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Review and Future Prospects for Lab-Grown Meat

[1]Tejas R. Suthar, [2]Riddhi V. Sanghvi, [3]Theertha K. Nair, [4]Anupama N. Devkatte and[5]Deepti N. Chaudhari

[1,2,3,4,5]*MIT College of Food Technology, MIT-ADT University, LoniKalbhor, Pune (India)*

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ABSTRACT

Demand for meat is increasing and supply of animals like cow, pigs, chickens used for meat, diminishing due to issues of commercial production of them. Commercial livestock production is becoming unviable on global scale in terms of animal welfare, environmental sustainability, and human health. Options for artificial meat are being explored using techniques like stem cell, satellite cells or myoblasts, Embryonic Stem Cells, Induced pluripotent stem cells, Totipotent stem cells, Pluripotent stem cells, etc. In this method animal tissue is grown in a controlled environment using cell culture technology. There are attempts made to develop different types of media and techniques like Self-organizing technique and Scaffold-based technique to grow the cell further. Cultured meat is also called as 'clean meat', 'in-vitro meat', 'artificial meat' or even 'alt-meat'. The concept is in infant stage of commercialization hence along with technical aspect there is lot of work is being done in environmental, commercial, social, economic and ethical aspect of the concept. The real benefits and cost as compared to current methods of meat production are still to be explored.

1. Introduction

Meat is a vital source of protein, iron and many other nutritional requirements of human diet since ages. Every year, more than 60 billion animals are reared in industrial conditions in order to produce meat. Animals grown in unnatural condition like poultry farms, goat farms, cattle farms etc. have resulted in numerous animal diseases like "Madcow", "Bird Flue", "Scombroid",which got transmitted to humans when such meat was consumed, forcing to slaughter these animals and disposal with care. Animal husbandry has another side effect that is choking the Earth by using about 70% of agricultural land for fodder of these animals which has adverse effects on natural resource such as depletion of fresh water reservoirs, biodiversity loss, soil erosion and destruction of habitats, etc. Livestock raising for meat, eggs and milk generates around 15% of global greenhouse gas emissions, the second highest source of emissions higher than all combined transport (Mr.Prabodh S. Halde and Ms. Diana Dsouza, 2019).

In 2016, it was seen that US consumed about 97.1 kg meat per capita, per year, followed by Australia 94.8 kg and then Argentina 86.1 kg. The only exception to this trend was India, where meat consumption per capita in 2013 was nearly exactly the same as in 1961 at less than 4 kg per person. To solve the problems related to meat consumption on a global scale as well as meet the meat demand, cultured meat or lab grown meat would play vital role in new era. The concept of cultured meat was popularized by Jason Matheny in the early 2000. In 2013, Mark Post created the first lab grown burger patty. Lab grown meat production involves multidisciplinary approach includes biotechnology, tissue engineering and molecular biology to create a new design to produce proteins and fats and tissues. The startups developing lab grown beef, pork, poultry and seafood are Mosa Meat, Memphis Meats, Super Meat and Finless Foods using modern techniques.

Global meat production is currently 263 million tons and is expected to almost double by 2050 to 445 million tons (Mr. Prabodh S. Halde and Ms. Diana Dsouza, 2019). Bulk of the demand will be met through cultured meat produced in factory under supervision of skilled workers. The major concerns for cultured meat include "trust", i.e. acceptance by the general public as it is perceived to be lab grown or unnatural. Theoretically lab grown meat contains no fat and would also contain no bones, which may compromise to the taste of consumers, as suggested by some scientists.

It is seen as carbon dioxide is produced by lab grown meat in contrast to methane that is produced by normal animal rearing, and carbon dioxide is said to be more harmful than methane as it remains in atmosphere for a longer time than methane. So, lab grown meat has both positive and negative sides therefore it depends on the consumers to ensure that they make a more sensible and rational judgement about the consumption and potential future of lab-grown-meat. While research is ongoing, the attempt was made to produce t he cultivated meat for space flights and protein requirement of inhabitants of space stations. NASA cultured muscle tissue obtained from common gold fish (*Carassiusauratus*), ranging 3-10 cm in length, in petri dishes (Benjaminson*et al.*,2002).

