# American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (6): 441-459, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.441.459


# Animal-free Meat Biofabrication

[1]Z.F. Bhat and [2]Hina Bhat
[1]Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Division of Livestock Products Technology, R.S. Pura, 181 102, India
[2]Department of Biotechnology, University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir, 190006, India

*Corresponding Author: Z.F. Bhat, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Division of Livestock Products Technology, R.S. Pura, 181 102, India Tel: +91-9697555990*

## ABSTRACT

Nutrition-related diseases, food borne illnesses, resource use and pollution and use of farm animals are some serious consequences associated with conventional meat production system and consumers have expressed growing concern over them. Biofabrication, production of complex living and non-living biological products, is a potential solution to reduce these ill effects of current meat production system. The industrial potential of biofabrication technology is far beyond the traditional medically oriented tissue engineering and organ printing and, in the long term, biofabrication can contribute to the development of novel biotechnologies that can dramatically transform traditional animal-based agriculture by inventing animal-free food, leather and fur products. In this study we review the possibility of producing *in vitro* meat using tissue-engineering techniques that may offer health and environmental advantages by reducing environmental pollution and land use associated with current meat production systems. Besides, reducing the animal suffering significantly, it will also ensure sustainable production of designer, chemically safe and disease free meat as the conditions in an *in vitro* meat production system are controlled and manipulatable. The techniques required to produce *in vitro* meat are not beyond imagination and the basic methodology of an *in vitro* meat production system (IMPS) involves culturing muscle tissue in a liquid medium on a large scale but 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. In the long term, tissue-engineered meat is the inescapable future of humanity. However, in the short term the extremely high prohibitive cost of the biofabrication of tissue-engineered meat is the main potential obstacle, although large-scale production and market penetration are usually associated with a dramatic price reduction.

**Key words:** Biofabrication, cultured meat, meat substitute, need, objections

## INTRODUCTION

Conventional meat production system is a major source of pollution and a significant consumer of fossil fuels, land and water resources. 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*., 2006). 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. World meat production at present is contributing between 15 and 24% of total current greenhouse gas emissions; a great proportion of this percentage is due to deforestation to create grazing land (Steinfeld *et al*., 2006). 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*., 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). 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 *et al*., 2006). 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). With a growing population and great proportion of which facing starvation, it no longer makes sense to contribute staple crops toward inefficient meat production, where 1 kg poultry, pork and beef requires 2, 4 and 7 kg of grain, respectively (Rosegrant *et al*., 1999). Thus, establishment of an *in vitro* meat production system (IMPS) is becoming increasingly justifiable in light of the sizable negative effects of current meat production system.

The idea of *in vitro* meat for human consumption is not new but was predicted long back by Winston Churchill in the 1920s (Churchill, 1932). In 1912, Alexis Carrel managed to keep a piece of chick heart muscle alive and beating in a Petri dish. 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 (Benjaminson *et al*., 2002).

Meat is already cultured on small and early scales using a variety of basic procedures, including techniques that use scaffolds and those that rely on self-organization (Edelman *et al*., 2005). The different design approaches for an *in vitro* meat production system, all of which are designed to overcome the diffusion barrier, range from those currently in use (scaffold/cell culture based and self organizing/tissue culture techniques) to the more speculative possibilities.

**Scaffolding techniques:** In scaffold-based techniques, embryonic myoblasts or adult skeletal muscle satellite cells are proliferated, attached to a scaffold or carrier such as a collagen meshwork or microcarrier beads and then perfused with a culture medium in a stationary or rotating bioreactor. By introducing a variety of environmental cues, these cells fuse into myotubes, which can then differentiate into myofibers (Kosnik *et al*., 2003). The resulting myofibers may then be harvested, cooked and consumed as meat (Fig. 1, 2).

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). However, Catts and Zurr (2002) appear to have been the first to have actually produced meat by this method. 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 forms of scaffolding could also be used, for example, growing muscle tissue on large sheets of edible or easily separable material. The muscle tissue could be processed after being rolled up to suitable thicknesses (Edelman *et al*., 2005). While, these kinds of techniques work for producing ground processed (boneless) meats with soft consistency, they do not lend themselves to highly structured meats like steaks. However, cells can also be grown in substrates that allow for the development of 'self-organizing constructs that produce more rigid structures.

**Self-organizing tissue culture:** To produce highly structured meats, one would need a more ambitious approach, creating structured muscle tissue as self-organizing constructs (Dennis and Kosnik, 2000) or proliferating existing muscle tissue *in vitro*, like Benjaminson *et al*. (2002) who cultured Gold fish (Carassius auratus) muscle explants. They took slices of goldfish tissue, minced and centrifuged them to form pellets, placed them in Petri dishes in a nutrient medium and grew them for 7 days. The explanted tissue grew nearly 14% when using fetal bovine serum as the nutrient medium and over 13% when using Maitake mushroom extract. When the explants were placed in a culture containing dissociated *Carassius* skeletal muscle cells, explant surface area grew a surprising 79% in a week's time. After a week, the explants and their newly grown tissue, which looked like fresh fish filets, were cooked (marinated in olive oil and garlic and deep-fried) and presented to a panel for observation. The panel reported that the fish looked and smelled good enough to eat (Benjaminson *et al*., 2002; Britt, 2002; Sample, 2002; Hukill, 2006).

