International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2017; 2(1): 34-39

www.veterinarypaper.com
Received: 09-11-2016
Accepted: 10-12-2016

Yogesh Kumar
Department of Livestock Products Technology, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Rajkumar Berwal
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Anurag Pandey
Department of Livestock Products Technology, Rajasthan University of Veterinary and Animal Sciences, Bikaner Rajasthan, India

Amit Sharma
Department of Livestock Production Management, Rajasthan University of Veterinary and Animal Sciences, Bikaner Rajasthan, India

Vikas Sharma
Department of Animal Genetics & Breeding; Post Graduate Institute of Veterinary Education & Research, Jaipur, Rajasthan, India

Correspondence
Yogesh Kumar
Department of Livestock Products Technology, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Hydroponics meat: An envisaging boon for sustainable meat production through biotechnological approach - A review

Yogesh Kumar, Rajkumar Berwal, Anurag Pandey, Amit Sharma and Vikas Sharma

Abstract
The most envisaging novel application of biotechnology in meat processing is the production of hydroponic meat. Hydroponic meat is an idea of manufacturing animal meat through tissue engineering technology without using an actual animal. All this would happen without any genetic manipulation in tissue i.e. without the need to interfere with the cells' genetic sequences. It is alleged to have financial, health, animal welfare, hygienic meat production and environmental advantages over conventional meat. Hydroponic meat production system envisages sustainable production of a new chemically safe, lean and hygienic meat besides significantly reducing the animal suffering. It requires an appropriate stem cell source scaffold for attachment, media for growth inside a bioreactor for proliferation and differentiation of cells into tissues. Stem cells are taken painlessly from live animal and transferred into a culture media in bioreactor; where, they start to proliferate and grow independently from the animal. Thus, hydroponic meat production may play an important role in the future for sustainable meat production, when natural resources become a limiting factor for meat production. It is not only about the substitute to meat production but also selective manipulation of meat quality.

Keywords: Hydroponic meat, tissue engineering, bioreactor, culture media, stem cell, hygienic meat

1. Introduction

Meat is an important nutritional and social factor i.e. consumer behaviour related to food animal species and slaughter process according to religion specific for the human being consumption and it is alleged that consumption of meat is expected to increase in years to come. Global meat consumption is estimated to be 311.8 million tonnes (FAO, 2014) [15] and is growing at the rate of 4.7 million tonnes per year. To meet this substantial increase in meat demand, it is difficult as already large portion of cultivated land about is under livestock management and feed production. Due to an expanding population and increasing meat consumption globally, it is predicted that meat consumption will double in the coming forty years (FAO, 2006) [13]. According to FAO (2011) [14] the capacity of conventional meat production was maximum or close to maximum. As a result meat will become scarce, more expensive and eventually a luxury food.

Animal meat production contributes considerable portion of gas emission, land usage, water and energy consumption. The contribution of livestock in the ratio of greenhouse gases to total emission of carbon dioxide, methane and nitrous oxide is 9%, 39% and 65%, respectively. Another motivation for livestock alternatives is the concern about animal welfare. Tonsor and Olynk (2011) [36] indicated that non-vegetarians decrease consumption of meat proportional with exposure to awareness campaigns of animal welfare through public media. Thus, public concerns about animal welfare affect consumer behavior thereby forcing the meat industry to continuously evaluate its practices. There are also public health problems surrounding animal production. These problems pertain to meat consumption not production i.e. cardiovascular diseases, diabetes and colorectal cancer are alleged to be associated with the consumption of red meat (Larsson and Wolk, 2006) [25]. Specific meta-analysis by Larsson and Wolk (2006) [25]; suggested that as little as 120 g of red meat/ day or 30 g of processed meat/ day significantly raise the relative risk of colorectal cancer.

In addition to these adverse health effects, food borne pathogens found in meat such as *Salmonella*, *Campylobacter* and *E. coli* are responsible for millions of episodes of illness every year. These pathogens and emerging diseases, such as avian and swine influenza are associated with the intensity of livestock farming and other anthropogenic developments in the bio-industry.