Muscle tissues cultured in crude cell extracts improved in cell mass. Such cells were later washed, dipped in olive oil and spices, coated with breadcrumbs and fried and tasted with a taste panel that concluded that the product was palatable(Churchill1932).One of the essential techniques of inv itro meat production was to obtain and grow muscle tissues in an acceptable medium and to harvest them (Benjaminson et al.,2002).

Invitro advanced meat engineering and tissue engineers h ave been involved in selection process, putting adult cells on scaffolds, developing them in bioreactors and using cultivated cells to produce muscle tissue (Bhat et al., 2017 and Catts et al., 2002).

Certain programs aimed at using proliferation of stem cells by putting them on top of each other (Kelland, 2012) and using inkjet techniques to spray cell materials on sheets or other structures (Bhat and Bhat, 2011). Researchers of the Cultured Beef Project remove muscle cells from the shoulder of a cow and feed the cells with a nutrient mixture in a petri dish. They grow into muscle tissue. It took three months to

grow the beef using stem cells from a cow`s shoulder. By tissue engineering technique it is possible to obtain tons of meat from a few starter cells. Mosa Meat, a Dutch company, launched its first hamburger in London in 2013 (Bertalan M. 2018).The burger included five ounces of grown meat (beef) patty, cooked and analyzed by a panel of sensory experts in London, who found it tasted close to a regular burger. For this burger the financial cost was over $330,000.The event encouraged consumers, particularly those concerned with animal welfare, to support the commercial introduction of such meat products (Zaraska, 2013).

The company ' Super Meats ' has been working in Israel with the Hebrew University of Jerusalem for several years, and recent news reports suggest that three Israeli artificial meat companies – Super Meat, Future Meat Technology and Meat the Future – will benefit from the $300 million trade agreement signed between China and Israel (Roberts, 2017). Although this deal has been reported in the press, it is not yet publicly clear what this will mean for these firms, and none of them have yet announced publicly any demonstration products (Stephensa et al. 2018) .

Image source: www.mosameat.com

2. Review of Materials, Methodology and Techniques

Materials: Cells used for lab grown cultured meat

For every organ and tissue in our body, stem cells are the cornerstone. There are many different types of stem cells that come from various places in the body or are developed vari ous stages of life. Embryonic stem cells, adult stem cells, adipose tissue-derived adult stem cells, myosatellite cells, or myoblasts are possible candidate for the initial stage of growing culturedmeat. However it is preferable to have a rapid rate of proliferation (high rate of cell reproduction) of stem cells that have the fastest rate of proliferation, but have not yet started to develop towards a particular type of cell, which poses the task of separating the cells and directing them to expand in a certain direction. Fully developed muscle cells are perfect in that they have already completed muscle development, but proliferate consequently, cells such as myosatellite and myoblast cells are often used because they tend to proliferate at an acceptable rate, but also vary significantly from other cell types (Edelman et al. 2005).

Is the focus of the current debate on which is the best source of cells for meat grown? There are two possible sources of cells to form cellular agricultural products engineered by tissue: primary cells derived from the initial tissue, or cell lines. Cell lines can be formed two ways (Stephensa et al. 2018). Usually, one approach is through induction (genetic or chemical engineering), which can program the cells to proliferate indefinitely (Eva et al., 2014). Another is choosing random mutations where the cell expresses immortality and culture the resulting population (ThermoFisher, 2017).

