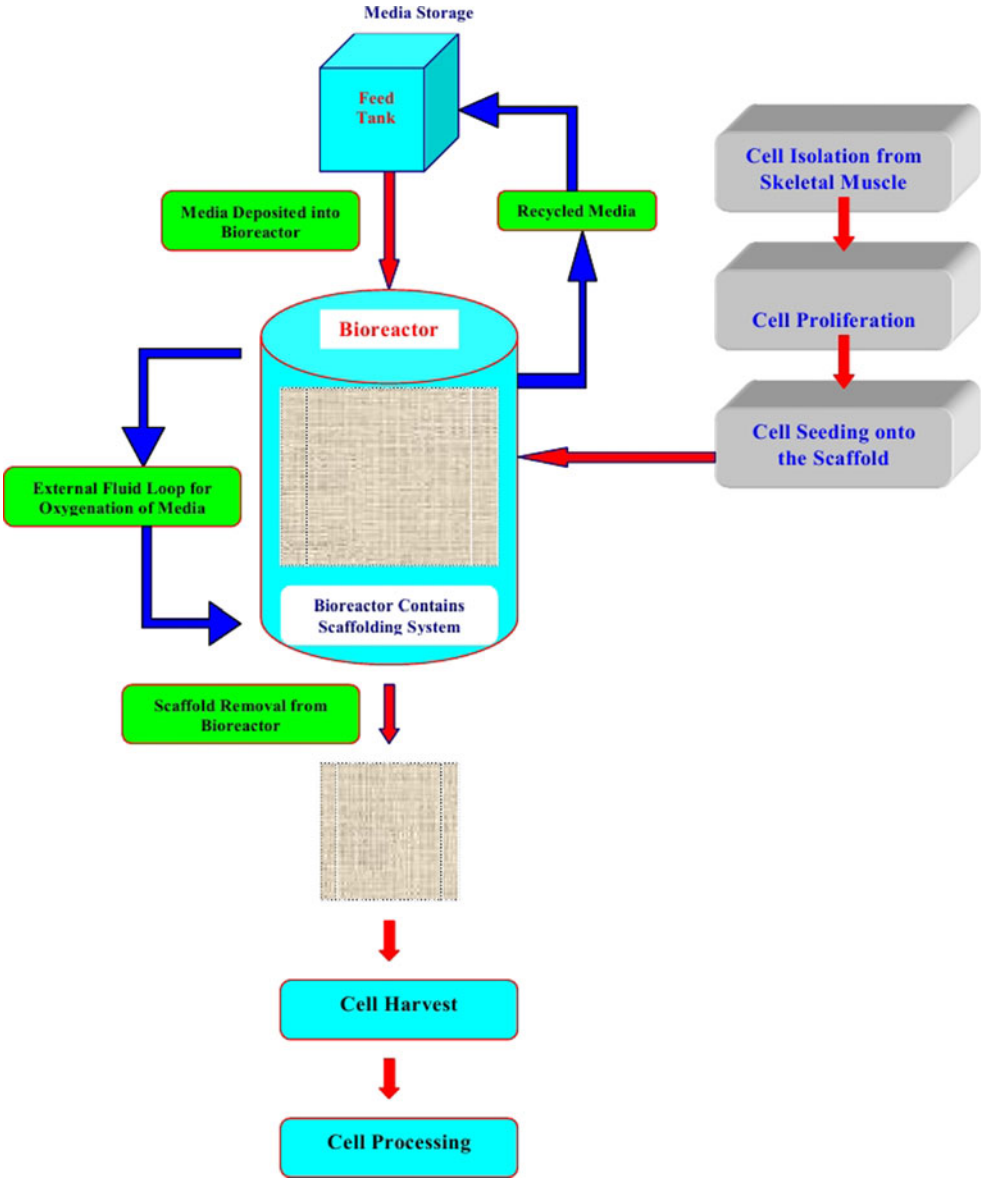

Fig. 2 Possible in vitro meat production scheme

explants so obtained were washed, dipped in olive oil with spices, covered in breadcrumbs and fried. A test-panel judged these processed explants and agreed that the product was acceptable as food (Benjaminson et al. 2002).

Cultured meat has the potential to greatly reduce animal suffering and make eating animals unnecessary, even while satisfying all the nutritional and hedonic requirements of meat eaters (Holmes and Dacey 2008). Furthermore, from a commercial perspective, animals are notoriously unreliable as a raw material for meat production, due to illness, stress and uneven growth. Cultured meat is potentially a much more reliable alternative. In comparison with animals, a product from a bioreactor could be attractive as it does not come with all the vicissitudes of animals. Furthermore, cultured meat is not bound to soil or place, which opens up possibilities for new places of production and for alternative land use. However, from a perspective of social acceptance, the technological character of cultured meat can have a negative value, and associations with Frankenstein, cloning, transgenesis and unknown risks are close at hand.

There are two different ideas regarding the concept of the cultured meat. Because people like meat and cultured meat is explicitly introduced as an alternative to the problems of normal meat, cultured meat should be as meat-like as possible in order to be a real alternative for 'traditional' meat from animals. It is therefore important that an alternative should have a similar taste and nutritional value. On the other hand, a new product needs a profile of its own; otherwise it will not be able to compete. From this perspective, it is not essential for the

product to resemble and should in fact be clearly distinctive from traditional meat.

Need of cultured meat

1. Designer meat: By manipulating the composition of the culture medium, the flavor and fatty acid composition of the cultured meat can be influenced. Moreover, health aspects of the meat can be enhanced by adding factors to the culture medium which might have an advantageous effect on the health, like certain types of vitamins (van Eelen et al. 1999). Co-culturing with other cell types might further enhance the meat quality, like coculturing with fat-producing adipocytes using the right culture medium can increase the fat content. Fat content can also be controlled by supplementation of fats after production and the ratio of saturated to poly-unsaturated fatty acids could be better controlled. Furthermore, with the advent of functional and enriched foods, consumers are more willing to try products that have been altered to have particular nutritional characteristics (Korhonen 2002; Burdock et al. 2006).
2. As laboratory produced meat does not come from a living animal, it therefore significantly minimizes the religious taboos like Jhatka, Halal etc. (Pathak et al. 2008; Bhat and Bhat 2011).
3. The incidence of food borne 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.
4. The global trade of meats from rare and endangered animals has reduced wild populations of many species in many countries. 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 cultures.
5. Theoretically, a single farm animal may be used to produce the world's meat supply and thus it reduces the animal use.
6. An animal kept for conventional meat production supports, in addition to muscle tissue, biological structures required for successful living, locomotion and reproduction. These include bones, respiratory system, digestive system, skin, and the nervous system. As they are not required to produce meat in an in vitro system, it reduces the amount of nutrients and energy needed for growth and maintenance of muscle tissue.
7. In vitro system takes significantly lower time to grow the meat than traditional meat production. It takes several weeks instead of months (for chickens) or years (for pigs and cows) before the meat can be harvested. This means that the time that tissue has to be maintained is much smaller and thus, the amount of feed and labor required per kg of in vitro cultured meat is much lower.
8. Unlike farm animals, bioreactors for in vitro meat production do not need extra space and can be stacked up in a fabric hall. For these reasons, the nutritional costs for in vitro cultured meat will be significantly lower than for traditionally cultured meat. The financial advantages are yet unclear and it might very well be that the decrease in costs of resources, labor, and land is compensated by the extra costs of a stricter hygiene regime, stricter control, computer management, etc.
9. The launch costs of long term manned space missions are currently very high that renders the prospect of launching the necessary food in preprocessed form unattractive. Moreover, astronauts often dislike the traditional space-foods and as a result, eat less of it than they normally would with variety in food choice (Zandstra et al. 2000). As for current space missions, supply and physiochemical regeneration (of water and oxygen) are the most cost-effective, but for longer periods and permanent bases, bioregeneration becomes more attractive (Drysdale et al. 2003). A controlled ecological life support system (celss) would not only provide fresh food to the astronauts, but also deal with waste, and provide oxygen and water (Saha and Trumbo 1996; Benjaminson et al. 1998; Drysdale et al. 2003).