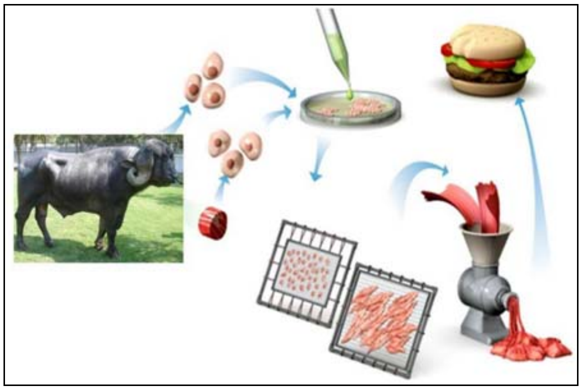

Fig 1: Model of farm to fork approach of hydroponic meat production

Hydroponic meat production could be one of the solution for all these above mentioned problems. It is also known as artificial meat, cultured meat, Hydroponic meat, Lab meat, In-vitro meat, Synthetic meat, Test tube meat, Tube steak and Victimless meat. The aim of this progressive technology is to produce meat through tissue-engineering without any genetic manipulation in tissue i.e. without the need to interfere with the cells' genetic sequences. In this technique, the stem cells of skeletal muscle also called as myosatellite are taken from the live animal and are put into a culture media in bioreactor at suitably maintained temperature and relative humidity; where, they start to multiply, grow and differentiate into muscle tissue.

2. History of hydroponic meat production

In 1912, Alexis Carrel managed to keep a piece of chick heart muscle alive and beating in a petri dish demonstrating that it may possible to keep muscle tissue alive outside the body (www.wikipedia.com). In 1950's Willem Van Eelen of Netherlands had idea of using tissue culture for the generation of hydroponic meat that was patented in 1999 (www.wikipedia.com).

Table 1: Timeline of biotechnological approach towards hydroponic meat development

Year	Scientist(s)	Experimental tools of different generation
1912	Alexis Carrel	Piece of chick heart muscle
1950	Willem Van Eelen	Tissue culture for hydroponic meat production
2002	Catts and Zurr	Muscle biopsies from frogs
2002	Benjaminson *et al.*	Muscle tissue from the common gold fish
2007	Keefer *et al.*	Ungulate stem cell lines
2007	Gimble *et al.*	Adipose tissue derived adult stem cells
2008	Talbot and Blomberg	Embryonic stem cell lines
2008	Wilschut *et al.*	Myosatellite cells
2013	Prof. Mark Post	In-vitro meat based burger

Source: www.wikipedia.com

SymbioticA harvested muscle biopsies from frogs and kept these tissues alive and growing in culture dishes (Catts and Zurr, 2002) [9]. Benjaminson *et al*. (2002) [4] cultured muscle tissue from the common gold fish (*Carassius auratus*) in petri dishes aiming to explore culturing animal tissues or muscle protein for space flights or habituation of space stations. These cultured muscle tissue were washed, dipped in olive oil with spices covered in bread crumbs and fried. A taste panel judged these processed explants and agreed that the product was acceptable as food.

In year 2013, Prof. Mark Post cooked world's first *in-vitro* meat based burger and was tasted by a sensory panel in London. The burger contained 15 g meat burger patty developed with laboratory grown beef. He alleged that hydroponic meat will be available in supermarkets in 10 - 20 years.

3. Techniques for production of hydroponic meat:

Hydroponic meat has been cultured by variety of techniques from using scaffolds to those which rely on self-organization but presently it is practiced at small scale (Edelman *et al*., 2005) [11] only in laboratory not at industrial level.