Cell types used for meat production

The meat consists mainly of skeletal muscles composed of several types of cells. Such skeletal muscle fibers develop from proliferation, embryonic myoblast differentiation, or satellite cells (Langelaan et al. 2010). Several types of cells proposed by several authors for invitro meat production are myosatellite cells, embryonic stem cells and adult stem cells. It may be beneficial to co-culture adipocytes (fat cells) with myofibrils to enhance the texture, flavor and tenderness of cultivated meat by effectively increasing intramuscular fat (Hocquette et al. 2010).There are different types of cells used for tissue culturing(Table 1).

Table 1 :Types of cells used for tissue culturing

Cell type	Advantage	Disadvantage	Reference
The satellite cells or myoblasts	Responsible for muscle regeneration and differentiate easily to form Myofibrils eventually.	May distinguish between various types of cells primarily found in organism development stages	Post 2012, Williams 2012
Embryonic Stem Cells	Unlimited regenerative potential	Over time, genetic mutation is likely to limit the production potential long term culturing results in loss of self-renewal capacity	Mattick and Allenby 2010, Datar and Betti 2010, Bhat and Bhat 2011
Myosatellite Cells	Most suitable source of cells for culturing of meat	Cells from different sources differ greatly in their proliferation and their differentiation capabilities	Edelman et al. 2005, Datar and Betti 2010, Post 2012, Bhat and Bhat 2011
Adult stem cells	They form the primary component of meat that is muscle	Differentiate into certain type of cell only or into a similar type of cell.	Williams 2012
Mesenchymal stem cells	Cells comes from the connective tissue that surround the body organ and other tissue, used to create	Decline in proliferative and differentiation capacity with age	Amos et al. 2013

	new body tissues, such as bone, cartilage and fat cells.		
Induced pluripotent stem cells	Abundant somatic cells of donor can be used, Very useful for drug development and development studies	Methods of ensured reproducibility and maintenance, as differentiated tissues are not certain	https://www.unmc.edu/stemcells/educational-resources/prosandcons.html
Totipotent stem cells	Relative potency is high ,Cells are capable of differentiating into any cell type	Ethical issues	Anna Macdonald 2018
Pluripotent stem cells	Relative potency is medium, Cells are capable of differentiate into cells from any of three germ layers	Ethical issues, teratoma formation	Anna Macdonald 2018
Multipotent stem cells	Relative potency is low, Cells are capable of differentiating into limited range of cells type	Hard to isolate, limited differentiation	Anna Macdonald 2018

Culture Media used for meat production:

Cultures of mammalian cells need a complex medium compared to prokaryotic cells which need simple growth conditions. Mammalian cells need a solid surface to be attached to eat food materials(Haagsman et al., 2009). To release and synthesize these growth factors, many cells have formed systems. Liver cells, for example, can provide growth factors in the medium (Edelman et al., 2005). It is necessary to provide serum and plasma beneficial for the proliferation of mammalian cells in liquid media. Generally, the fetal calf serum is applied to the medium at the final concentration of 5-20 percent. Serum-free media can delay the development of culture; therefore, serum provision is compulsory to achieve good results (Jochems et al., 2002). In addition to sufficient growth factors, cell culture includes the culture medium. The medium used to grow cells typically comes from animal sources; serum-based medium from adults, newborns or fetuses (Coecke 2005).

The culture media surrounding the developing culture cells and fibers must contain adequate levels of all the necessary nutrients and oxygen, but it should also contain growth factors and bioactive compounds which are essential for normal muscle production. Some of these are actually derived from animal tissues, but it would eventually be ideal for the media to be free of all animal products (Isam et al. 2015). Ultroser G is one of many commercially available serum replacements designed to substitute fetal bovine serum for anchorage-dependent cell growth because it contains all the necessary components required for eukaryotic cell growth (Duque et al. 2003).