There are other situations also 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 and bunkers designed for long-term survival of personnel following a nuclear or biological attack. 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.

10. Need for other protein sources demands production of cultured meat. Cultured meat may be the preferred alternative because it is, unlike the other products, animal-derived and with respect to composition most like meat.
11. A definite market is available for meat substitutes. Examples are legume-based and mycoprotein-based meat substitutes.

12. A small market comprising the vegetarians that do not eat meat for ethical reasons is also available.
13. Like the growing population, demand for meat is increasing at present and it will not be possible to produce all that meat in an environmental and animal friendly way. Thus, there is a rather conventional meat market for in vitro meat.
14. Animal proteins can be produced using plants and fungi. They are animal friendly, sustainable and have been used to make a variety of good products that are not expensive. The disadvantage of these products is the lack of a good texture and a taste and such products are however no solution for the craving for meat.
15. Cultured meat will be safer than conventional meat and due to the non-sustainability of traditional meat production there is a huge market for this. If the processing technology becomes very advanced one may think that the actual protein sources can be from bacteria, algae, plants, yeast as well as well as from tissue culture.
16. There is definitely a need and a market for cultured meat on a society level, but there is no need for a different new taste. One of the most important reasons to produce in vitro meat would be consumer demand. More and more people are interested in cultured meat, and it can be a very successful product.
17. Other factors like potential impact on reducing cardiovascular diseases and greenhouse gas emissions, liberation of land for nature (including wild animals), prevention of animal suffering and prevention of food scarcity that can be expected with an increasing world population.

Culturing of in vitro meat

Commonly meat may be defined as the flesh part of farm animals that mostly contains the skeletal muscle composed of bundles of muscle fibers. During embryological development, committed muscle tissue formation begins with mononucleated myoblasts of limited proliferation capacity (Benjaminson et al. 2002). Myoblasts fuse with each other and form multinucleated myotubes, which mature into a non-proliferative myofiber (Campion 1984). Postnatally, increases in number of myofibers and number of nuclei per myofiber are kept minimal, except in instances requiring repair or regeneration. In these cases, myosatellite cells are responsible for generating new myofibers or contributing additional myonuclei to existing ones (Le Grand and Rudnicki 2007). Located between the basal lamina and sarcolemma of an associated myofiber, mononucleated myosatellite cells are normally in a quiescent, non-dividing state (Hill et al. 2003). When activated in vivo by weight-bearing stress or injury, myosatellite cells asymmetrically divide into self-renewing myoblasts and committed myofibers (Benjaminson et al. 2002; Le Grand and Rudnicki 2007).

Some efforts have already been put into culturing artificial meat but obviously, small biopsies will not be practical for large–scale meat production. Therefore, it is proposed to use tissue engineering to produce in vitro cultured meat. With the help of tissue engineering it is attempted to mimic neo-organogenesis ex vivo for the treatment of various diseases and surgical reconstruction. It is a powerful technique that is mainly being designated for regenerative medicine in a wide variety of tissues and organs (Bach et al. 2003; Mol et al. 2005). In particular, tissue engineering of skeletal muscle has many applications, ranging from in vitro model systems for drug–screening (Vandenburgh et al. 2008), pressure sores (Gawlitta et al. 2007) and physiology to in vivo transplantation to treat muscular dystrophy and muscular defects (Boldrin et al. 2008). Obviously, tissue engineering could also be employed for the in vitro production of skeletal muscle tissue from farm animals for consumption purposes (Edelman et al. 2005).

Production of tissue in vitro necessitates the use of large quantities of cells, but differentiated cells exhibit a limited proliferative capacity. In contrast, there are stem cells that maintain or regain the capacity to self-renew, which means that these cells continue to proliferate. Stem cells are unique in their capacity to remain in a rather undifferentiated state for a substantial amount of population doublings while retaining the ability to differentiate into at least one specific cell type (Roelen and Lopes 2008). Muscle fibers can be cultured in vitro, however, they do not proliferate and as an alternative, satellite cells (stem cells) can be cultured. Myosatellite cells are a very small proportion (1–5%) of the cell population of muscle tissue, and this percentage is dependent on muscle fiber composition and organism age (Allen et al. 1997). The proliferative capacity of satellite cells decreases with the age of the donor and also depends on the species and disease state of the donor. Furthermore, myosatellite cells are much more abundant in muscles of young animals than in muscles of older animals and thus, typically, neonatal individuals are selected for the isolation of these cells (Hawke and Garry 2001) that offer optimal regenerative potential and myofiber morphology (longer myofibers and greater myofiber density) in vitro (Delo et al. 2008). For isolation, mincing of complete muscles is followed by enzymatic treatment and satellite cells are thereafter separated by differential centrifugation, preplating, precoll gradients, or a combination thereof. Besides many additional techniques can be used as well (Burton et al. 2000). On the removal of growth factors from the culture medium, these myoblasts fuse to form myofibers and after fusion, they start to contract randomly (Wolpert et al. 1998).

The only aim to develop an in vitro meat production system (imps) is the proliferation of animal muscle tissue. Small muscle-like organs have been demonstrated to grow from co-cultures of myoblasts and fibroblasts. These organs, termed myooids, are able to contract both spontaneously and by electrical stimulation, albeit with only a fraction of the force observed in control muscles probably because of lack of innervation (Dennis and Kosnik 2000; Dennis et al. 2001; Kosnik et al. 2001). The diameter of myooids is limited at most to 1 mm (Kosnik et al. 2001) due to the lack of perfusion which is probably the biggest problem to overcome in designing an in vitro meat production system. There are different design approaches for an in vitro meat production system, all of which are designed to overcome the diffusion barrier and can be roughly divided into cell culture and tissue culture.

Cell culture

Currently there are two detailed proposals based on emerging field of tissue engineering (Boland et al. 2003; Zandonella 2003) for using cell culture for producing in vitro meat. Both these proposals are similar in nature and neither of the two has been tested. One of the two proposals to create an in vitro meat production system has been written by Vladimir Mironov for the NASA (Wolfson 2002) while the other proposal has been written by Willem van Eelen who also holds a worldwide patent for this system (van Eelen et al. 1999). Both of these systems work by growing myoblasts in suspension in a culture medium. Mironov proposal uses a bioreactor in which cells are grown together with collagen spheres to provide a substrate onto which the myoblasts can attach and differentiate whereas van Eelen's proposal uses a collagen meshwork and the culture medium is refreshed from time to time or percolated through the meshwork. Once differentiated into myofibers, the mixture of collagen and muscle cells can be harvested and used as meat. Other edible proteins or artificial substrates can be used in place of collagen. Alternatively, van Eelen's proposal may use two-dimensional monolayers of muscle cells sandwiched onto each other after harvesting. Both of these cell culture based proposals will provide muscle proteins which will not be structured as meat but may be used in processed meat products.

Tissue culture

Unlike cell culture techniques, tissue culture based techniques aim at creating meat which is structured as such. This can be done by creating muscle tissue de novo, like in tissue engineering, or by proliferating existing muscle tissue, like Benjaminson et al. (2002) who cultured Gold fish (Carassius auratus) muscle explants. Benjaminson in another experiment kept chicken muscles alive in a Petri dish for at most 2 months before it got necrosed (Wolfson 2002). Tissue culture techniques have the advantages that explants contain all the tissues which make up meat in the right proportions and closely mimics in vivo situation but the control over the production process is limited. According to Vladimir Mironov entirely artificial muscle can be created with tissue engineering techniques by a branching network of edible porous polymer through which nutrients are perfused and myoblasts and other cell types can attach (Wolfson 2002). Such a design using the artificial capillaries for the purpose of tissue-engineering has been proposed (Technology Review 2003; Zandonella 2003). Like the myooids, it is possible to co-culture the myoblasts with other cell types to create a more realistic muscle structure which can be organized in much the same way as real muscles (Dennis and Kosnik 2000; Dennis et al. 2001; Kosnik et al. 2001).