Table 2: Development of different techniques for hydroponic meat production

Year	Scientist(s)	Technique
2002	Catts and Zurr	Scaffolding technique
2002	Wolfson	Scaffolding technique
2007	Gimble *et al*.	Adipose tissue derived adult stem cells
2007	Matsumoto *et al*.	Dedifferentiated fat cells
2008	Kazama *et al*.	Skeletal myocytes

All of the different design approaches for an in-vitro meat production system are designed to overcome the diffusion barrier (Scaffold/ cell culture based and self-organizing/ tissue culture based techniques) into the more speculative possibilities such as organ printing, bio photonics, nanotechnology (Bhat and Bhat, 2011) [6, 7], scaffolding technique (Catts and Zurr (2002) [9]; Wolfson (2002) [40]. In this technique meat is produced by isolation of embryonic myoblasts or adult skeletal muscle satellite cells from animals. These cells are ideal for culturing since these cells have an almost infinite self-renewal capacity. But these cells may be specifically stimulated to differentiate into myoblasts and may in accurately recapitulate my genesis (Bach *et al*., 2003) [2]. However, establishing ungulate stem cell lines over the past decades have been generally unsuccessful with difficulties arising in the recognition, isolation and differentiation of these cells (Keefer *et al*., 2007) [20]. Until now true embryonic stem cell lines have only been generated from mouse, rhesus monkey, human and rat embryos (Talbot and Blomberg, 2008) [34]. Myosatellite cells have been isolated and characterized from the skeletal muscle tissue of fish (Wilschut *et al*., 2008) [39]. Adult stem cells from animals can be used as myosatellite cells; adult stem cell type with multilineage potential (Asakura *et al*., 2001) [1]. Adult stem cells have been isolated from several different adult tissues but their hydroponic proliferation capacity is not unlimited and they can proliferate *in vitro* for several months at most. These cells have the capacity to differentiate into skeletal muscle cells although not very efficiently at present. However, there is possibility that these adult stem cells are prone to malignant transformation in long term culture.

Adipose tissue derived adult stem cells (ADSCs) is another relevant cell type for in-vitro meat production (Gimble *et al*., 2007) [16]; which can be obtained from subcutaneous fat and subsequently Trans differentiated to myogenic, osteogenic, chondrogenic or adipogenic cell lineages. Adipose tissue derived stem cells immortalize at high frequency and undergo spontaneous transformation in long term culturing (Rubio *et al*., 2005) [32]. Matsumoto *et al*. (2007) [28] reported that mature adipocytes can be dedifferentiated hydroponically into a multipotent preadipocyte cell line known as dedifferentiated fat (DFAT) cells. These DFAT cells are capable of transdifferentiation into skeletal myocytes (Kazama *et al*., 2008) [19]. Myoblasts are not able to produce contractile proteins but produce little extracellular matrix too. Fibroblasts are responsible for production of extra cellular matrix. Currently only thin tissues are produced because of passive diffusion limitations. To overcome tissue thickness limit of 100-200 µm, a vasculature needs to be created (Jain *et al*., 2005) [18].

3.1 Scaffolds/ Substratum

A substratum or scaffold must be provided for proliferation and differentiation of myoblasts as they are anchorage dependent cells. Scaffolding mechanisms differ in shape, composition, characteristics and 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 in order to optimize muscle cell and tissue morphology. 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) [12] and its by-products must be edible and natural and may be derived from non-animal sources. Edelman *et al*. (2005) [11] proposed porous beads made of edible collagen as a substrate while Van Eelen *et al*. (1999) [37] proposed a collagen meshwork described as a collagen sponge of bovine origin. Other scaffolds include large elastic sheets or an array of long thin filaments. Cytodex-3 microcarrier beads have been used as scaffolds in rotary bioreactors.