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. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if separated for long periods by more than 0.5 mm from a nutrient supply (Dennis and Kosnik, 2000). 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 (Zandonella, 2003). Like the

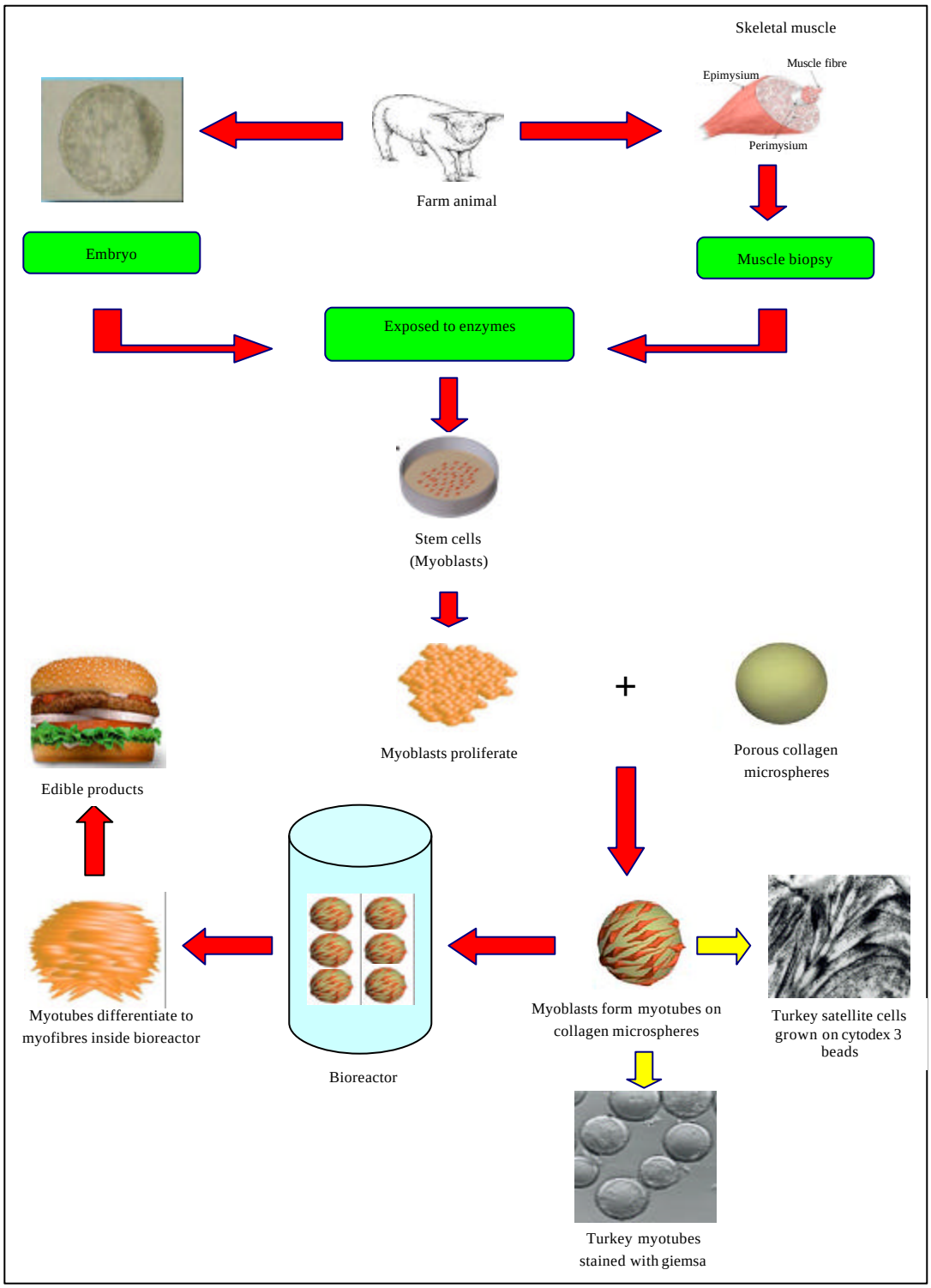


Fig. 1: Scaffold-based cultured meat production

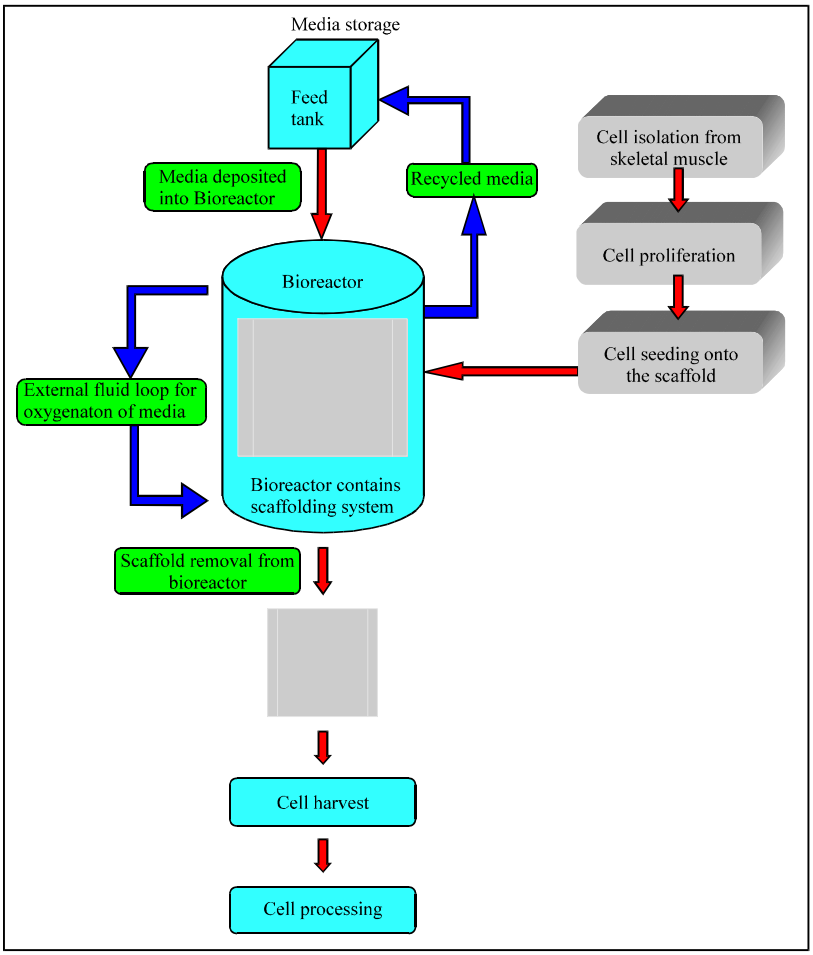


Fig. 2: Possible *in vitro* meat production scheme

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)

**Organ printing:** The various problems associated with the current tissue engineering techniques are that they cannot provide consistency, vascularization, fat marbling or other elements of workable and suitably-tasting meat that are not simply versions of ground soft meat. A potential solution to such problems comes from research on producing organs for transplantation procedures known as organ printing.

Organ printing uses the principles of ordinary printing technology-the technology used by inkjet printers to produce documents. Researchers use solutions containing single cells or balls of cells and spray these cell mixtures onto gels that act as printing paper. The paper can actually be removed through a simple heating technique or could potentially be automatically degradable. What happens is essentially that live cells are sprayed in layers to create any shape or structure desired. After spraying these three dimensional structures, the cells fuse into larger structures, such as rings and tubes or sheets. As a result, researchers argue that the feasibility of producing entire organs through printing has been proved. The organs would have not only the basic cellular