Considering the benefits of an in vitro meat production system, it is not surprising that a number of parties have proposed and patented the methodology for actualizing this idea (Vein 2004; Van Eelen et al. 1999; Edelman et al. 2005). As of yet none of these processes, though detailed, have been tested, this review introduces the techniques so far proposed. This is partially because livestock animal cell lines have not been well-established in vitro (Talbot and Blomberg 2008) and because growing muscle cells ex vivo on a large scale is certainly a vast and unexplored undertaking. The technical demands of large scale production are unseen in the world of medical research, where most efforts in growing tissue ex vivo have been directed. The nutritional composition of ex vivo engineered muscle tissue has not yet been paid much attention. As a result, establishment of an in vitro meat production system is faced with many unique challenges so far unexplored in the field of tissue engineering.

Challenges for the production of cultured meat on commercial scale

Cultured meat technology is still in its infancy and the most important challenge is sufficient knowledge of the biology of the stem cell and its differentiation into muscle cells. Tissue engineering on a very large scale is the second requirement along with the maintenance of constant conditions around all individual cells in a large-scale reactor with sophisticated instrumentation for measuring and controlling conditions. Need of cell growth and differentiation and subsequent release from support without damage upon harvesting is third requirement along with the need for on-site cleaning and sterilization systems in the

large-scale reactors. Studies are required to determine the consumer preferences and marketing strategies. When meat from animals is available why would a consumer prefer cultured meat and if it is all about sustainability or animal welfare issues, than eating more plant proteins and less animal protein is a good alternative. The following challenges have to be met before cultured meat can be produced on a commercial scale:

Generation of suitable stem cell lines from farm animal species In vitro meat can be produced by culturing the cells from farm animal species in large quantities starting from a relatively small number. Culturing embryonic stem cells would be ideal for this purpose since these cells have an almost infinite self-renewal capacity and theoretically it is being said that one such cell line would be sufficient to literally feed the world. In theory, after the embryonic stem cell line is established, its unlimited regenerative potential eliminates the need to harvest more cells from embryos however; the slow accumulation of genetic mutations over time may determine a maximum proliferation period for a useful long-term ES culture (Amit et al. 2000). While embryonic stem cells are an attractive option for their unlimited proliferative capacity, these cells must be specifically stimulated to differentiate into myoblasts and may inaccurately recapitulate myogenesis (Bach et al. 2003). Although embryonic stem cells have been cultured for many generations but so far it has not been possible to culture cell lines with unlimited self-renewal potential from pre-implantation embryos of farm animal species. Until now, true embryonic stem cell lines have only been generated from mouse, rhesus monkey, human and rat embryos (Talbot and Blomberg 2008) but the social resistance to cultured meat obtained from mouse, rat or rhesus monkey will be considerable and will not result in a marketable product. The culture conditions required to keep mouse and human embryonic cells undifferentiated are different from the conditions that will be required for embryonic cells of farm animal species and fundamental research on the early development of embryos of these species can provide clues.

However, different efforts invested into establishing ungulate stem-cell lines over the past two decades have been generally unsuccessful with difficulties arising in the recognition, isolation and differentiation of these cells (Keefer et al. 2007). According to Bach et al. (2003) myosatellite cells are the preferred source of primary myoblasts although, they have the disadvantage of being a rare muscle tissue cell type with limited regenerative potential because they recapitulate myogenesis more closely than immortal myogenic cell lines. Myosatellite cells isolated from different animal species have different benefits and limitations as a cell source and that isolated from different muscles have different capabilities to proliferate, differentiate, or be regulated by growth modifiers (Burton et al. 2000). Myosatellite cells have been isolated and characterized from the skeletal muscle tissue of cattle (Dodson et al. 1987), chicken (Yablonka-Reuveni et al. 1987), fish (Powell et al. 1989), lambs (Dodson et al. 1986), pigs (Blanton Blanton et al. 1999, Wilschut et al. 2008), and turkeys (McFarland et al. 1988). Porcine muscle progenitor cells have the potential for multilineage differentiation into adipogenic, osteogenic and chondrogenic lineages, which may play a role in the development of co-cultures (Wilschut et al. 2008). Advanced technology in tissue engineering and cell biology offer some alternate cell options having practical applications and multilineage potential allowing for co-culture development with suitability for large-scale operations.

Alternatively, we can use adult stem cells from farm animal species and myosatellite cells are one example of an adult stem-cell type with multilineage potential (Asakura et al. 2001). Adult stem cells have been isolated from several different adult tissues (Wagers and Weissman 2004) but their in vitro proliferation capacity is not unlimited and can proliferate in vitro for several months at most. These cells also have the capacity to differentiate into skeletal muscle cells, although not very efficiently but for now, these are the most promising cell type for use in the production of cultured meat. A rare population of multipotent cells found in adipose tissue known as adipose tissue-derived adult stem cells (ADSCs) is another relevant cell type for in vitro meat production (Gimble et al. 2007) which can be obtained from subcutaneous fat and subsequently transdifferentiated to myogenic, osteogenic, chondrogenic or adipogenic cell lineages (Kim et al. 2006). However, adult stem cells are prone to malignant transformation in long-term culture (Lazennec and Jorgensen 2008) that is the greatest matter of debate. It has been observed that adipose tissue-derived adult stem cells immortalize at high frequency and undergo spontaneous transformation in long-term (4–5 months) culturing (Rubio et al. 2005), while evidence of adult stem cells remaining untransformed have also been reported (Bernardo et al. 2007). To minimize the risk of spontaneous transformation, re-harvesting of adult stem cells may be necessary in an in vitro meat production system and as such obtaining ADSCs from subcutaneous fat is far less invasive than collection of myosatellite cells from muscle tissue.

Matsumoto et al. (2007) reported that mature adipocytes can be dedifferentiated in vitro into a multipotent preadipocyte cell line known as dedifferentiated fat (DFAT) cells, reversion of a terminally differentiated cell into a multipotent cell type. These DFAT cells are capable of being transdifferentiated into skeletal myocytes (Kazama et al. 2008) and appear to be an attractive alternative to the use of stem cells. This process known as “ceiling culture method” certainly seems achievable on an industrial scale but

Rizzino (2007) has put forth the argument that many of the claims of transdifferentiation, dedifferentiation and multipotency of once terminally differentiated cells may be due to abnormal processes resulting in cellular look-alikes.

Safe media for culturing of stem cells In vitro meat would need an affordable medium system to enjoy its potential advantages over conventional meat production and that medium must contain the necessary nutritional components available in free form to myoblasts and accompanying cells. Myoblast culturing usually takes place in animal sera, a costly media that does not lend itself well to consumer acceptance or large-scale use. Animal sera are from adult, newborn or fetal source, with fetal bovine serum being the standard supplement for cell culture media (Coecke et al. 2005). Because of its in vivo source, it can have a large number of constituents in highly variable composition and potentially introduce pathogenic agents (Shah 1999). The harvest of fetal bovine serum also raises ethical concern and for the generation of an animal-free protein product, the addition of fetal calf serum to the cells would not be an option and it is therefore essential to develop a serum-free culture medium. Commercially available serum replacements and serum-free culture media offer some more realistic options for culturing mammalian cells in vitro. Serum-free media reduce operating costs and process variability while lessening the potential source of infectious agents (Froud 1999). Improvements in the composition of commercially available cell culture media have enhanced our ability to successfully culture many types of animal cells and serum-free media have been developed to support in vitro myosatellite cell cultures from the turkey (McFarland et al. 1991), sheep (Dodson and Mathison 1988) and pig (Doumit et al. 1993). Variations among different serum-free media outline the fact that satellite cells from different species have different requirements and respond differentially to certain additives (Dodson et al. 1996). Ultroser G is an example of a commercially available serum substitute containing growth factors, binding proteins, adhesin factors, vitamins, hormones, mineral trace elements and has been designed specially to replace fetal bovine serum for growth of anchorage-dependent cells in vitro (Duque et al. 2003). Benjaminson et al. (2002) succeeded in using a serum-free medium made from maitake mushroom extract that achieved higher rates of growth than fetal bovine serum and recently it has been shown that lipids such as sphingosine-1-phosphate can replace serum in supporting the growth and differentiation of embryonic tissue explants. In most cases, serum-free media are supplemented with purified proteins of animal origin (Merten 1999).