3.2 Bioreactor

Commercial production of hydroponic meat based products requires large bioreactors for large scale culturing for the generation of sufficient number of muscle cells. The designing of a bioreactor 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 3-dimensional culture submerged in the medium in a perpetual free fall state, which improves diffusion with high mass transfer rates at minimal levels of shear stress, producing 3-dimensional tissues with structures very similar to those in-vivo (Martin *et al*., 2004) [27]. Direct perfusion bioreactors are used for high density uniform myocyte cell seeding (Radisic *et al*., 2003) [30]. It is necessary to have adequate oxygen perfusion during cell seeding and cultivation on the scaffold (Radisic *et al*., 2008) [31]. Adequate oxygen perfusion is mediated by bioreactors which increase mass transport between culture medium and cells by the use of oxygen carriers to mimic hemoglobin provided oxygen supply to maintain high oxygen concentrations in solution's similar to that of blood. Artificial perfluoro chemicals are used as oxygen carriers (Lowe, 2006) [26].

3.3 Culture media and growth factors

Hydroponic meat needs an affordable system containing the necessary nutritional components available in free form to myoblasts and accompanying cells. Myoblast culturing usually takes place in animal serum. Foetal bovine serum is being the standard supplement for cell culture media because of its hydroponic source.

Serum free media have been developed to support in-vitro myosatellite cell cultures from the turkey, sheep and pig. A sort of paracrine signaling system can be arranged so that co-cultured cell types can secrete growth factors necessary for growth and proliferation of neighboring cells. Appropriate co-culturing with 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, as well as myosatellite cell proliferation in several meat animal species hydroponically.

3.4 Atrophy and exercise

Hydroponic meat can undergo atrophy or wasting due to reduction of cell size caused by lack of use and denervation, or one of a variety of diseases. Regular contraction is a necessity for skeletal muscle and promotes differentiation and healthy myofiber morphology while preventing atrophy. Proliferation and differentiation of myoblasts have been found to be affected by the mechanical, electromagnetic, gravitational, and fluid flow fields (Kosnik *et al*., 2003) [23]. Repetitive stretch and relaxation equal to 10% of length, six times per hour increase differentiation into myotubes (Powell *et al*., 2002). Electrical stimulation also contributes to differentiation as well as sarcomere formation within established myotubes (Kosnik et al., 2003) [23]. Neuronal activity can be mimicked by applying appropriate electrical stimuli in-vitro cultures (Stern-Straeter *et al*., 2005) [33] and has proven to be pivotal in the development of mature muscle fibers.

Mechanotransduction is the process through which cells react to mechanical stimuli and is a complex mechanism (Burkholder, 2007) [8] that is another important bio-physical stimulus in myogenesis. Cyclic strain activates quiescent satellite cells (Tatsumi *et al*., 2001) and increases proliferation of myoblasts (Kook *et al*., 2008) [135].

3.5 Self organizing techniques

This technique utilizes explanted animal tissue to produce highly structured meats creating structured muscle tissue a self-organizing construct or proliferating existing muscle tissue hydroponically. (Benjaminson *et al*., 2002) [4]; cultured gold fish (*Carassius auratus*) muscle explants by taking slices of gold fish tissue, minced and centrifuged them to form pellets, placed them in a petridish in a nutrient medium and grew them for 7 days. They used mature skeletal muscle explants because they contain muscle fibers as well as all the cell types generally associated with muscle in-vivo (Benjaminson *et al*., 2002) [4]. The explanted tissue grew nearly 14% when foetal bovine serum was used as the nutrient medium and grew over 13% when using maitake mushroom extract but when the explants were placed in a culture containing dissociated *Carassius auratus* skeletal muscle cells, the explants surface area grew 79% in period of 7 days. Self-organizing in-vitro meat production may be used to produce highly structured meat; however, lack of blood supply makes growth impossible as cells become necrotic if separated for long periods by more than 0.5 mm from a nutrient supply (Kosnik *et al*., 2001) [24].

4. Merits of hydroponic meat production

Some important merits of hydroponic meat production over conventional meat production are discussed in this section of the article.

