Newer trends and techniques adopted for manufacturing of *In vitro* meat through "tissue-engineering" technology: A review

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Abstract

Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological tissues. Current meat production methods have many health, environmental and other problems associated with them like high risk of infectious animal diseases, nutrition-related diseases, resource use and environmental pollution through green house gas emissions, decrease in the fresh water supply, erosion and subsequent habitat and biodiversity loss besides the use of farm animals and non-sustainable meat supply. A new approach to produce meat and thereby reducing these risks is probably feasible with existing tissue engineering techniques and has been proposed as a humane, safe and environmentally beneficial alternative to slaughtered animal flesh. The growing demand for meat and the shrinking resources available to produce it by current methods also demand a new sustainable production system. In vitro meat production system ensures sustainable production of a new chemically safe and disease free meat besides reducing the animal suffering significantly. This review discusses the discuss about in-vitro meat production systems by involving various techniques. It is a concept in which edible animal tissues can be produced by the culturing through tissue engineering techniques like Scaffold-based techniques and self-organizing techniques.

Keywords: *In vitro* meat, tissue-engineering, Scaffold Based Technique, Self-organizing Techniques, Biophotonics, Organ Printing, culture media, bioreactors

Introduction

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve biological tissue. Tissue engineering has an emerging role in manufacturing of in vitro meat (Edelman et al., 2005). The traditional model of meat production involves raising non-human animals to a certain age, feeding them, housing them, and ultimately slaughtering them in order to produce steaks, fillets, cutlets and other products for our consumption. But Post's demonstration shows that we could cut out the middle-man, so to speak — produce meat without involving any actual non-human animals (for simplicity, henceforth referred to as 'animals'). Meat could be produced *in vitro* (that is, in a laboratory environment) instead of a farm (Steinfeld et al., 2006). *In vitro* meat development is an alternative meat production system driven by the growing demand for meat and the shrinking resources available to produce it by current methods. Implementation of an in vitro meat production system (IMPS) to complement existing meat production practices creates the opportunity for meat products of different characteristics to be put onto the market (I. Datar *et al*., 2009).

In vitro meat production system is the production of meat outside the food animals by culturing the stem cells derived from farm animals inside the bioreactor by using advanced tissue engineering techniques. Besides winning the favour of animal rights activists for its humane production of meat, *in vitro* meat production system also circumvents many of the issues associated with conventional meat production systems, like excessively brutal slaughter of food animals, nutrition-related diseases, food borne illnesses, resource use, antibiotic-resistant pathogen strains, and massive emissions of methane that contribute to global warming. As the conditions in an *in vitro*

meat production system are controlled and manipulatable, it will be feasible to produce designer, chemically safe and disease free meat on sustainable basis (Zuhaib Fayaz Bhat *et al*,. 2014). In vitro produced meat products resembling the processed and comminuted meat products of today will be sooner to develop than those resembling traditional cuts of meat. While widening the scope of the meat industry in practices and products, the IMPS will reduce the need for agricultural resources to produce meat. Meat produced in vitro has been proposed as a humane, safe and environmentally beneficial alternative to slaughtered animal flesh as a source of nutritional muscle tissue. The basic methodology of an in vitro meat production system (IMPS) involves culturing muscle tissue in a liquid medium on a large scale. Each component of the system offers an array of options which are described taking into account recent advances in relevant research. A major advantage of an IMPS is that the conditions are controlled and manipulatable (I. Datar *et al*., 2009).

The term "cultured meat" will be used here as it seems to be the most widely used and accepted term, but alternative terms used for the same product include "synthetic meat", "*in vitro* meat", and sometimes "artificial meat". These are generally interchangeable, but are distinct from the term "simulated meat", which encompasses products that are similar in some respects to meat, but are made from non-animal proteins such as those from plants (especially soya bean) and fungi (e.g. Quorn). The technique to generate cultured muscle tissues from stem cells was described long ago, but has not yet been developed for the commercial production of cultured meat products. The technology is at an early stage and prerequisites of implementation include a reasonably high level of consumer acceptance, and the development of commercially-viable means of large scale production. Recent advancements in tissue culture techniques suggest that production may be economically feasible, provided it has physical properties in terms of colour, flavour, aroma, texture and palatability that are comparable to conventional meat. Although considerable progress has been made during recent years, important issues remain to be resolved, including the characterization of social and ethical constraints, the fine-tuning of culture conditions, and the development of culture media that are cost-effective and free of animal products. Consumer acceptance and confidence in *in vitro* produced cultured meat might be a significant impediment that hinders the marketing process (KADIM Isam *et al*., 2014).