Indeed such media have already been generated and are available from various companies for biomedical purposes; however, their price is incompatible with the generation of an affordable edible product. Therefore, a cell culture medium has to be developed that does not contain products of animal origin and enables culturing of cells at an affordable price.

Safe differentiation media to produce muscle cells For stem cell culturing it is important that these cells remain undifferentiated and maintain their capacity to proliferate and for the production of cultured meat a specific and efficient differentiation process initiated with specific growth factors is needed. An appropriate array of growth factors is required to growing muscle cells in culture in addition to proper nutrition and these 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 myosatellite cells of different species respond differentially to the same regulatory factors (Burton et al. 2000) and as such extrinsic regulatory factors must be specific to the chosen cell type and species. Furthermore, formulation may be required to change over the course of the culturing process from proliferation period to the differentiation and maturation period, requiring different set of factors. A multitude of regulatory factors have been identified as being capable of inducing myosatellite cell proliferation (Cheng et al. 2006), and the regulation of meat animal-derived myosatellite cells by hormones, polypeptide growth factors and extracellular matrix proteins has also been investigated (Dodson et al. 1996; Doumit et al. 1993). Purified growth factors or hormones may be supplemented into the media from an external source such as transgenic bacterial, plant or animal species which produce recombinant proteins (Houdebine 2009). Alternatively, a sort of synthetic paracrine signalling system can be arranged so that co-cultured cell types can secrete growth factors which can promote cell growth and proliferation in neighbouring cells. Appropriate co-culture systems like hepatocytes may be developed to provide growth factors necessary for cultured muscle production that provide insulin-like growth factors which stimulate myoblast proliferation and differentiation (Cen et al. 2008) as well as myosatellite cell proliferation in several meat-animal species in vitro (Dodson et al. 1996). Typically, investigators initiate differentiation and fusion of myoblasts by lowering the levels of mitogenic growth factors and the proliferating cells then commence synthesis of insulin-like growth factor-II, which leads to differentiation and formation of multinucleated myotubes (Florini et al. 1991) and stimulate myocyte maturation (Wilson et al. 2003). So, the successful system must be capable of changing the growth factor composition of the media. Currently the most efficient method to let (mouse) stem cells differentiate into skeletal muscle cells is to culture them in a medium that contains 2% horse serum instead of 10 or

20% fetal calf serum. However, for the generation of cultured meat, it is essential that the cells are cultured and differentiated without animal products, so a chemically defined culture medium has to be developed that enables the differentiation of stem cells to skeletal muscle cells.

Tissue engineering of muscle fibers The possibility to form a 3-dimensional structure of cells is restricted in the absence of blood flow that provides oxygen and nutrients to the cells and removes metabolic end products. Because of the limitations in nutrient diffusion, the in vitro culturing of cells is limited to only a few layers of cells. A solution to this problem may be provided by culture of cells on edible or biodegradable synthetic or biological scaffolds which would provide shape and structure to the engineered tissue. Another solution would be the processing of these thin layers of cells into a meat based product. Alternatively, deformable micro-carrier beads of edible (non-animal) material may be developed that enable production of secondary myotubes in suspension which may be used as an animal protein ingredient in a wide variety of products. Alternatively, products of animal origin with a meat-like appearance and texture can be made by addition of fibroblasts (for firmness) and fat cells (for taste) to the myotubes.

Scaffolds As myoblasts are anchorage-dependent cells, a substratum or scaffold must be provided for proliferation and differentiation to occur (Stoker et al. 1968). Scaffolding mechanisms differ in shape, composition and characteristics to optimize muscle cell and tissue morphology. An ideal scaffold must have a large surface area for growth and attachment, be flexible to allow for contraction as myoblasts are capable of spontaneous contraction, maximize medium diffusion and be easily dissociated from the meat culture. A best scaffold is one that mimics the in vivo situation as myotubes differentiate optimally on scaffold with a tissue-like stiffness (Engler et al. 2004) and its by-products must be edible and natural and may be derived from non-animal sources, though inedible scaffold materials cannot be disregarded. New biomaterials may be developed that offer additional characteristics, such as fulfilling the requirement of contraction for proliferation and differentiation (De Deyne 2000). Thus, challenge is to develop a scaffold that can mechanically stretch attached cells to stimulate differentiation and a flexible substratum to prevent detachment of developing myotubes that will normally undergo spontaneous contraction.

Edelman et al. (2005) proposed porous beads made of edible collagen as a substrate while as Van Eelen et al. (1999) proposed a collagen meshwork described as a "collagen sponge" of bovine origin. The tribeculate structure of the sponge allows for increased surface area and diffusion, but may impede harvesting of the tissue culture. Other possible scaffold forms include large elastic sheets or an array of long, thin filaments. Cytodex-3 micro-carrier beads have been used as scaffolds in rotary bioreactors but these beads have no stretching potential. One elegant approach to mechanically stretch myoblasts would be to use edible, stimuli-sensitive porous micro-spheres made from cellulose, alginate, chitosan, or collagen (Edelman et al. 2005) that undergo, at minimum, a 10% change in surface area following small changes in temperature or pH. Once myoblasts attach to the spheres, they could be stretched periodically provided such variation in the pH or temperature would not negatively affect cell proliferation, adhesion, and growth. Jun et al. (2009) have found that growing myoblasts on electrically conductive fibers induces their differentiation, forming more myotubes of greater length without the addition of electrical stimulation but use of such inedible scaffolding systems necessitates simple and nondestructive techniques for removal of the culture from the scaffold.

Furthermore, there are greater technical challenges in developing a scaffold for large and highly structured meats due to the absence of vascular system. There is a need to build a branching network from an edible, elastic, and porous material, through which nutrients can be perfused and myoblasts and other cell types can then attach to this network. Edelman et al. (2005) acknowledge that a cast of an existing vascularization network, such as that in native muscle tissue, can be used to create a collagen network mimicking native vessel architecture. Taking this a step further, Borenstein et al. (2002) has proposed an approach to create such a network by creating a cast onto which a collagen solution or a biocompatible polymer is spread and after solidification seeding the network with endothelial cells. Following dissolution of the polymer mold, successful proliferation could theoretically leave behind a network of endothelial tissue, a branched network of micro-channels, which can be stacked onto each other to form a three-dimensional network onto which one could grow myocytes. A synthetic vascular system would then require a circulation pumping system and a soluble oxygen carrier in the medium to be fully functional. But at this moment creation of these artificial vascular networks does not translate well into mass production due to the microfabrication processes required. Alternatively, Benjaminson et al. (2002) proposed an attempt to create a highly structured meat without a scaffold by solving the vascularization problem through controlled angiogenesis of explants.