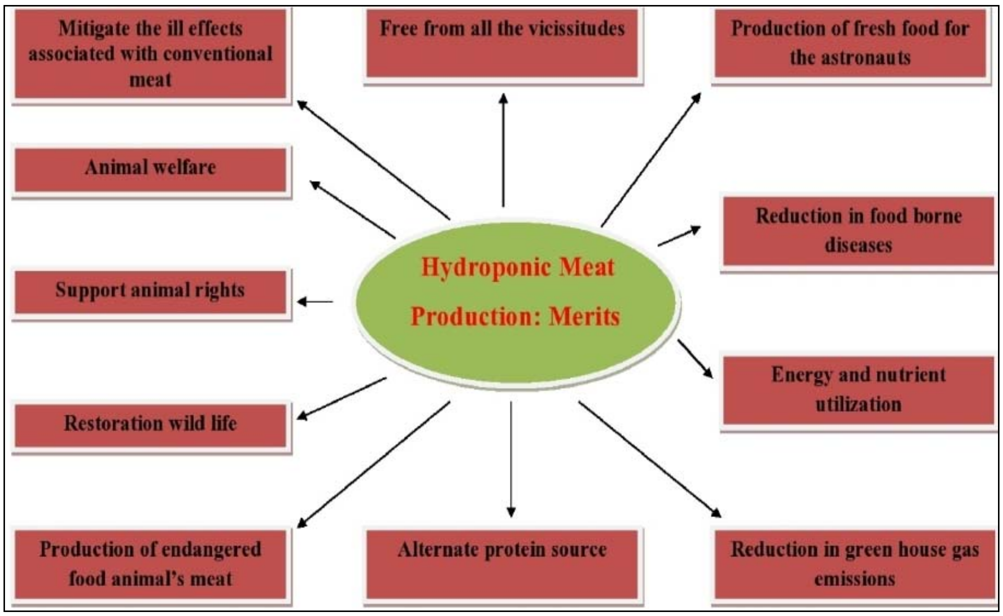

Fig 2: merits of hydroponic meat production

4.1 Mitigate the ill effects associated with conventional meat

Hydroponic meat production can mitigate the ill effects associated with conventional meat production. Hopkins and Dacey (2008) [17] suggested that hydroponic meat has the potential to greatly reduce animal suffering and make eating animals unnecessary. In comparison to the conventional meat the hydroponic meat can be engineered to be healthier and functional by manipulating the composition of the culture medium, the fat content and fatty acid composition of the cultured meat. Harmful fatty acids could be replaced by healthy fats like omega-3 fatty acids.

4.2 Animal welfare

Hydroponic meat bypasses the moral, ramifications of conventional meat production, by avoiding animal death thus giving due respect to the animal welfare. If ten stem cells divide and differentiate continually for two months, they could yield 50000 metric tons of meat. Culturing embryonic cells or stem cells would be ideal for this aim since these cells have an almost infinite self-renewal capacity. Theoretically, one such cell line would be sufficient to literally feed the world (Bartholet, 2011) [3].

4.3 Reduction in food borne diseases

Reduction in zoonotic and food borne illness; due to strict quality control rules, such as GMP and GHP, the incidence of food borne diseases could be significantly reduced as the chance of meat contamination would be lower in absence of potentially hazardous organisms.

Also, the risk of exposure to other hazardous associated with conventional meat production systems like pesticides, arsenic, dioxins and hormones could be significantly reduced.

4.4 Energy and nutrient utilization

Conventional meat production systems are inefficient in terms of energy and nutrient utilization and also takes long conversion time with months for chickens and years for pigs and cows before the meat can be harvested and commercially available. Hydroponic meat takes lesser time than conventional meat production. Hydroponic meat production reduces resource use and ecological foot print. Hydroponic meat production reduces the carbon foot print of meat products. In conventional meat 75-95% of the feed given to an animal is lost because of metabolism and in edible structures like skeleton and neurological tissue. In-vitro meat utilizes all the energy and nutrients in the production of lean meat.

4.5 Reduction in greenhouse gas emissions

Hydroponic meat production systems could reduce greenhouse gas emissions from raising livestock by as much as 90% and reduce use of land and water resources for raising meat by up to 80%.