Methods of cultured meat production :

In 1930 Frederick Edwin Smith and in 1932 Winston Churchill postulated that we will not need to grow cow or chicken to have specific part of it as meat for our food. We should have techniques developed down the line to have specific parts grown to meet our need of meat (Birkenhead and Smith 1930 & Churchill 1932). Since then, for the actualization of the idea, two major Smith 1930 & Churchill 1932). Since then, for the actualization of the idea, two major technologies have been developed. The center of the scheme is the biotechnological approach which primarily includes cell culture and tissue culture / tissue engineering techniques and is also popularly known as ' scaffold-based ' and ' self-organizing techniques ' in the jargon of biotechnology, respectively. (Edelman et al. 2005).

Self-organizing technique:

The first technique involves using an explant from the recipient animal's muscle, which is then proliferated in a reservoir of nutrients. In 1912, Alexis Carrel managed to keep a piece of chicken heart muscle alive and beat in a petri dish, showing that tissue could be kept alive outside the body, provided it was fed with appropriate and all suitable nutrients (Bhat and Bhat 2011). But in the real-time sense, the genesis of the concept has its origins in the early 21st century when Benjaminson, Gilchriest and Lorenz used tissue-engineering techniques to produce meat. (Benjaminson et al. 2002). They placed goldfish (Carassiusauratus) skeletal muscle explants in a variety of culture media and observed a varied pattern of growth in terms of surface area development over 7 days. The medium-based result was as: fetal bovine serum:13.8%, fishmeal extract:7.1%, shiitake extract:4.8%, maitake extract:15.6%. Benjamison also made it possible in other research projects to retain muscle tissue in a fungal medium; the chicken muscle could be stored in the petri plate for a period of two months. (Wolfson 2002). The technique of self-organization helps to create organized food, i.e. meat created will have a well-defined 3D structure just like a natural meat confirmation. The same can be achieved using tissue engineering concepts for de novo muscle tissue synthesis (Edelman 2003).

Scaffold-based technique:

The second method of growing and culturing meat contains sufficient stem cells that can be collected from a variety of tissues (Hamburger junction 2012). In this process, embryonic myoblast or adult skeletal satellite muscle cells are proliferated, linked to a scaffold or carrier and then perfused into a suitable bioreactor with a culture medium. (Figure 1) (Kosnik et al. 2003). The theory of this technique is that in the presence of a culture medium in the bioreactor, sufficient muscle cells are proliferated on a carrier called scaffold. This cultivation leads to myofibers that can be harvested, processed and eaten as meat or its products (Bhat and Fayaz 2011).