structure of the organ but would also include, built layer-by-layer, appropriate vascularization providing a blood supply to the entire product. For applications focused on producing meat, fat marbling could be added as well, providing taste and structure. Essentially, sheets and tubes of appropriate cellular components could create any sort of organ or tissue for transplantation or for consumption (Mironov *et al*., 2003; Aldhous, 2010; Hopkins and Dacey, 2008).

**Biophotonics:** Biophotonics is a new field that relies on the effects of lasers to move particles of matter into certain organizational structures, such as three-dimensional chessboard, or hexagonal arrays. In general, biophotonics refers to the process of using light to bind together particles of matter and the mechanisms of this field are still poorly understood. A surprising property of interacting light, this phenomenon produces so called 'optical matter in which the crystalline form of materials (such as polystyrene beads) can be held together by nets of infrared light that will fall apart when the light is removed. This is a phenomenon a step-up from optical tweezers that have been used for years to rotate or otherwise move tiny particles in laboratories. This has a binding effect among a group of particles that can lead them not only to be moved one by one to specific locations but that can coax them to form structures. Although, primarily sparking interest in medical technologies such as separating cells, or delivering medicine or other microencapsulized substances to individual cells, there is an intriguing possibility that such a technology could be used for the production of tissues, including meat (Hopkins and Dacey, 2008). Arrays of red blood cells and hamster ovaries have already been created using this technology (Mullins, 2006). Given the success of creating two-dimensional arrays, there is the possibility of producing tissue formations that use only light to hold the cells together, thus eliminating the need for scaffoldings (Hopkins and Dacey, 2008).