Another important factor is the texture and microstructure of scaffolds as texturized surfaces can attend to specific requirements of muscle cells, one of which is myofiber alignment. This myofiber organization is an important determinant for the functional characteristics of muscle

and the textural characteristics of meat. Lam et al. (2006) cultured myoblasts on a substrate with a wavy micropatterned surface to mimic native muscle architecture and found that the wave pattern aligned differentiated muscle cells while promoting myoblast fusion to produce aligned myotubes. While using scaffold-based techniques for meat culturing, micropatterned surfaces could allow muscle tissue to assume a two dimensional structure more similar to that of meat of native origin. Riboldi et al. (2005) utilized electrospinning, a process that uses electrical charge to extract very fine fibers from liquids, by using electrospun microfibrous meshwork membranes as a scaffold for skeletal myocytes. These membranes offer high surface area to volume ratio in addition to some elastic properties. Electrospinning creates very smooth fibers, which may not translate well into a good adhesive surface and coating electrospun polymer fibers with extracellular matrix proteins, such as collagen or fibronectin, promotes attachment by ligand–receptor binding interactions (Riboldi et al. 2005). Electrospinning shows promise for scaffold formation because the process is simple, controllable, reproducible and capable of producing polymers with special properties by co-spinning (Riboldi et al. 2005).

Production of meat by the scaffold-based technique faces a technical challenge of removal of the scaffolding system. Confluent cultured cell sheets are conventionally removed enzymatically or mechanically, but these two methods damage the cells and the extracellular matrix they may be producing (Canavan et al. 2005). However, thermoresponsive coatings which change from hydrophobic to hydrophilic at lowered temperatures can release cultured cells and extracellular matrix as an intact sheet upon cooling (da Silva et al. 2007). This method known as "thermal liftoff," results in undamaged sheets that maintain the ability to adhere if transferred onto another substrate (da Silva et al. 2007) and opens the possibility of stacking sheets to create a three-dimensional product. Lam et al. (2009) have presented a method for detaching culture as a confluent sheet from a non-adhesive micropatterned surface using the biodegradation of selective attachment protein laminin. However, culturing on a scaffold may not result in a two-dimensional confluent "sheet" of culture. The contractile forces exerted after scaffold removal by the cytoskeleton of the myocyte are no longer balanced by adhesion to a surface that causes the cell lawn to contract and aggregate, forming a detached multicellular spheroid (da Silva et al. 2007). To remove the culture as a sheet, a hydrophilic membrane or gel placed on the apical surface of the culture before detachment can provide physical support and use of a fibrin hydrogel is ideal for skeletal muscle tissue because cells can migrate, proliferate and produce their own extracellular matrix within it while degrading excess fibrin (Lam et al. 2009). These two-dimensional sheets could be stacked to create a three-dimensional product as suggested by Van Eelen et al. (1999).

Industrial bioreactors Production of in vitro meat for processed meat based products will require large-scale culturing in large bioreactors as stem cells and skeletal muscle cells require a solid surface for culturing and a large surface area is needed for the generation of sufficient number of muscle cells. Cultured meat production is likely to require the development of new bioreactors that maintain low shear and uniform perfusion at large volumes. The bioreactor designing is intended to promote the growth of tissue cultures which accurately resemble native tissue architecture and provides an environment which allows for increased culture volumes. A laminar flow of the medium is created in rotating wall vessel bioreactors by rotating the cylindrical wall at a speed that balances centrifugal force, drag force and gravitational force, leaving the three-dimensional culture submerged in the medium in a perpetual free fall state (Carrier et al. 1999) that improves diffusion with high mass transfer rates at minimal levels of shear stress, producing three dimensional tissues with structures very similar to those in vivo (Martin et al. 2004). Direct perfusion bioreactors appear more appropriate for scaffold based myocyte cultivation allowing flow of medium through a porous scaffold with gas exchange taking place in an external fluid loop (Carrier et al. 2002). Besides offering high mass transfer they also offer significant shear stress, so determining an appropriate flow rate is essential (Martin et al. 2004). Direct perfusion bioreactors are also used for high-density, uniform myocyte cell seeding (Radisic et al. 2003). Another method of increasing medium perfusion is by vascularizing the tissue being grown. Levenberg et al. (2005) had induced endothelial vessel networks in skeletal muscle tissue constructs by using a co-culture of myoblasts, embryonic fibroblasts and endothelial cells co-seeded onto a highly porous biodegradable scaffold. Research size rotating bioreactors have been scaled up to three liters and, theoretically, scale up to industrial sizes should not affect the physics of the system.

Adequate perfusion of the cultured tissue is required to produce large culture quantities and it is necessary to have adequate oxygen perfusion during cell seeding and cultivation on the scaffold as cell viability and density positively correlate with the oxygen gradient in statically grown tissue cultures (Radisic et al. 2008). Adequate oxygen perfusion is mediated by bioreactors which increase mass transport between culture medium and cells and by the use of oxygen carriers to mimic hemoglobin provided oxygen supply to maintain high oxygen concentrations in solution, similar to that of blood. Oxygen carriers are either modified versions of hemoglobin or artificially produced perfluorochemicals (PFCs) that are chemically inert (Lowe 2006). Many

chemically modified hemoglobins have been developed but their bovine or human source makes them an unfit candidate and alternatively, human hemoglobin has been produced by genetically modified plants (Dieryck et al. 1997) and microorganisms (Zuckerman et al. 1998).

Atrophy and Exercise One of the potential problems associated with cultured meat is that of atrophy or muscle wasting due to a reduction of cell size (Fox 1996) caused by lack of use, denervation, or one of a variety of diseases (Charge et al. 2002; Ohira et al. 2002). Regular contraction is a necessity for skeletal muscle and promotes differentiation and healthy myofiber morphology while preventing atrophy. Muscle in vivo is innervated, allowing for regular, controlled contraction whereas in vitro system would necessarily culture denervated muscle tissue, so contraction must be stimulated by alternate means. It might be possible that mechanical or electrical stimulation can promote growth and structure of the cultured meat as newly formed myotubes in culture start to contract spontaneously (Wolpert et al. 1998) or as a matter of fact, myooids also contract spontaneously at approximately 1 Hz. once formed (Dennis and Kosnik 2000). So exercise by electrical stimulation might be a viable solution to overcome atrophy in an in vitro meat production system. Cha et al. (2006) have found that administration of cyclic mechanical strain to a highly porous scaffold sheet promotes differentiation and alignment of smooth muscle cells. Edelman et al. (2005) and Van Eelen et al. (1999) proposed mechanical stretching of scaffolds and expandable scaffold beads to fulfill the requirement of providing contraction. de Deyne (2000) noted that external mechanical contraction is less effective than electrical stimulation in promoting muscle development. Electrical stimulation, feasible on a large scale, induces contraction internally as opposed to passively and aids in differentiation and sarcomere formation. Even growth on electrically conductive fibers without application of electrical stimulation sufficed in reaping the benefits of induced contraction (Jun et al. 2009).

Senescence In vitro meat production system based on satellite cells still imposes a challenge of senescence that can be tackled either by starting fresh cell culture whenever needed or by immortalizing cell culture or by using embryonic stem cell cultures. Fresh satellite cells can be extracted without harming the animal donors (van Eelen et al. 1999) from time to time to start new cell cultures, although animal slaughter is a more common practice (Burton et al. 2000). Second approach involves modification of the cells in culture so that senescence can be overcome by involving the ectopic expression of the gene for the telomerase enzyme (Alberts et al. 1994). An additional expression of an oncogene may be required to overcome senescence (Prowse and Greider 1995; Counter et al. 1998; Lustig 1999; O'Hare et al. 2001) but this method falls within the domain of genetic modification, which might severely hinder consumer acceptance. Third approach involves embryonic stem cells which are pluripotent and apparently have an unlimited capability for division (Burdon et al. 2002) and thus, embryonic stem cell culture derived from a single donor can be theoretically propagated unlimited but embryonic stem cells have to differentiate to muscle cells before they can be used.

Food processing technology New food processing technologies need to be developed to make in vitro meat based products attractive and that will depend on the starting material whether suspensions of small myotubes, myofibers on scaffolds or microspheres, etc. are utilized. The first in vitro meat based product may be developed using small pieces of cultured muscle fiber as raw material.