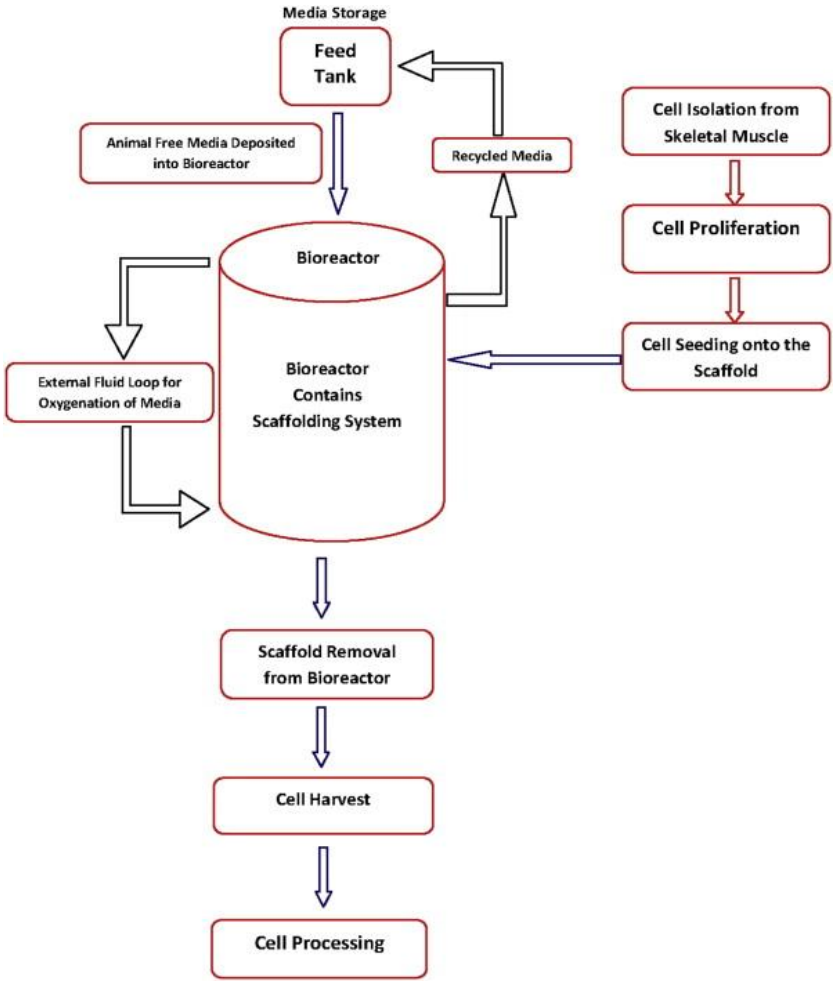

Figure 1: Scaffold-based technique

Further development in techniques was introduced by Dr. Gabor Forgacs in 2011 (Lu 2012). Meat is generally a mixture of billions of muscle cells, fat cells, blood cells, and so on. Cells can be inserted and injected into a surface by using a 3-D printer in the form of an ' ink, ' usually a piece of removable paper. (Aldous 2006; Mironov et al. 2003).

Printed cells including muscle cells, fat cells and blood cells can then be grown in a suitable medium similar to the steps taken in scaffold techniques.

Table 2:showing a summary of the content and conclusions of recent reviews about the production of cultured meat from stem cells.

References	Title of article	Topics covered and Conclusions
*Langelaan et al. (2010)	"Meet the new meat: Tissue engineered skeletal muscle"	• A review for the manufacturing of cultured meat economically which is feasible from engineering point of view • Main hurdles to find the better source of stem cell, and developed the commercially feasible methods of 3-dimensional structure inside the bio-reactor
*Stephens (2010)	"In vitro meat: zombies on the menu?"	• Possibility the production of in vitro meat with reference to emerging regulative, moral and social matters • The author did not use the terminology conducive to others, in vitro meat is "zombie meat"
*Bhat and Fayaz (2011)	"Prospectus of cultured meat-Advancing meat alternatives	• The authors noted that the manufacturing of meat-products is good nutritional value, disease free as well as its chemically-safe and they said that it will be easily to achieve as compare to the raw-meat by-products production along with all organoleptic and physical properties
*Tuomisto and Teixeira de Mattos (2011)	"Environmental impacts of cultured meat production"	• Modeling method used by authors and they assume to differentiate the production of cultured meat with different conventional methods such as chicken, beef, sheep and pork related to the land usage, outflow of greenhouse gas, energy usage and H2O used/kilogram of eatable meat • Result proved that all of these factors was superior for the production of cultured meat except that the production of