**Nanotechnology:** The ability of optical tweezers to rotate or move tiny particles has intrigued nanotechnologists, who have inventive plans for what to do with the molecular scale sized robots they would like to create (but so far, having few tools with which to make them). Nanotechnology (the production and alteration of materials at the level of the atom and molecule) holds out enormous possibilities and the holy grail of nanotechnology is some version of an assembler, a robot the size of a molecule that would allow moving matter at the atomic and molecular level. The obvious power of such a technology given that everything is made of the same basic atoms but simply arranged in different ways is that we would be able to construct virtually any substance we wanted from scratch by putting together exactly the molecules we wanted. Interestingly, one of the first examples given of the speculative technology of nanotechnology was that of synthesized meat. Thus, technologies ranging from the actual to the speculative promise a variety of ways to create real meat without killing animals. Though still commercially infeasible at the moment or in some cases technologically infeasible for several years to come, the point here is not to be distracted by the fact that we cannot yet make use of these technologies but rather to decide whether we should support the development of these technologies (Hopkins and Dacey, 2008).

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

**Cells:** *In vitro* meat can be produced by culturing the cells from farm animal species in large quantities starting from a relatively small number and 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 *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 (Askura *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*. (2008) 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.

**Fields:** Proliferation and differentiation of myoblasts have been found to be affected by the mechanical, electromagnetic, gravitational and fluid flow fields (Kosnik *et al*., 2003, De Deyne, 2000). Repetitive stretch and relaxation equal to 10% of length, six times per hour increase differentiation into myotubes (Powell *et al*., 2002). Myoblasts seeded with magnetic microparticles induced differentiation by placing them in a magnetic field without adding special growth factors or any conditioned medium (Yuge and Kataoka, 2000). Electrical stimulation also contributes to differentiation, as well as sarcomere formation within established myotubes (Kosnik *et al*., 2003).

**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 microspheres 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 (Pathak *et al*., 2008). 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) which 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 and flow 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 key 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).

**Culture media and growth factors**

**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.

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 but for the generation of cultured meat, however, 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.

**Need of cultured meat:** Meat composition and quality can be controlled by manipulating the composition of the culture medium and co-culturing with other cell types. The flavor, fatty acid composition, fat content and ratio of saturated to poly-unsaturated fatty acids of the cultured meat can be better controlled. 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).

- As laboratory produced meat does not come from a living animal, it therefore significantly minimizes the religious taboos like Jhatka, Jewish and Halal etc. (Pathak *et al*., 2008)
- 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, the chance of meat contamination and incidence of food borne disease could be significantly reduced. In addition, the risks of exposure to pesticides, arsenic, dioxins and hormones associated with conventional meat could also be significantly reduced
- 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 and thus a sustainable alternative to global trade of meats from rare and endangered animals will help in increasing wild populations of many species in many countries
- *In vitro* system reduces animal use in the meat production system as theoretically a single farm animal may be used to produce the world's meat supply

- As the biological structures in addition to muscle tissue are not required to produce meat in an *in vitro* system, it reduces the amount of nutrients and energy needed for their growth and maintenance
- *In vitro* system significantly lowers time to grow the meat and takes several weeks instead of months (for chickens) or years (for pigs and cows) before the meat can be harvested and thus, the amount of feed and labor required per kg of *in vitro* cultured meat is much lower
- Bioreactors for *in vitro* meat production, unlike farm animals, do not need extra space and can be stacked up in a fabric hall. Thus, nutritional costs for *in vitro* cultured meat will be significantly lower and the decrease in costs of resources, labor and land may be compensated by the extra costs of a stricter hygiene regime, stricter control, computer management, etc.
- The 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. 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
- Need for other protein sources demands production of cultured meat and because it is, unlike the other products, animal-derived and with respect to composition most like meat, it may be the preferred alternative

A definite market is available for meat substitutes. Examples are legume-based and mycoprotein-based meat substitutes. A small market comprising the vegetarians that do not eat meat for ethical reasons is also available. However, it is still unclear if animal suffering minimized through *in vitro* meat production actually convinces all the vegetarians (Pathak *et al*., 2008).

- It will not be possible to produce all the meat in an environmental and animal friendly way for the growing population and thus, there is a rather conventional meat market for *in vitro* meat
- The proteins produced using plants and fungi are animal friendly, sustainable and have been used to make a variety of good chief products but they lack a good texture and taste and such products are no solution for the craving for meat
- 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
- 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
- The comparatively minimal land requirement of an *in vitro* meat production system allows meat production and processing to take place domestically in countries which would normally rely on imported meats. By bringing the stages of the meat production process closer together spatially and temporally, meat supply can be better determined by demand
- Other factors like potential impact on reducing cardiovascular diseases, greenhouse gas emissions and liberation of land for nature (including wild animals), prevention of animal suffering and food scarcity that can be expected with an increasing world population

**Objections to cultured meat:** Hopkins and Dacey (2008) defined the following objections to cultured meat:

**Danger:** Worry about the danger of consuming untested (or even tested) novel materials.

**Cannibalism:** The ability to culture human muscle tissue leading to victimless cannibalism (Peterson, 2006; Mcilroy, 2006).