4.6 Support animal rights
Hydroponic meat production would not involve in the killing of the animals and has potentially profound environmental benefits. It will have strong basis of support in the scientific, animal rights and environmental communities (Hopkins and Dacey, 2008) [17].

4.7 Restoration wild life
Reforestation and wild life would be restored as reduction in land usage will make it possible to the return of wilderness and may help in be restoration of many endangered species.

4.8 Production of endangered food animal's meat
Hydroponic meat production system can also make the exotic meat available to us i.e. cells from endangered food animal species could be used to produce exotic meat in bioreactors.

4.9 Free from all the vicissitudes
Hydroponic meat being free from all the vicissitudes of animals may be suitable for people who are vegetarian due to ethical reasons.

4.10 Production of fresh food for the astronauts
Hydroponic meat for space missions and settlements; a controlled ecological life support system cells would not only provide fresh food to the astronauts, but also deal with waste and provide oxygen and water (Drysdale *et al*., 2003) [10].

4.11 Alternate protein source
Hydroponic meat may act as alternate protein source and other reasons for its production would be consumer demand as more and more people are interested in newly proposed meat, other factors like food scarcity and increasing world population also favours the hydroponic meat production. Hydroponic meat production is predicted to become available in the next 5-10 years at competitive prices.

5. Limitations
There are some limitations in the production of hydroponic meat till now as discussed in this section of the article.

5.1 Consumer acceptance
The color and appearance of the hydroponic meat have some difficulties in competing with the conventional meat. The cultured meat produced and tasted by a sensory panel in London in 2013 was reported to be colorless as it was devoid of natural pigment, myoglobin. The color of the meat was improved by adding red beet juice and saffron (Zaraska M., 2013) [42]. Scaffolds developed and produced by using scaffolds natural and biomaterials like collagen that allow for 3-D tissue culture and complex structuring of meat have also been proposed and attempted (Hopkins and Dacey, 2008) [17].

5.2 Alienation to nature
Hydroponic meat can alienate us from nature and animals that can be a step in our retreat from nature to live in cities. Cultured meat fits it with an increasing dependence on technology and the worry is that this comes with an ever greater estrangement from nature.

5.3 Economic disturbance
The extremely high prohibitive cost and poses as the main obstacle, although large scale production and market penetration are usually associated with dramatic price reduction (Bhat, Z. F. and Fayaz, H., 2011) [6, 7]. Hydroponic meat production will affect the economies of conventional meat producing countries; it will also affect the employment in the agricultural sector in countries with a large scale introduction of cultured meat production.

5.4 Social acceptance
Unnaturalness of meat is being perceived as one of the strongest barriers for public acceptance. Consumers may worry about its unnatural character. People may feel hydroponic meat as artificial meat and not the real as such they depreciate the value of the meat in the same way as they look down on artificial flowers or synthetic diamonds (Hopkins and Dacey 2008) [17]. It is apprehended that in-vitro meat production could result in victimless cannibalism by its ability to culture human muscle tissue.

Another argument is that hydroponic meat shall use original cells gathered from some animal in a morally suspect way and that use of such cells will morally taint all future generations of regarding tissue culture (Hopkins and Dacey, 2008) [17]. Nutritionally, hydroponic meat may not be in match with conventional meat e.g. the cultured meat is devoid of important minerals. This is also a major challenge for consumption of hydroponic meat.

Presently development in techniques for production of hydroponic meat is limited to laboratory level. There is a great need of time to implement these techniques at industrial level, which could be an envisaging boon for meat industry to sustainable meat production.

6. Conclusion
Conventional meat production systems require a relatively high proportion of land, energy and water besides contributing to the emission of greenhouse gases, pollution of water and soil significantly. Nutrition related diseases, food borne illnesses, development of antibiotic resistant, pathogen strains and animal welfare issues are other factors along with deforestation and ecological disturbances associated with conventional meat production. With the increasing global human population, meat consumption is expected to double with 50% increase in global population during the next forty years; it will be associated with almost doubling of the greenhouse gas emissions and aggravating repercussions of raising livestock. Production of hydroponic meat seems to be one of the prospective solutions. Besides reducing the use of animals, it may combine a favorable ecological foot print with similar nutritional values and sensory qualities as that of the conventional meat.