		chicken was better with energy use factor
*Dodson et al. (2012)	"Cell supermarket: Adipose tissue as a source of stem cells"	• Cultured meat is not directly concerned in this review, but the success of cultured meat would be desirable to involve adipocytes in order to assure good palatability • It can be induced from the description of the type of cultured cells derived from adipose tissue
*Post (2012)	"Cultured meat from stem cells"	• A need of this review to overcome the problems of cultured meat • Three main motives were identified for the production of commercial cultured meat such as: (1) to meet the projected demand for meat increases; (2) concern about the environmental impact of production of meat from cattle; and (3) concern about ethics • Emphasizes the product needs to mimic the meat which produced conventionally as close as possible
*Welin, Gold, and Berlin (2012)	"In vitro meat: What are the moral issues?"	• They noted that the culture meat development is an important aspect of medical concern in "tissue engineering"
*Young et al. (2013)	"Novel aspects of health-promoting compounds in meat"	• As shown in the title, it focuses on health supporting "functional" or "biologically-active" composite present in meat, also briefly studied the cultured meat • Authors believe that 4 primary challenges, in vitro or cultured meat manufacturing such as: (1) identification of the better seed cells sources and an appropriate growth medium cost-efficient; (2) Suitable framework for the development of cell growth and differentiation; (3) The program scaling-up to industrial levels; (4) To ensure that the consumer acceptance, nutritional value, and health-promoting attributes, at least equal to conventional meat
*Goodwin and Shoulders (2013)	"The future of meat: A qualitative analysis of cultured meat media coverage"	• Discussion and summary, in many countries the media have been running stories about cultured meat, its potential and problems
*Post (2014)	"Cultured beef: Medical technology to produce food"	• After greatly-advertised on television this review was written, sampling the 85-grams of meat-pie which is made from muscles fibers of in vitro grown cultured bovine from satellite cells • In this demonstration, stages of proof-of-concept overview • It's deduced that there are still many challenges, but the meat demands increase in the coming decades according to the research point of view
*Van der Weele and Tramper (2014)	"Cultured meat: Every village its own factory"	• The authors suggested that in the future, culture meat production is probable to be technologically achievable, and that cultured meat production has some certain benefits as compared to the production system of conventional meat • They suggested that the cultured meat production at small-scale may prove effective when it will consume
Isam et al. (2015)	"Cultured meat from muscle stem cells: A review of challenges and prospects"	• A flow diagram illustrating in general terms some of the steps in the production of cultured meat product are outlined • Conclude with the list of characteristics of several types of mammalian stem cells • Questions concerning cultured meat are not only about technology and science, they are also about its acceptability as a food.
Mohammad et al. (2017)	"Cultured Meat in Islamic Perspective"	• The authors discussed about Islamic perspective on cultured meat based on the original scripture in the Qur'an and interpretations by authoritative Islamic jurists. • The authors expressed their views on Islamic Ruling on Cultured Meat Based on the (1) Use of ESCs as the Source for Stem Cells (2) Use of ASCs as the Source for Stem Cell (3) Use of Serum as Culture Medium
Neil et al. (2018)	"Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture"	• What is distinctabout this paper is a willingness to engage in articulating the practicalchallenges facing the field and the call for extending the socio-politicaldebate on cultured meat beyond 'ethics' and 'consumer acceptance' toinclude complex policy issues like food transitions and practical regulatory mechanisms. • This paper aims to review and survey the current scenario of cultured meat industry and talks about future prospects and technology upcoming in this field.

Christopher Bryant and Courtney Dillard (2019)	"The Impact of Framing on Acceptance of Cultured Meat"	• In this paper it is demonstrated that the framing of cultured meat has a significant effect on many attitudes and beliefs about the product, as well as behavioral intentions toward it. It is found out that technical descriptions of cultured meat lead to it's lower acceptance rate, this is because 'artificial' meat term causes a sense of perceived unnaturalness in minds of consumers. • This paper also highlights on an experiment whose results conclude that there is a substantial potential for cultured meat market and provides evidence that cultured meat could displace a considerable amount of conventional meat in market.

*Source: Derived from (Kadim, Mahgoub, Baqir, Faye and Purchas, 2015).

Advantages and Disadvantages of Cultured Meat :