**Reality of meat:** Some people may feel that *in vitro* meat is not real meat and they will think of it as artificial meat or synthetic meat and not the real thing. As such, they depreciate the value of the meat in the same way they would look down on artificial flowers or synthetic diamonds.

**Naturalness:** The quality of being unnatural is the primary objection to cultured meat.

**Yuck factor:** Ever since Leon Kass's call for making human cloning illegal largely as a result of the wisdom of repugnance or the less nobly known yuck factor, people have paid more attention to the reaction of disgust in trying to judge whether a new and especially biotechnological, process is morally permissible and whether it should be legally permissible (Kass, 1997).

**Technological fix is moral cowardice:** One argument against this whole approach is about seeking a technological fix at all.

**Wrong moral motivations:** A related argument against promoting cultured meat is that this would be motivated by selfishness when we should be self-sacrificing and virtuous. That is, refusing to stop eating meat until a new technology relieves our discomfort is hardly morally laudable.

**Delay moral change:** Another worry might be that hoping for a technological fix will make people more at ease with meat-eating now, will make them think they do not need to change anything now because in the near future technology will solve their difficulties.

**The lives of food animals are better than nothing:** One objection that is already familiar from critiques of ethical vegetarianism is that animals' lives will go better, paradoxically, in a world with something like the present meat industry, than in a world with universal or widespread vegetarianism.

**Taint of the source:** Another argument is that *in vitro* meat will use original cells gathered from some animal in a morally suspect way and that the use of such cells will morally taint all future generations of tissue (Hawthorne, 2005).

**Animal integrity:** Novel consideration brought up for the purposes of exonerating some people's moral intuitions that animals should not be biotechnologically altered is that 1 of animal integrity. In regards to a suggestion that chickens might be genetically altered into insentient lumps of flesh, producing eggs and meat, some have argued that such alterations are intuitively wrong and this sense of wrong can be captured by the idea that the integrity of the animal has been violated.

**Dominion versus reverence:** A final, more sweeping version of the objection from respect and dignity, which applies to many different types of technological manipulations, goes like this. To revere a creature (or perhaps the world in general) we must accept what is given about it rather than transforming its nature.

## CONCLUSIONS

*In vitro* meat production is a sustainable and safer system and could offer a number of benefits. With cultured meat, the composition, flavour and functional role of meat could be better controlled; the incidence of food borne disease could be significantly reduced; and resources could be used more efficiently. Cultured meat has the potential to make eating animals unnecessary, even while satisfying all the nutritional and hedonic requirements of meat eaters (Hopkins and Dacey, 2008). It also has the potential to greatly reduce animal suffering. 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. *In vitro* meat production on an industrial scale is feasible only when a relatively cost-effective process creating a product qualitatively competitive with existing meat products is established and provided with governmental subsidization like that provided to other agribusinesses.

## REFERENCES

Aldhous, P., 2010. Print me a heart and a set of arteries. New Scientist.

Amit, M., M.K. Carpenter., M.S. Inokuma., C.P. Chiu and C.P. Harris *et al*., 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev. Biol., 227: 271-278.

Askura, A., M. Komaki and M.A. Rudnicki, 2001. Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic and adipogenic differentiation. Differentiation, 68: 245-253.

Asner, G.P., A.J. Elmore, L.P. Olander, R.E. Martin and A.T. Harris, 2004. Grazing systems, ecosystem responses and global change. Annu. Rev. Environ. Resour., 29: 261-299.

Bach, A.D., J. Stem-Straeter, J.P. Beier, H. Bannasch and G.B. Stark, 2003. Engineering of muscle tissue. Clin. Plast. Surg., 30: 589-599.

Barnard, N.D., A. Nicholson and J.L. Howard, 1995. The medical costs attributable to meat consumption. Prev. Med., 24: 646-655.

Benjaminson, M.A., J.A. Gilchriest and M. Lorenz, 2002. *in vitro* edible muscle protein production system (MPPS): Stage 1, fish. Acta Astronaut., 51: 879-889.

Bernardo, M.E., N. Zaffaroni, F. Novara, A.M. Cometa and M.A. Avanzini *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: 9142-9149.

Blanton, J.R., A.L. Grand, D.C. McFarland, J.P. Robinson and C.A. Bidwell, 1999. Isolation of two populations of myoblasts from porcine skeletal muscle. Muscle Nerve, 22: 43-50.

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

Borenstein, J.T., H. Terai, K.R. King, E.J. Weinberg, M.R. Kaazempur-Mofrad and J.P. Vacanti, 2002. Microfabrication technology for vascularized tissue engineering. Biomed. Microdevices, 4: 167-175.