Techniques and Procedures Adopted for manufacturing of *In vitro* meat through "tissue-engineering" technology

In vitro meat production involves culturing of stem cells outside the food animal from which it is derived. The techniques required to produce *in vitro* meat are not beyond imagination and the basic methodology of an *in vitro* meat production system involves culturing muscle tissue in a liquid medium on a large scale. By culturing loose myosatellite cells on a substrate, it is probably possible to produce cultured meat by harvesting mature muscle cells after differentiation and processing them into various meat products (Bhat and Bhat 2011a). Tissue engineering can be employed to produce cultured meat (Edelman et al., 2005) and a number of demands need to be met for using tissue engineering techniques for meat production. Firstly, a cell source is required that can proliferate indefinitely and also differentiate into functional skeletal muscle tissue. Secondly, these cells need to be embedded in a three dimensional matrix that allows for muscle growth, while keeping the delivery of nutrients and release of waste products undisturbed and lastly, muscle cells need to be conditioned adequately in a bioreactor to get mature, functional muscle fibers for processing to various meat products. The different design approaches for an *in vitro* meat production system can be roughly divided into scaffold/cell culture based and self organizing/tissue culture techniques.

Scaffold Based Technique

A scaffold based *in vitro* meat production system would involve isolation of embryonic myoblasts or adult skeletal muscle satellite cells from the farm animals like cattle, sheep, pig, etc which would be allowed to grow inside a stationary or rotating bioreactor using a plant origin growth medium. These cells would divide and redivide for weeks and months together and would be finally differentiated into the muscle fibers onto a scaffold inside the bioreactor. Attached to a scaffold or carrier such as a collagen meshwork or microcarrier beads, stem cells fuse into myotubes, which can then differentiate into myofibers by introducing a variety of environmental cues (Kosnik *et al.* 2003). The resulting myofibers may then be harvested, processed, cooked, and consumed as emulsion or ground meat products. In scaffold-based techniques, embryonic myoblasts or mature skeletal muscle satellite cells are proliferated, attach to a scaffold or carrier, such as a collagen meshwork or microcarrier beads, and then perfuse with a culture medium in a stationary or rotating bioreactor. By introducing a variety of environmental cues, these cells fused into myotubes, which can then differentiate into

myofibers. The resulting myofibers may then harvested, cooked, and consumed as meat. Van Eelen, Van Kooten, and Westerhof embrace a Dutch patent for this general approach to producing cultured meat (Van Eelen et al., 1999). However, Catts and Zurr appear to have been the first to have actually produced meat using the method (Catts and Zurr, 2002).

These scaffold-based techniques cannot produce highly structured meats like steaks but can be used to produce ground and boneless meats with soft consistency. However, cells can also be grown in substrates that allow for the development of ‘‘self-organizing constructs’’ that produce more rigid structures. A scaffold-based technique may be appropriate for producing processed meats, such as hamburger or sausage. But it is not suitable for producing highly structured meats, such as steaks. To produce these, one would need a more ambitious approach, creating structured muscle tissue as self-organizing constructs (Dennis and Kosnik, 2000) or proliferating existing muscle tissue *in vitro*.

Self-organizing Techniques

This technique was employed by Benjaminson, Gilchriest and Lorenz (Benjaminson et al.,2002). They placed skeletal muscle explants from goldfish (*Carassius auratus*) in diverse culture media for seven days and observed an increase in surface area between 5.2 and 13.8 percent. When the explants were placed in a culture containing dissociated *Carassius* skeletal muscle cells, explant surface area grew by 79 percent. Explants have the advantage of containing all the cells that make up muscle in their corresponding proportions, thus closely mimicking an *in vivo* structure. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if separated for long periods by more than 0.5 mm from a nutrient supply (Dennis and Kosnik, 2000). Thus without vascularization, the production of large, highly structured meats will not be possible. Future efforts in culturing meat will have to be the limitations of modern techniques through advances that make cultured cells, scaffolds, culture media, and growth factors both edible and affordable.

Self-organizing *in vitro* meat production may hold the promise to produce the highly structured meats as the 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). Vladimir Mironov suggested a branching network of edible porous polymer through which nutrients could be 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 already been proposed (Zandonella 2003).