Aspects	Advantages of Cultured Meat	Disadvantages of cultured Meat
Environmental pollution and water consumption		
	Cultured meat involves 7-45% less energy, lower greenhouse gas emission by 78-96%, 99% lower use of land and 82-95% lower water consumption as compared to conventional meat. These figures shows that cultured meat holds great environmental promise. (Hanna et al. 2011, StellanWelin 2013)	About 14.5% of world's total Greenhouse Gas Emission is due to animal agriculture as stated by UN Food And Agriculture Organization (FAO) (Gerber et al., 2013). About 44% of animal industry's total emission consists of Methane which is one of the leading reasons for global warming. (Climate change division 2012).
Social issues: Employment		
	Skilled man power utilization resulting in increase of the human capital thus affecting the GDP of the country	**Job Losses/ Unemployment** From farmers to the laborers in the meat processing industry would lose their jobs if lab grown meat gets popularized. If the population chooses lab grown meat over the traditional normal meat, farmers would be discouraged to involve in animal husbandry.
Ethical issues:		
	Sterile production – The production of meat from cell cultures is safer than traditional production by animal husbandry due to the aseptic and strictly controlled environment needed for its production. (Nicolas et al., 2011) Through embracing cultivated meat technology, already developed meat producers will find an opportunity to improve public understanding of their product in terms of food security and animal welfare. (Robert et al., 2013)	**Boneless Meat**- Lab grown meat does not contain bones so it can be used for burgers, sandwiches or as any other processed food sources, it is the best and preferred. Indian population on the other hand would not prefer boneless meat. The consumption of meat in India is in the forms of curry, roasted/fried or in biriyani. The consumers also can argue about the compromise in taste brought in due to the lack of bones. The biggest obstacle so far is not engineering but the taste, i.e. unlike what people are used to because there is no blood, fat or connective tissue, so scientists are trying to improve it. (Bertalan M 2018).
	Infectious disease transmissions: Approximately 60% of all known human diseases and 75% of the most harmful emerging diseases were initially zoonotic (animal-transmitted). The overall risk of transmission of zoonotic diseases between livestock and humans is increased (Henning et al., 2006, Jay P et al., 2008). This data can be declined with the use of cultured meat.	

3. Challenges to cultured meat development (Rorheim et al., 2016) :

- **Funding for basic research:** Not a lot of funding is available for this initiative as this subject is relatively in its infant stages.
- **Lack of regulatory preparedness**: The current food industry regulations are not suitable enough for commercial production of cutured meat at a significant stage .
- **Genetic modification:** Public should be well informed and aware of use of GM in the production of cultured meat, transparency and openness to public should be key aspects for public enquiry.
- **Product mimicry**: Achieving textures similar to steak,chicken breast and bacon has been difficult for researchers to achieve.
- **Culture medium**: Development in this field is significantly hampered by the fact that ideal cell lines have not yet been identified, because individual cell lines still require separate medium formulations to proliferate. (Isha and Daan 2016, M A Lawson et al., 2000).
- **Concerns over culture medium:** A culture media is required which supplies all the essential macro amd

micro nutrients for growth and one of the key elements of media is foetal bovine serum(FBS).A pregnant cow is slaughtered and blood is drained from heart of its live,an-anesthetized fetus to obtain Foetal bovine serum and this inhumane process of extraction has been a major barrier for ethical profile of cultured meat.

- **Energy requirements**: Although water requirements needed to produce cultured meat are low compared to other forms of meat production but the energy required to produce cultured is estimated to be very high compared to previous estimates. According to some researchers the production of cultured meat ends up using more energy than livestock production(Matt Simon 2018).

4. Conclusion

It seems that by slowly replacing animal farming, largescale agricultural meat production can significantly reduce animal suffering, risk of human disease, and environmental problems. Government subsidies and increased national budget for bio- and agro technology research may be able to accelerate the production of cultivated products. Cultured meat would provide healthy, nutritious and sustainable food for future generations with a rapidly growing global population. It would reduce food shortages, reduce food-borne diseases, reduce pollution, and increase the production of cultured meat. Cultured meat would minimize relying on natural resources and land resources, providing the opportunity to use that land for other recreational and beneficial purposes. Cultured meat technology is still in its experimental stage and has so far been limited to producing a small number of processed meat items in laboratory settings for demonstrative purposes. Recently the research is motivated on streamlining the production methods in order to reduce the cost, advancement of scalability and minimize requirement of animal as a source. Further research can be done on launching and marketing of these products to build consumer`s trust, acceptance and familiarity with the product.

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