Risks of contamination Cultured meat will be safer and more sustainable than conventional meat but production may be less safe because of risks of contamination. Cultured meat may have a completely different risk profile than conventional meat and much attention would require to be paid to the safety of added substrates and other compounds of the culture medium. So, there are fewer risks with respect to microbial contamination but more risk of contamination of substrates.

Discussion

It is anticipated that during the next 40 years global meat consumption will double with 50% increase in global population and if no actions are taken, it will be accompanied with an almost doubling of the greenhouse gas emissions. Meat production requires a relatively high proportion of land, energy, fresh water use, and moreover, livestock contributes significantly to the emission of greenhouse gases and, in many countries, to the pollution of water and soil. Reducing the use of products of animal origin and replacement of dietary animal (vertebrate) proteins by plant, fungal, or even insect proteins particularly in societies where the consumption of animal proteins is very high seems one of the solutions. Production of in vitro meat by culturing large amounts of muscle cells derived from stem cells of farm animal species is yet another prospective possibility. No animals are needed in the preparation of the meat products by this technology which may combine a favorable ecological footprint with similar nutritional values and sensory qualities as some of

the conventional products. The essential requirements for the production of cultured meat may be summarized as:

1. A suitable, bona fide, stem cell line that can proliferate indefinitely. Both embryonic stem cells and adult stem cells having a minimal self-renewal capacity may be suitable.
2. Separate culture media must be developed for growth and for the differentiation of the stem cells that do not contain products of animal origin at prices that are compatible with food production.
3. Formation of a three-dimensional structure of fused muscle cells (myofibers) with edible non-animal origin scaffolds or hydrogels to circumvent the absence of vascularization. Many factors that are important for culturing muscle tissue together with biochemical and biophysical stimuli (electrical and mechanical) are important in the differentiation and maturation of muscle tissue.
4. Cost effectiveness of the cultured meat: it must be produced at prices that are comparable with factory gate prices for cheap meat.
5. A favorable ecological footprint compared to the conventional production of meat.
6. Consumer acceptance: no scientific study has been conducted about the ethical and societal aspects of cultured meat production and consumption.

Conclusion

In vitro meat holds great promises as an alternative to traditionally produced meat, if consumer resistance can be overcome. Since crucial knowledge is still lacking on the biology and technology, it may be concluded that commercial production of cultured meat is as yet not possible and the focus must be on filling these gaps in knowledge. Furthermore, knowledge is lacking with respect to ethical and societal issues and a great body of research has to be performed before this kind of meat can be produced on an industrial scale.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular biology of the cell, 3rd edn. Garland, New York

Allen RE, Temm-Grove CJ, Sheehan SM, Rice G (1997) Skeletal muscle satellite cell cultures. Methods Cell Biol 52:155–176

Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA et al (2000) Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev Biol 227(2):271–278

Asakura A, Komaki M, Rudnicki M (2001) Muscle satellite cells are multi-potential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation 68(4–5):245–253

Asner GP, Elmore AJ, Olander LP, Martin RE, Harris AT (2004) Grazing systems, ecosystem responses and global change. Annu Rev Environ Resour 29:261–299

Avery A (1997) Red meat production can be part of an environmentally sound future. J Am Diet Assoc 97(11):1249–1250

Bach AD, Stem-Straeter J, Beier JP, Bannasch H, Stark GB (2003) Engineering of muscle tissue. Clin Plast Surg 30(4):589–599

Barnard ND, Nicholson A, Howard JL (1995) The medical costs attributable to meat consumption. Prev Med 24:646–655

Bender A (1992) Meat and meat products in human nutrition in developing countries. Food and Agriculture Organization of the United Nations (FAO), Food and Nutrition Paper 53, Italy, Rome

Benjaminson M, Lehrer S, Macklin D (1998) Bioconversion systems for food and water on long term space missions. Acta Astronaut 43(3–6):329–348

Benjaminson MA, Gilchriest JA, Lorenz M (2002) In vitro edible muscle protein production system (MPPS): Stage 1, fish. Acta Astronaut 51(12):879–889

Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A et al (2007) Human bone marrow-derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 67(19):9142–9149

Bhat ZF, Bhat H (2011) Animal-free meat biofabrication. Am J Food Technol 6(6):441–459

Blanton JR, Grand AL, McFarland DC, Robinson JP, Bidwell CA (1999) Isolation of two populations of myoblasts from porcine skeletal muscle. Muscle Nerve 22(1):43–50

Boland T, Mironov V, Gutowska A, Roth E, Markwald R (2003) Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. Anat Rec 272A(2):497–502

Boldrin L, Malerba A, Vitiello L, Cimetta E, Piccoli M, Messina C et al (2008) Efficient delivery of human single fiber derived muscle precursor cells via biocompatible scaffold. Cell Transplant 17(5):577–584

Borenstein JT, Terai H, King KR, Weinberg EJ, Kaazempur-Mofrad MR, Vacanti JP (2002) Microfabrication technology for vascularized tissue engineering. Biomed Microdevices 4(3):167–175

Burdock GA, Carabin GI, Griffiths GC (2006) The importance of GRAS to the functional food and nutraceutical industries. Toxicology 221(1):17–27

Burdon T, Smith A, Savatier P (2002) Signalling, cell cycle and pluripotency in embryonic stem cells. Trends Cell Biol 12 (9):432–438

Burton NM, Vierck JL, Krabbenhoft L, Byrne K, Dodson MV (2000) Methods for animal satellite cell culture under a variety of conditions. Methods Cell Sci 22(1):51–61

Campion DR (1984) The muscle satellite cell: a review. Int Rev Cytol 87:225–251

Canavan HE, Cheng X, Graham DJ, Ratner BD, Castner DG (2005) Cell sheet detachment affects the extracellular matrix: a surface science study comparing thermal liftoff, enzymatic, and mechanical methods. J Biomed Mater Res 75A(1):1–13

Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R et al (1999) Cardiac tissue engineering: cell seeding, cultivation parameters and tissue construct characterization. Biotechnol Bioeng 64(5):580–589

Carrier RL, Rupnick M, Langer R, Schoen FJ, Freed LE, Vunjak-Novakovic G (2002) Perfusion improves tissue architecture of engineered cardiac muscle. Tissue Eng 8(2):175–188

Catts O, Zurr I (2002) Growing semi-living sculptures: Tissue culture and art project. In: Leonardo 35(4):365–370

Cen S, Zhang J, Huang F, Yang Z, Xie H (2008) Effect of IGF-I on proliferation and differentiation of primary human embryonic myoblasts. Chinese Journal of Reparative and Reconstructive Surgery 22(1):84–87

Cha JM, Park SN, Noh SH, Suh H (2006) Time-dependent modulation of alignment and differentiation of smooth muscle cells seeded on a porous substrate undergoing cyclic mechanical strain. Artif Organs 30(4):250–258

Charge S, Brack A, Hughes S (2002) Aging-related satellite cell differentiation defect occurs prematurely after Ski-induced muscle hypertrophy. Am J Physiol Cell Physiol 283(4):C1228–C1241

Cheng L, Gu X, Sanderson JE, Wang X, Lee K, Yao X et al (2006) A new function of a previously isolated compound that stimulates activation and differentiation of myogenic precursor cells leading to efficient myofiber regeneration and muscle repair. Int J Biochem Cell Biol 38(7):1123–1133

Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T et al (2005) Guidance on good cell culture practice: a report of the second ECVAM Task Force on good cell culture practice. Altern Lab Anim 33(3):261–287

Costales A, Gerber P, Steinfeld H (2006) Underneath the livestock revolution. Livestock report, Rome, Italy: Food and Agriculture Organization of the United Nations

Counter C, Hahn W, Wei W, Caddle S, Beijersbergen R, Lansdorp P, Sedivy J, Weinberg R (1998) Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Proc Natl Acad Sci USA 95(25):14723–14728