Britt, R.R., 2002. Food of the future: Fish flesh grown without the fish. http://www.space.com/scienceastronomy/generalscience/fish_food_020329.html.

Burton, N.M., J.L. Vierck, L. Krabbenhoft, K. Byrne and M.V. Dodson, 2000. Methods for animal satellite cell culture under a variety of conditions. Methods Cell Sci., 22: 51-61.

Canavan, H.E., X. Cheng, D.J. Graham, B.D. Ratner and D.G. Castner, 2005. Cell sheet detachment affects the extracellular matrix: A surface science study comparing thermal liftoff, enzymatic and mechanical methods. J. Biomed. Mater. Res., 75: 1-13.

Carrier, R.L., M. Papadaki, M. Rupnick, F.J. Schoen and N. Bursac *et al.*, 1999. Cardiac tissue engineering: Cell seeding, cultivation parameters and tissue construct characterization. Biotechnol. Bioeng., 64: 580-589.

Carrier, R.L., M. Rupnick, R. Langer, F.J. Schoen, L.E. Freed and G. Vunjak-Novakovic, 2002. Perfusion improves tissue architecture of engineered cardiac muscle. Tissue Eng., 8: 175-188.

Catts, O. and I. Zurr, 2002. Growing semi-living sculptures: The tissue culture and art project. Leonardo, 35: 365-370.

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

Cheng, L., X. Gu, J.E. Sanderson, X. Wang and K. Lee *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: 1123-1133.

Churchill, W., 1932. Fifty Years Hence. In Thoughts and Adventures. Thornton Butterworth, London, pp: 24–27.

Coecke, S., M. Balls, G. Bowe, J. Davis and G. Gstraunthaler *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: 261-287.

Da Silva, R.M.P., J.F. Mano and R.L. Reis, 2007. Smart thermoresponsive coatings and surfaces for tissue engineering: Switching cell-material boundaries. Trends Biotechnol., 25: 577-583.

De Deyne, P.G., 2000. Formation of sarcomeres in developing myotubes: Role of mechanical stretch and contractile activation. Am. J. Physiol. Cell Physiol., 279: 1801-1811.

Dennis, R. and P. Kosnik, 2000. Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered *in vitro*. *in vitro* Cell Dev. Biol. Anim., 36: 327-335.

Dennis, R.G., P.E. Kosnik, M.E. Gilbert and J.A. Faulkner, 2001. Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines. Am. J. Physiol. Cell Physiol., 280: 288-295.

Dieryck, W., J. Pagnier, C. Poyart, M.C. Marden and V. Gruber *et al.*, 1997. Human haemoglobin from transgenic tobacco. Nature, 386: 29-30.

Dodson, M.V. and B.A. Mathison, 1988. Comparison of ovine and rat muscle-derived satellite cells: Response to insulin. Tissue Cell, 20: 909-918.

Dodson, M.V., D.C. McFarland, A.L. Grant, M.E. Doumit and S.G. Velleman, 1996. Extrinsic regulation of domestic animal-derived satellite cells. Domestic Anim. Endocrinol., 13: 107-126.

Dodson, M.V., D.C. McFarland, E.L. Martin and M.A. Brannon, 1986. Isolation of satellite cells from ovine skeletal muscles. J. Tissue Cult. Meth., 10: 233-237.

Dodson, M.V., E.L. Martin, M.A. Brannon, B.A. Mathison and D.C. McFarland, 1987. Optimization of bovine satellite cell derived myotube formation *in vitro*. Tissue Cell, 19: 159-166.

Doumit, M.E., D.R. Cook and R.A. Merkel, 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: 326-332.

Duque, P., E. Gomez, E. Diaz, N. Facal, C. Hidalgo and C. Diez, 2003. Use of two replacements of serum during bovine embryo culture *in vitro*. Theriogenology, 59: 889-899.

Edelman, P.D., D.C. McFarland, V.A. Mironov and J.G. Matheny, 2005. Commentary: *in vitro*-cultured meat production. Tissue Eng., 11: 659-662.

Engler, A.J., M.A. Griffin, S. Sen, C.G. Bonnemann, H.L. Sweeney and D.E. Discher, 2004. Myotubes differentiate optimally on substrates with tissue-like stiffness, pathological implications for soft or stiff microenvironments. J. Cell Biol., 166: 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, I.S., S. Meakens and Enter-Net Participants, 2006. Surveillance of enteric pathogens in Europe and beyond: Enter-net annual report for 2004. Eur. Surveill., 11: E060824.3-E060824.3.

Florini, J.R., K.A. Magri, D.Z. Ewton, P.L. James, K. Grindstaff and P.S. Rotwein, 1991. Spontaneous differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. J. Biol. Chem., 266: 15917-15923.

Froud, S.J., 1999. The development, benefits and disadvantages of serum-free media. Dev. Biol. Stand., 99: 157-166.