Thus, hydroponic meat holds great promise as an alternative to conventional meat production systems, provided consumer resistance can be overcome. However, more research is needed to be done with respect to the cost effectiveness of the technology, societal and ethical issues before effective large scale production can be achieved.

7. References

1. Asakura A, Komaki M, Rudnicki M. Muscle satellite cells are multi-potential stem cells that exhibit yogenic, osteogenic, and adipogenic differentiation. Differentiation. 2001; 68(4-5): 245-253.
2. Bach AD, Stem-Straeter J, Beier JP, Bannasch H, Stark GB. Engineering of muscle tissue. Clinics in Plastic Surgery. 2003; 30(4):589-599.
3. Bartholet J. Inside the meat lab. Scientific American. 2011; 305:65-69.
4. Benjaminson MA, Gilchriest JA, Lorenz M. *In vitro* edible muscle protein production system (MPPS): Stage 1, fish. Acta astronautica. 2002; 51(12):879-889.

5. Bhat ZF, Bhat H. Animal-free meat bio fabrication. American Journal of Food Technology. 2011; 6:441-459.
6. Bhat ZF, Fayaz H. Prospectus of cultured meat-advancing meat alternatives. Journal of food science and technology. 2011; 48(2):125-140.
7. Bhat ZF, Bhat H. Tissue engineered meat-future meat. Journal of Stored Products and Postharvest Research. 2011; 2:1-10.
8. Burkholder TJ. Mechanotransduction in skeletal muscle. Frontiers in bioscience: a journal and virtual library. 2007; 12:174-191.
9. Catts O, Zurr I. Growing semi-living sculptures: The tissue culture & art project. Leonardo. 2002; 35(4): 365-370.
10. Drysdale A, Ewert M, Hanford A. Life support approaches for mars missions. Advances in Space Research. 2003; 31: 51-61.
11. Edelman PD, McFarland DC, Mironov VA, Matheny JG. Commentary: *In vitro*-cultured meat production. Tissue Engineering. 2005; 11(5):659-662.
12. Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, Discher DE. *Et al.* Myotubes differentiate optimally on substrates with tissue like stiffness, pathological implications for soft or stiff microenvironments. Journal of Cell Biology. 2004; 166(6):877-887.
13. FAO. Food and Agricultural Organization of the United Nations, Rome. 2006.
14. FAO. Food and Agricultural Organization of the United Nations, Rome. 2011.
15. FAO. Food and Agricultural Organization of the United Nations, Rome. 2014.
16. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Journal of Clinical Investigation. 2007; 100(9):1249-1260.
17. Hopkins PD, Dacey A. Vegetarian meat: Could technology save animals and satisfy meat eaters? Journal of Agricultural and Environmental Ethics. 2008; 21: 579-96.
18. Jain RK, Au P, Tam J, Duda DG, Fukumura D. Engineering vascularized tissue. Nature Biotechnology. 2005; 23(7):821-823.
19. Kazama T, Fujie M, Endo T, Kano K. Mature adipocyte-derived dedifferentiated fat cells can trans-differentiate into skeletal myocytes *in vitro*. Biochemical and Biophysics Research. 2008; 377(3):780-785.
20. Keefer CL, Pant D, Blomberg L, Talbot NC. Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. Animal Reproduction Science. 2007; 98(1-2):147-168.
21. Key TJ, Davey GK, Appleby PN. Health benefits of a vegetarian diet. Proceedings of the Nutrition Society. 1999; 58(2):271-275.