Crok M (2003) Bij de beesten af. Natuur and Techniek 71(4):46–49

Da Silva RMP, Mano JF, Reis RL (2007) Smart thermoresponsive coatings and surfaces for tissue engineering: switching cell-material boundaries. Trends Biotechnol 25(12):577–583

De Deyne PG (2000) Formation of sarcomeres in developing myotubes: role of mechanical stretch and contractile activation. Am J Physiol Cell Physiol 279(6):C1801–C1811

de Haan C, Steinfeld H, Blackburn H (1997) Livestock and the environment: finding a balance. Food and Agriculture Organization of the United Nations, World Bank and US Agency for International Development

Delgado CL (2003) Rising consumption of meat and milk in developing countries has created a new food revolution. J Nutr 133:3907S–3910S

Delo DM, Eberlie D, Williams JK, Anderrson KE, Atala A, Soker S (2008) Angiogenic gene modification of skeletal muscle cells to compensate for ageing induced decline in bioengineered functional muscle tissue. BJU Int 102(7):878–884

Dennis R, Kosnik P 2nd (2000) Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. In Vitro Cell Dev Biol Anim 36(5):327–335

Dennis R, Kosnik P 2nd, Gilbert M, Faulkner J (2001) Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines. Am J Physiol Cell Physiol 280(2):C288–C295

Dieryck W, Pagnier J, Poyart C, Marden M, Gruber V, Bournat P et al (1997) Human haemoglobin from transgenic tobacco. Nature 386 (6620):29–30

Dodson MV, Mathison BA (1988) Comparison of ovine and rat muscle-derived satellite cells: response to insulin. Tissue Cell 20 (6):909–918

Dodson MV, McFarland DC, Martin EL, Brannon MA (1986) Isolation of satellite cells from ovine skeletal muscles. J Tissue Cult Methods 10(4):233–237

Dodson MV, Martin EL, Brannon MA, Mathison BA, McFarland DC (1987) Optimization of bovine satellite cell derived myotube formation in vitro. Tissue Cell 19(2):159–166

Dodson MV, McFarland DC, Grant AL, Doumit ME, Velleman SG (1996) Extrinsic regulation of domestic animal-derived satellite cells. Domest Anim Endocrinol 13(2):107–126

Doumit ME, Cook DR, Merkel RA (1993) Fibroblast growth factor, epidermal growth factor, insulin-like growth factor and platelet-derived growth factor-BB stimulate proliferate of clonally derived porcine myogenic satellite cells. J Cell Physiol 157 (2):326–332

Drysdale A, Ewert M, Hanford A (2003) Life support approaches for Mars missions. Adv Space Res 31(1):51–61

Duque P, Gómez E, Diaz E, Facal N, Hidalgo C, Diez C (2003) Use of two replacements of serum during bovine embryo culture in vitro. Theriogenology 59(3–4):889–899

Edelman PD, McFarland DC, Mironov VA, Matheny JG (2005) Commentary: in vitro-cultured meat production. Tissue Eng 11 (5):659–662

Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, Discher DE (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness, pathological implications for soft or stiff microenvironments. J Cell Biol 166(6):877–887

European Food Safety Authority (2006) The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and food borne outbreaks in the European Union in 2005. EFSA J 94:2–288

Fisher IS, Meakens S (2006) Surveillance of enteric pathogens in Europe and beyond: enter-net annual report for 2004. Euro Surveillance: Bulletin Européen sur les Maladies Transmissibles 11, E060824.060823. Available at: http://www.hpa.org.uk/hpa/inter/enter-net/Enter-net%20annual%20report%202004.pdf, (26 March 2007 last date accessed)

Florini JR, Magri KA, Ewton DZ, James PL, Grindstaff K, Rotwein PS (1991) Spontaneous differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. J Biol Chem 266(24):15917–15923

Fox SI (1996) Human physiology. Wim C, Brown, Boston

Froud SJ (1999) The development, benefits and disadvantages of serum-free media. Dev Biol Stand 99:157–166

Gawlitta D, Oomens CW, Bader DL, Baaijens FP, Bouten CV (2007) Temporal differences in the influence of ischemic factors and deformation on the metabolism of engineered skeletal muscle. J Appl Physiol 103(2):464–473

Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. Circ Res 100(9):1249–1260

Hawke T, Garry D (2001) Myogenic satellite cells: physiology to molecular biology. J Appl Physiol 91(2):534–551

Hill M, Wernig A, Goldspink G (2003) Muscle satellite (stem) cell activation during local tissue injury and repair. J Anat 203(1):89–99

Holmes PD, Dacey A (2008) Vegetarian meat: could technology save animals and satisfy meat eaters? J Agric Environ Ethics 21:579–596

Houdebine LM (2009) Production of pharmaceutical proteins by transgenic animals. Comp Immunol Microbiol Infect Dis 32 (2):107–121

Jun I, Jeong S, Shin H (2009) The stimulation of myoblast differentiation by electrically conductive sub-micron fibers. Biomaterials 30(11):2038–2047

Kazama T, Fujie M, Endo T, Kano K (2008) Mature adipocyte-derived dedifferentiated fat cells can transdifferentiate into skeletalmyocytes in vitro. Biochem Biophys Res Commun 377(3):780–785

Keefer CL, Pant D, Blomberg L, Talbot NC (2007) Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. Anim Reprod Sci 98(1–2):147–168

Kim MJ, Choi YS, Yang SH, Hong HN, Cho SW, Cha SM et al (2006) Muscle regeneration by adipose tissue-derived adult stem