Gimble, J.M., A.J. Katz and B.A. Bunnell, 2007. Adipose-derived stem cells for regenerative medicine. Circ. Res., 100: 1249-1249.

Hawthorne, M., 2005. From fiction to fork. SATYA September: 2005 (The New Taste of Vegan). http://www.satyamag.com/sept05/hawthorne.html.

Hopkins, P.D. and A. Dacey, 2008. Vegetarian meat: Could technology save animals and satisfy meat eaters. J. Agric. Environ. Ethics, 21: 579-596.

Houdebine, L.M., 2009. Production of pharmaceutical proteins by transgenic animals. Comp. Immunol. Microbiol. Infect. Dis., 32: 107-121.

Hukill, T., 2006. Would you eat lab-grown meat. Environment. http://www.alternet.org/environment/38755.

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

Kass, L., 1997. The wisdom of repugnance. New Republic, 216: 17-26.

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

Keefer, C.L., D. Pant, L. Blomberg and N.C. Talbot, 2007. Challenges and prospects for the establishment of embryonic stem cell lines of domestic ungulates. Anim. Reprod. Sci., 98: 147-168.

Kim, M.J., Y.S. Choi, S.H. Yang, H.N. Hong and S.W. Cho*et al*., 2006. Muscle regeneration by adipose tissue-derived adult stem cells attached to injectable PLGA spheres. Biochem. Biophys. Res. Commun., 348: 386-392.

Kosnik, P.E., J.A. Faulkner and R.G. Dennis, 2001. Functional development of engineered skeletal muscle from adult and neonatal rats. Tissue Eng., 7: 573-584.

Kosnik, P.E., R.G. Dennis and H.H. Vandenburgh, 2003. Tissue Engineering Skeletal Muscle. In: Functional Tissue Engineering, Guilak, F. (Ed.). Springer-Verlag, New York, pp: 377–392.

Lam, M.T., S. Sim, X. Zhu and S. Takayama, 2006. The effect of continuous wavy micropatterns on silicone substrates on the alignment of skeletal muscle myoblasts and myotubes. Biomaterials, 27: 4340-4347.

Lam, M.T., Y.C. Huang, R.K. Birla and S. Takayama, 2009. Microfeature guided skeletal muscle tissue engineering for highly organized three-dimensional free-standing constructs. Biomaterials, 30: 1150-1155.

Lazennec, G. and C. Jorgensen, 2008. Concise review: Adult multipotent stromal cells and cancer: Risk or benefit. Stem Cells, 26: 1387-1394.

Levenberg, S., J. Rouwkema, M. Macdonald, E.S. Garfein and D.S. Kohane *et al.*, 2005. Engineering vascularized skeletal muscle tissue. Nat. Biotechnol., 23: 879-884.

Lowe, K.C., 2006. Blood substitutes: From chemistry to clinic. J. Mater. Chem., 16: 4189-4196.

Martin, I., D. Wendt and M. Herberer, 2004. The role of bioreactors in tissue engineering. Trends Biotechnol., 22: 80-86.

Matsumoto, T., K. Kano, D. Kondo, N. Fukuda and Y. Iribe *et al.*, 2008. Mature adipocyte-derived dedifferentiated fat cells exhibit multilineage potential. J. Cell. Physiol., 215: 210-222.

McFarland, D.C., J.E. Pesall, J.M. Norberg and M.A. Dvoracek, 1991. Proliferation of the turkey myogenic satellite cell in a serum-free medium. Comp. Biochem. Physiol., 99: 163-167.

McFarland, D.C., M.E. Doumit and R.D. Minshall, 1988. The turkey myogenic satellite cell: Optimization of *in vitro* proliferation and differentiation. Tissue Cell, 20: 899-908.

Mcilroy, A., 2006. Will consumers have a beef with test-tube meat?. http://www.theglobeandmail.com/servlet/story/LAC.20060327.MEAT27/TPStory/?query=meat+starter+cells&pageRequested=all&print=true.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig and J.S. Bresee *et al.*, 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Merten, O.W., 1999. Safety issues of animal products used in serum-free media. Dev. Biol. Stand., 99: 167-180.

Mironov, V., T. Boland, T. Trusk, G. Forgacs and R.R. Markwald, 2003. Organ printing: Computer-aided jet-based 3D tissue engineering. Trends Biotechnol., 21: 157-161.

Mullins, J., 2006. The stuff of beams: Building with light. New Scientist, 2551: 44-47.

Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.

Nicholson, F.A., M.L. Hutchison, K.A. Smith, C.W. Keevil, B.J. Chambers and A. Moore, 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.

Pathak, V., S.A.A. Bukhari, Z.F. Bhat and N. Sharma, 2008. Prospectus of *in vitro* meat. Indian Food Ind., pp: 33-35.