Cell sources for manufacturing of *In vitro* meat

In vitro meat can be produced by culturing embryonic stem cells from farm animal species and are ideal for culturing since these cells have an almost infinite selfrenewal capacity. But these cells must be specifically stimulated to differentiate into myoblasts and may inaccurately recapitulate myogenesis (Bach et al., 2003). 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). Although embryonic stem cells have been cultured for many generations but so far it has not been possible to culture cell lines with unlimited selfrenewal 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.

Culturing of Muscle Cell

It is possible to grow or culture muscle fibre *in-vitro* however, the problem of its proliferation may occur. As an alternate satellite cells can be cultured. Typically neonate individuals are selected for the isolation of Myo-satellite cells because these cells are much more abundant in the muscle of the young animals than the older animals. The capacity of differentiation into variety of cells is more in young than older ones. Most of the cells are taken from still born pig foetus. There are always some dead ones. The cells are taken from the semitendinosus muscle of the pig hamstring. After its freezing in liquid nitrogen it can be utilized for years together. Its isolation requires the mincing of complete muscle followed by enzymatic treatment or separation of the satellite cells by differential centrifuge. Preplating, precell gradients or combination of these on removal of the growth factor from the culture medium these myoblasts fuse from myofibers. Growth factors contain hormones, growth may have basic amino acids, glucose, minerals, serum harvested from animals usually calves and artificial serum has been produced from the limp muscle (Singh VP et al., 2014).

Co-culturing Muscle Cell

Myoblasts cell are specialized to produce contractile proteins but produce only little extracellular matrix and as such other cells likely need to be introduced to engineer muscle. Fibroblasts residing in the muscle are mainly responsible for the production of extracellular matrix which could be beneficial to add to the culture system (Brady et al., 2008). However, due to the difference in growth rate, co-culturing involves the risk of fibroblasts overgrowing the myoblasts. Meat also contains fat and a vasculature and possibly, co-culture with fat cells should also be considered (Edelman et al., 2005). The problem of vascularisation is a general issue in tissue engineering and currently we can only produce thin tissues because of passive diffusion limitations. To overcome the tissue thickness limit of 100 to 200 um, a vasculature needs to be created (Jain et al., 2005).

Cell Sequencing

There are two subsequent stages involved in the dying of the old cell culture. First one is senescence-in which cell normally die and second crisis-which occurs when cells for some reason have survival senescence. Senescence can be overcome by start fresh cell culture when needed, immobilize cell culture and by the use of an embryonic stem cell (Singh VP et al., 2014).

Perfusion of Growing Muscle Tissue

Actual growth of muscle tissue in culture is problematic because of the absence of blood circulation. It has been approved that it is possible to grow small muscle like organs termed as myoids denovo from co-culture of myoblast and fibroblast. These organs are able to contract both spontaneously and by electrical stimulation. An electrode ensures an electrical current about 1 Hz passing through the cells to make these skeletal muscle cells develop into muscle. They need to be constantly exercised just like into body (Singh VP et al., 2014).

Requisites for *in-vitro* Meat Production

Fields

For the growth and proliferation of cells there is a need of optimum field. Mechanical, electromagnetic, gravitational, and fluid flow fields have been found to influence the proliferation and differentiation of myoblasts (Kosnik et al., 2003 and De Deyne, 2000). Yuge and Kataoka seeded myoblasts with magnetic micro particles and induced differentiation by placing them in a magnetic field, without adding special growth factors or any conditioned medium (Yuge and Kataoka,2000). Powell and others found that repetitive stretch and relaxation equal to 10 percent of length, 6 times per hour, increased 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 and De Deyne, 2000).

Culture Media and Growth Factors

For the growth of any substance affordable medium is required. Such medium must contain the necessary nutritional components and be presented in a form freely available to myoblasts and complementary cells, as no digestive system are involved. In this concern, McFarland and others developed a serum-free medium that supported the proliferation of turkey satellite cells in culture (McFarland et al., 1991). Kosnik, Dennis, and Vandenburgh refer to serum-free media developed by Allen et al., Dollenmeier et al., and Ham et al (Kosnik et al., 2003). Benjaminson and others succeeded in using a serum-free medium made from maitake mushroom extract that achieved higher rates of growth than fetal bovine serum (Benjaminson et al., 2002

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. In general biophotonics refers in general to the process of using light to bind together particles of matter. A new field, and one in which the mechanisms are still poorly understood, biophotonics relies on the effects of lasers to move particles of matter into definite organizational structures, such as three-dimensional chessboard, or hexagonal arrays. A amazing 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 microencapsulated substances to individual cells, there is an intriguing possibility that such a technology could be used for the production of tissues, including meat. A main researcher in bio photonics, Kishan Dholakia,

reports in an interview that he and colleagues are already using the technology to create arrays of red blood cells and hamster ovaries (Mullins 2006).