cells attached to injectable PLGA spheres. Biochem Biophys Res Commun 348(2):386–392
Korhonen H (2002) Technology options for new nutritional concepts. Int J Dairy Technol 55(2):79–88
Kosnik P, Faulkner J, Dennis R (2001) Functional development of engineered skeletal muscle from adult and neonatal rats. Tissue Eng 7(5):573–584
Lam MT, Sim S, Zhu X, Takayama S (2006) The effect of continuous wavy micropatterns on silicone substrates on the alignment of skeletal muscle myoblasts and myotubes. Biomaterials 27 (24):4340–4347
Lam MT, Huang YC, Birla RK, Takayama S (2009) Microfeature guided skeletal muscle tissue engineering for highly organized three-dimensional free-standing constructs. Biomaterials 30 (6):1150–1155
Lazennec G, Jorgensen C (2008) Concise review: adult multipotent stromal cells and cancer: risk or benefit? Stem Cells 26(6):1387–1394
Le Grand F, Rudnicki MA (2007) Skeletal muscle satellite cells and adult myogenesis. Curr Opin Cell Biol 19(6):628–633
Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC et al (2005) Engineering vascularized skeletal muscle tissue. Nat Biotechnol 23(7):879–884
Lowe K (2006) Blood substitutes: from chemistry to clinic. J Mater Chem 16(43):4189–4196
Lustig A (1999) Crisis intervention: the role of telomerase. Proc Natl Acad Sci USA 96(7):3339–3341
Martin I, Wendt D, Herberer M (2004) The role of bioreactors in tissue engineering. Trends Biotechnol 22(2):80–86
Matsumoto T, Kano K, Kondo D, Fukuda N, Iribe Y, Tanaka N et al (2007) Mature adipocyte-derived dedifferentiated fat cells exhibit multilineage potential. J Cell Physiol 215(1):210–222
McFarland DC, Doumit ME, Minshall RD (1988) The turkey myogenic satellite cell: optimization of in vitro proliferation and differentiation. Tissue Cell 20(6):899–908
McFarland DC, Pesall JE, Norberg JM, Dvoracek MA (1991) Proliferation of the turkey myogenic satellite cell in a serum-free medium. Comp Biochem Physiol 99(1–2):163–167
Mead P, Slutsker L, Dietz A, McCaig L, Bresee J, Shapiro C, Griffin P, Tauxe R (1999) Food-related illness and death in the United States. Emerg Infect Dis 5(5):607–625
Merten OW (1999) Safety issues of animal products used in serum-free media. Dev Biol Stand 99:167–180
Mol A, Driessen NJ, Rutten MC, Hoerstrup SP, Bouten CV, Baaijens FP (2005) Tissue engineering of human heart valve leaflets: a novel bioreactor for a strain-based conditioning approach. Ann Biomed Eng 33(12):1778–1788
Nataro JP, Kaper JB (1998) Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11:142–201
Nestle M (1999) Animal vs. plant foods in human diets and health: is the historical record unequivocal? Proc Nutr Soc 58:211–218
Nicholson FA, Hutchison ML, Smith KA, Keevil CW, Chambers BJ, Moore A (2000) A study on farm manure applications to agricultural land and an assessment of the risks of pathogen transfer into the food chain. Project Number FS2526, Final report to the Ministry of Agriculture, Fisheries and Food, London
O'Hare M, Bond J, Clarke C, Takeuchi Y, Atherton A, Berry C, Moody J, Silver A, Davies D, Alsop A, Neville A, Jat P (2001) Conditional immortalization of freshly isolated human mammary fibroblasts and endothelial cells. Proc Natl Acad Sci USA 98 (2):646–651
Ohira Y, Yoshinaga T, Nomura T, Kawano F, Ishihara A, Nonaka I, Roy R, Edgerton V (2002) Gravitational unloading effects on muscle fiber size, phenotype and myonuclear number. Adv Space Res 30(4):777–781
Pathak V, Bukhari SAA, Bhat ZF, Sharma N (2008) Prospectus of in-vitro meat. Indian Food Industry Nov-Dec:33–35
Powell RE, Dodson MV, Cloud JG (1989) Cultivation and differentiation of satellite cells from skeletal muscle of the rainbow trout Salmo gairdneri. J Exp Zool 250(3):333–338
Prowse K, Greider C (1995) Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci USA 92(11):4818–4822
Radisic M, Euloth M, Yang L, Langer R, Freed LE, Vunjak-Novakovic G (2003) High-density seeding of myocyte cells for cardiac tissue engineering. Biotechnol Bioeng 82(4):403–414
Radisic M, Marsano A, Maidhof R, Wang Y, Vunjak-Novakovic G (2008) Cardiac tissue engineering using perfusion bioreactor systems. Nat Protoc 3(4):719–738
Reay D (2002) Intensive farming, US-style, is not sustainable worldwide. Nature 417(6884):15
Riboldi SA, Sampaolesi M, Neuenschwander P, Cossu G, Mantero S (2005) Electrospun degradable polyesterurethane membranes: potential scaffolds for skeletal muscle tissue engineering. Biomaterials 26(22):4606–4615
Rizzino A (2007) A challenge for regenerative medicine: proper genetic programming, not cellular mimicry. Dev Dyn 236 (12):3199–3207
Roelen BA, Lopes SM (2008) Of stem cells and gametes: similarities and differences. Curr Med Chem 15:1249–1256
Rosegrant M, Leach N, Gerpacio R (1999) Alternative futures for world cereal and meat consumption. Proc Nutr Soc 58(2):219–234
Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC et al (2005) Spontaneous human adult stem cell transformation. Cancer Res 65(8):3035–3039
Saha P, Trumbo P (1996) The nutritional adequacy of a limited vegan diet for a controlled ecological life-support system. Adv Space Res 18(4–5):63–72
Sanders T (1999) The nutritional adequacy of plant-based diets. Proc Nutr Soc 58(2):265–269
Savadogo P, Sawadogo L, Tiveau D (2007) Effects of grazing intensity and prescribed fire on soil physical and hydrological properties and pasture yield in the savanna woodlands of Burkina Faso. Agricult Ecosys Environ 118:80–92
Shah G (1999) Why do we still use serum in production of biopharmaceuticals? Dev Biol Stand 99:17–22
Solomon R, Johnston C (1997) Production of red meat should be curbed in order to conserve natural resources. J Am Diet Assoc 97(11):1249
Speedy AW (2003) Global production and consumption of animal source foods. J Nutr 133:4048S–4053S
Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, De Haan C (2006a) Livestock's long shadow: environmental issues and options (Rome: Food and Agriculture Organization of the United Nations, p. xxi). Available at: www.virtualcentre.org/en/library/key_pub/longshad/A0701E00.pdf. Accessed March 7, 2008
Steinfeld H, Wassenaar T, Jutzi S (2006b) Livestock production systems in developing countries: status, drivers, trends. Revue Scientifique et Technique de l'Office International des Epizooties, Paris 25(2):505–516
Stoker M, O'Neil C, Berryman S, Waxman V (1968) Anchorage and growth regulation in normal and virus-transformed cells. Int J Cancer 3:683–693
Talbot NC, Blomberg LA (2008) The pursuit of ES cell lines of domesticated ungulates. Stem Cell Reviews and Reports 4 (3):235–254
Technology Review (2003) Fractals support growing organs. Technology Research News. http://www.trnmag.com/Stories/2003/073003/Fractals_support_growing_organs_073003. Accessed July 30/August 6, 2003

Thornton P, Kruska R, Henninger N, Kristjanson P, Reid R, Atieno F, Odero A, Ndegwa T (2002) Mapping poverty and livestock in the developing world. International Livestock Research Institute, Nairobi, p 124

Van Eelen WF, van Kooten WJ, Westerhof W (1999) WO/1999/031223: industrial production of meat from in vitro cell cultures. Patent Description http://www.wipo.int/pctdb/en/wo.jsp?wo=1999031223 Accessed Mar. 2009

Vandenburgh H, Shansky J, Benesch-Lee F, Barbata V, Reid J, Thorrez L et al (2008) Drug screening platform based on the contractility of tissue engineered muscle. Muscle Nerve 37(4):438–447

Vein J (2004) Method for producing tissue engineered meat for consumption. US Patent Number 6835390, http://www.freepatentsonline.com/6835390.html-12/28/2004

Verma AK, Banerjee R (2010) Dietary fibre as functional ingredient in meat products: a novel approach for healthy living: a review. J Food Sci Technol 47(3):247–257

Wagers AJ, Weissman IL (2004) Plasticity of adult stem cells. Cell 116(5):639–648

Webster R (2002) The importance of animal influenza for human disease. Vaccine 20(Suppl 2):S16–S20

WHO (2001) Global burden of disease estimates for 2001. Geneva, World Health Organization. http://www3.who.int/whosis/menu.cfm?path=evidence,burden,burden_estimates,burden_estimates_2001&language=english, accessed 9 April 2004

Wilschut KJ, Jaksani S, Van Den Dolder J, Haagsman HP, Roelen BAJ (2008) Isolation and characterization of porcine adult muscle-derived progenitor cells. J Cell Biochem 105(5):1228–1239

Wilson EM, Hsieh MM, Rotwein P (2003) Autocrine growth factor signaling by insulin-like growth factor-II mediates myod-stimulated myocyte maturation. J Biol Chem 278(42):41109–41113

Wolfson W (2002) Raising the steaks. New Sci 176:60–63

Wolpert L, Beddington R, Brockes J, Jessel T, Lawrence P, Meyerowitz E (1998) Principles of development. Current Biology, 1st edn. Oxford University Press

Yablonka-Reuveni Z, Quinn LS, Nameroff M (1987) Isolation and clonal analysis of satellite cells from chicken pectoralis muscle. Dev Biol 119(1):252–259

Zandonella C (2003) Tissue engineering: the beat goes on. Nature 421(6926):884–886

Zandstra E, de Graaf C, van Trijp H (2000) Effects of variety and repeated in-home consumption on product acceptance. Appetite 35(2):113–119

Zuckerman SH, Doyle MP, Gorczynski R, Rosenthal GJ (1998) Preclinical biology of recombinant human hemoglobin, rHb1.1. Artif Cells Blood Substit Immobil Biotechnol 26(3):231–257