Peterson, D., 2006. The *in vitro* cultured meat: No cows needed. Special to the Catalyst. http://www.musc.edu/catalyst/archive/2006/co1-20invitro.html.

Powell, R.E., M.V. Dodson and J.G. Cloud, 1989. Cultivation and differentiation of satellite cells from skeletal muscle of the rainbow trout *Salmo gairdneri*. J. Exp. Zool., 250: 333-338.

Powell, C.A., B.L. Smiley, J. Mills and H.H. Vandenburgh, 2002. Mechanical stimulation improves tissue-engineered human skeletal muscle. Am. J. Physiol. Cell Physiol., 283: 1557-1565.

Radisic, M., M. Euloth, L. Yang, R. Langer, L.E. Freed and G. Vunjak-Novakovic, 2003. High-density seeding of myocyte cells for cardiac tissue engineering. Biotechnol. Bioeng., 82: 403-414.

Radisic, M., A. Marsano, R. Maidhof, Y. Wang and G. Vunjak-Novakovic, 2008. Cardiac tissue engineering using perfusion bioreactor systems. Nat. Protocols, 3: 719-738.

Riboldi, S.A., M. Sampaolesi, P. Neuenschwander, G. Cossu and S. Mantero, 2005. Electrospun degradable polyesterurethane membranes: Potential scaffolds for skeletal muscle tissue engineering. Biomaterials, 26: 4606-4615.

Rizzino, A., 2007. A challenge for regenerative medicine: Proper genetic programming, not cellular mimicry. Dev. Dyn., 236: 3199-3207.

Rosegrant, M.W., N. Leach and R.V. Gerpacio, 1999. Alternative futures for world cereal and meat consumption. Proc. Nutr. Soc., 58: 219-234.

Rubio, D., J. Garcia-Castro, M.C. Martin, R.D. La-Fuente, J.C. Cigudosa, A.C. Lloyd and A. Bernad, 2005. Spontaneous human adult stem cell transformation. Cancer Res., 65: 3035-3039.

Sample, I., 2002. Fish fillets grow in tank. New Scientist. http://www.newscientist.com /article.ns?id=dn2066.

Sanders, T.A.B., 1999. The nutritional adequacy of plant-based diets. Proc. Nutr. Soc., 58: 265-269.

Savadogo, P., L. Savadogo and D. Tiveau, 2007. Effects of grazing intensity and prescribed fire on soil physical and hydrological properties and pasture yield in the savanna woodlands of Burkina Faso. Agric. Ecosyst. Environ., 118: 80-92.

Shah, G., 1999. Why do we still use serum in production of biopharmaceuticals. Dev. Biol. Stand., 99: 17-22.

Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales and C. De Haan, 2006. Livestock's Long Shadow: Environmental Issues and Options. Food and Agricultural Organization, Rome.

Stoker, M., C. O'Neil, S. Berryman and V. Waxman, 1968. Anchorage and growth regulation in normal and virus-transformed cells. Int. J. Cancer, 3: 683-693.

Talbot, N.C. and L.A. Blomberg, 2008. The pursuit of ES cell lines of domesticated ungulates. Stem Cell Rev. Rep., 4: 235-254.

Van Eelen, W.F., W.J. van Kooten, W. Westerhof and C. Mummery, 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.

WHO, 2001. Global Burden of Disease Estimates for 2001. World Health Organization, Geneva.

Wagers, A.J. and I.L. Weissman, 2004. Plasticity of adult stem cells. Cell, 116: 639-648.

Webster, R.G., 2002. The importance of animal influenza for human disease. Vaccine, 2: 16-20.

Wilschut, K.J., S. Jaksani, J.V. Den Dolder, H.P. Haagsman and B.A. Roelen, 2008. Isolation and characterization of porcine adult muscle-derived progenitor cells. J. Cell Biochem., 105: 1228-1239.

Wilson, E.M., M.M. Hsieh and P. Rotwein, 2003. Autocrine growth factor signaling by insulin-like growth factor-II mediates myod-stimulated myocyte maturation. J. Biol. Chem., 278: 41109-41113.

Wolfson, W., 2002. Raising the steaks. New Scientist, 176: 60-63.

Yablonka-Reuveni, Z., L.S. Quinn and M. Nameroff, 1987. Isolation and clonal analysis of satellite cells from chicken pectoralis muscle. Dev. Biol., 119: 252-259.

Yuge, L. and K. Kataoka, 2000. Differentiation of myoblasts accelerated in culture in a magnetic field. *in vitro* Cell. Dev. Biol. Anim., 36: 383-383.

Zandonella, C., 2003. Tissue engineering: The beat goes on. Nature, 421: 884-886.

Zuckerman, S.H., M.P. Doyle, R. Gorczynski and G.J. Rosenthal, 1998. Preclinical biology of recombinant human hemoglobin, rHb1.1. Artif. Cells Blood Substit. Immobil. Biotechnol., 26: 231-257.