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).Cultured meat production is probable to require the development of new bioreactors that sustain low shear and uniform perfusion at large volumes. The bioreactor designing is intended to promote the growth of tissue cultures which accurately resemble native tissue architecture and provides an environment which allows for increased culture volumes. A laminar flow of the medium is created in revolving wall vessel bioreactors by revolving the cylindrical wall at a speed that balances centrifugal force, draw force and gravitational force, leaving the 3-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 *(*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 builds by using a co-culture of myoblasts, embryonic fibroblasts and endothelial cells coseeded onto a highly porous biodegradable scaffold. Research size revolving bioreactors have been scaled up to three litters and, theoretically, scale up to industrial sizes should not affect the physics of the system. As cell feasibility and density positively correlate with the oxygen gradient in statically grown tissue cultures, it is necessary to have adequate oxygen perfusion throughout cell seeding and cultivation on the scaffold (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.

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. Not surprisingly, given the confluence of technologies, some of the same people who are working on culturing meat are also working on research in organ printing. Organ printing is a simple yet astounding idea. Using the principles of ordinary printing technology—the kind of technology that inkjet printers use to produce documents like this one—researchers have essentially been able to use solutions containing single cells or balls of cells rather than ink 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 you would like— whether for transplantation or for consumption (Mironov et al. 2003).

CONCLUSIONS

The aim of in-vitro meat production is to grow fully developed muscle organs, but the first generation will most likely be minced meat products. It is a concept in which edible animal tissues can be produced by the culturing through tissue engineering techniques. These techniques offer health and environmental advantages over existing meat production systems. Though, the production of highly-structured, unprocessed meats faces considerably greater technical challenges in respect to its safety and quality. Through the use of various techniques like Scaffold-based techniques

and self-organizing techniques we can produce edible tissues. The hindrances in this field are the structural integrity as the whole animal tissues and its safety for human consumption. *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

1. Bach AD, Stem-Straeter J, Beier JP, Bannasch H, Stark GB (2003). Engineering of muscle tissue. Clin. Plast. Surg., 30(4): 589-599.
2. Benjaminson M A, Gilchriest J A, Lorenz M. 2002. In vitro edible muscle protein production system (MPPS): Stage 1, fish. *Acta Astronaut*, 51, 879-889.
3. Bhat Z F, Bhat H. 2011a. Prospectus of Cultured Meat-Advancing Meat Alternatives. *Journal of Food Science and Technology*, 48, 125-140.
4. Brady MA, Lewis MP and Mudera V. 2008. Synergy between myogenic and non myogenic cells in a 3D tissue_engineered craniofacial skeletal muscle construct. J. Tissue Eng. Regen. M., 2(7): 408_417.
5. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N and Langer R. 1999. Cardiac tissue engineering: cell seeding, cultivation parameters and tissue construct characterization. Biotechnol. Bioeng., 64(5): 580-589.
6. Carrier RL, Rupnick M, Langer R, Schoen FJ, Freed LE and Vunjak- Novakovic G. 2002. Perfusion improves tissue architecture of engineered cardiac muscle. Tissue Eng., 8(2):175-188.
7. Catts O, Zurr I. 2002. Growing semi-living sculptures: The tissue culture project. *Leonardo*, 35, 365-370.
8. De Deyne PG. 2000. Formation of sarcomeres in developing myotubes: role of mechanical stretch and contractile activation. Am J Physiol Cell Physiol .,279: C1801.
9. Dennis R, Kosnik 2nd P. 2000. Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. In Vitro and Cellular Developmental Biology-Animal, 36, 327-35.
10. Edelman PD, McFarland DC, Mironov VA, Matheny JG (2005). Commentary: *In vitro*-cultured meat production. Tissue Eng., 11(5): 659-662.
11. Edelman PD, McFarland DC, Mironov VA, Matheny JG (2005). Commentary: *In vitro*-cultured meat production. Tissue Eng., 11(5): 659-662.