22. Kook SH, Lee HJ, Chung WT, Hwang IH, Lee SA, Kim BS, *et al.* Cyclic mechanical stretch stimulates the proliferation of C_2C_{12} myoblasts and inhibits their differentiation via prolonged activation of p38 MAPK. Molecules and cells. 2008; 25(4):479-486.
23. Kosnik PE, Dennis RG, Vandenburgh HH. Tissue engineering skeletal muscle. In Functional tissue engineering. Springer New York. 2003; 377-392.
24. Kosnik PE, Faulkner JA, Dennis RG. Functional development of engineered skeletal muscle from adult and neonatal rats. Tissue engineering. 2001; 7(5):573-584.
25. Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. International journal of cancer. 2006; 119(11):2657-2664.
26. Lowe K. Blood substitutes: From chemistry to clinic. Journal of Materials Chemistry. 2006; 16(43):4189-4196.
27. Martin I, Wendt D, Herberer M. The role of bioreactors in tissue engineering. Trends in Biotechnology. 2004; 22(2):80-86.
28. Matsumoto T, Kano K, Kondo D, Fukuda N, Iribe Y, Tanaka N. *et al.* Mature adipocyte-derived dedifferentiated fat cells exhibit multilineage potential. Journal of Cell Physiology. 2007; 215(1):210-222.
29. Powell CA, Smiley BL, Mills J, Vandenburgh HH. Mechanical stimulation improves tissue-engineered human skeletal muscle. American Journal of Physiology-Cell Physiology. 2002; 283:1557-1564.
30. Radisic M, Euloth M, Yang L, Langer R, Freed LE, Vunjak-Novakovic G. *et al.* High-density seeding of myocyte cells for cardiac tissue engineering. Biotechnology and Bioengineering. 2003; 82(4):403-414.
31. Radisic M, Marsano A, Maidhof R, Wang Y, Vunjak-Novakovic G. Cardiac tissue engineering using perfusion bioreactor systems. Nature Protocols. 2008; 3(4):719-738.
32. Rubio D, Garcia-Castro J, Martín MC, dela-Fuente R, Cigudosa JC, Lloyd AC, *et al.* Spontaneous human adult stem cell transformation. Cancer research. 2005; 65(8):3035-3039.
33. Stern-Straeter J, Bach AD, Stangenberg L, Foerster VT, Horch RE, Stark GB, *et al.* Impact of electrical stimulation on three-dimensional myoblast cultures-a real-time RT-PCR study. Journal of Cellular and Molecular Medicine. 2005; 9(4):883-892.
34. Talbot NC, Blomberg LA. The pursuit of ES cell lines of domesticated ungulates. Stem Cell Reviews and Reports. 2008; 4(3):235-254.
35. Tatsumi R, Sheehan SM, Iwasaki H, Hattori A, Allen RE. Mechanical stretch induces activation of skeletal muscle satellite cells *in vitro*. Experimental cell research. 2001; 267(1):107-114.
36. Tonsor GT, Olynk NJ. Impacts of Animal Well-Being and Welfare Media on Meat Demand. Journal of Agricultural Economics. 2011; 62(1):59-72.
37. Van-Eelen WF, Van-Kooten WJ, Westerhof W. Industrial production of meat from in-vitro cell cultures. Patent Description; WO/1999/031223.
38. Wagers AJ, Weissman IL. Plasticity of adult stem cells. Cell. 1999; 116(5):639-648.
39. Wilschut KJ, Jaksani S, Van-Den-Dolder J, Haagsman HP, Roelen BAJ. Isolation and characterization of porcine adult muscle-derived progenitor cells. Journal of Cell Biochemistry. 2008; 105(5):1228-1239.
40. Wolfson W. raising the steaks. New Scientist. 2002; 176:60-63.
41. www.wikipedia.com//Hydroponic/ In-vitro meat. Accessed on 15-01-2016.
42. Zaraska M. Lab-grown beef taste test: Almost like a burger. http://www.washingtonpost.com/ national/health-science/lab-grown-beef-taste-test- almost like a burger/2013/08/05/921a5996-fdf4-11e2-96a8-d3b921c0924a_story.html 2013.