I. Datar and M. Betti. 2009. Possibilities for an in vitro meat production system. *Innovative Food Science & Emerging Technologies*, 11(1): 13-22,.

12. Jain RK, Au P, Tam J, Duda DG and Fukumura D. 2005. Engineering vascularized tissue. Nat. Biotechnol., 23(7): 821-823.
13. KADIM Isam, MAHGOUB Osman, BAQIR Senan, FAYE Bernard, PURCHAS Roger (2014). Cultured Meat from Muscle Stem Cells: A Review of Challenges and Prospects. *Journal of Integrative Agriculture, Doi :* 10.1016/S2095-3119(14)60881-9
14. Keefer CL, Pant D, Blomberg L, Talbot NC (2007). Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. Anim. Reprod. Sci., 98(1-2): 147-168.
15. Kosnik PE, Dennis RG and Vandenburgh HH. 2003. Tissue Engineering Skeletal Muscle. In: Guilak, F., ed. Functional Tissue Engineering. New York: Springer, pp. 377-392.
16. Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS and Darland DC. 2005. Engineering vascularized skeletal muscle tissue. Nat. Biotechnol., 23(7): 879-884.
17. Martin I, Wendt D and Herberer M. 2004. The role of bioreactors in tissue engineering. Trends Biotechnol., 22(2): 80-86.
18. McFarland DC, Pesall JE, Norberg JM and Dvoracek MA. 1991. Proliferation of the turkey myogenic satellite cell in a serum-free medium. Comparative Biochemistry and Physiology, 99A: 163.
19. Mironov V, Boland T, Trusk T, Forgacs G and Markwald R. 2003. Organ printing: Computer-aided jet-based 3D tissue engineering. Trends in Biotechnology, 21(4), April.
20. Mulligan R C. 1999. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature, 401(6751): 390-394.
21. Pathak, V., S.A.A. Bukhari, Z.F. Bhat and N. Sharma, 2008. Prospectus of *in vitro* meat. Indian Food Ind., pp: 33-35.
22. Powell CA, Smiley BL, Mills J, Vandenburgh HH (2002). Mechanical stimulation improves tissue-engineered human skeletal muscle. Am. J. Physiol. Cell Physiol., 283:C1557.
23. Radisic M, Euloth M, Yang L, Langer R, Freed LE and Vunjak-Novakovic G. 2003. High density seeding of myocyte cells for cardiac tissue engineering. Biotechnol. Bioeng. 82(4): 403−414.
24. Radisic M, Marsano A, Maidhof R, Wang Y. and Vunjak-Novakovic G. 2008. Cardiac tissue engineering using perfusion bioreactor systems. Nat. Protoc., 3(4): 719-738.
25. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, De Haan C (2006). Livestock's long shadow: environmental issues and options (Rome: Food and Agriculture Organization of the United Nations, p. xxi). Available at: www.virtualcentre.org/en/library/key_pub/longshad/A0701E00.pdf. Accessed March 7, 2008
26. Talbot NC, Blomberg LA (2008). The pursuit of ES cell lines of domesticated ungulates. Stem Cell Rev. Reports, 4(3): 235-254.
27. V.P. Singh, Sachan, N. and Akhilesh K. Verma (2014). *In Vitro* Meat - The Start of New Era in Meat Production. *International Journal of Livestock Research*, 2(1):38-51
28. Van Eelen W F, van Kooten W J, Westerhof W. 1999. Industrial production of meat from in vitro cell cultures. WO/1999/031223: Patent Description http://www.wipo.int/pctdb/en/wo.jsp?wo=1999031223 Accessed Mar. 2009.
29. Wolfson W. 2002. Raising the steaks. *New Scientist*, **176**, 60-3.
30. Yuge L, Kataoka K (2000). Differentiation of myoblasts accelerated in culture in a magnetic field. *In vitro* Cell Dev. Biol. Anim., 36: 383.
31. Zandonella C. 2003. Tissue engineering: The beat goes on. *Nature*, **421**, 884-6.

32. Zuhaib Fayaz Bhat, Sunil Kumar, Hina Fayaz (2014). *In Vitro* Meat Production: Challenges and Benefits Over Conventional Meat Production. *Journal of Integrative Agriculture, Doi : 10.1016/S2095-3119(14)60887-X*