International Journal of Animal Science, Husbandry and Livestock Production ISSN: 2141-5191 Vol. 2 (1), pp. 123-132, January, 2015. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Review

Tissue built meat- Future meat

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Accepted 30 December, 2014

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 (Asner et al., 2004; Savadogo et al., 2007) 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 requirements that need to be met to increase the feasibility of *in vitro* meat production, which includes finding an appropriate stem cell source, their growth inside a bioreactor and providing essential cues for proliferation and differentiation.

Key words: *In vitro* meat, tissue engineering, future meat substitute.

INTRODUCTION

The present meat production methods have some serious consequences associated with them and the consumers are showing serious concern over them since last few years. Currently, 70% of all agricultural land, corresponding to 30% of the total global surface, is being used for livestock production; with 33% of arable land being used for growing livestock feed crops and 26% being used for grazing (FAO, 2006; Steinfeld et al., 2006). World meat production at present is contributing between 15 and 24% of total current greenhouse gas emissions, which is more than the total emission of the transportation sector, a great proportion of this percentage is due to deforestation to create grazing land (FAO, 2006, Steinfeld et al., 2006). Food production directly or indirectly involves about 70% of the fresh water use and 20% of the energy consumption of mankind, of which a considerable proportion is used for the production of meat. The water use for livestock and accompanying feed crop production also has a dramatic effect on the environment such as a decrease in the fresh water supply, erosion and subsequent habitat and biodiversity loss (Asner et al., 2004; Savadogo et al., 2007). In addition, other consequences associated with current meat production methods are emergence of multi-drug-resistant strains of pathogenic bacteria (Sanders, 1999), animal disease epidemics or even pandemics, which can kill millions of people (Webster, 2002), nutritionally related diseases, such as cardiovascular diseases and diabetes, associated with the over-consumption of animal fats which are now responsible for a third of global mortality (WHO, 2001) and food-borne illnesses with a six fold increase in gastro-enteritis and food poisoning in industrialized countries in the last 20 years (Nicholson et al., 2000) with contaminated meats

and animal products being the most common causes of food borne diseases (Barnard et al., 1995; Mead et al., 1999; Nataro and Kaper, 1998; European Food Safety Authority, 2006; Fisher and Meakens, 2006).

Global population is anticipated to increase to 9 billion by the year 2050 and the demand for meat continues to grow worldwide (Steinfeld et al., 2006). Annual global meat production will rise to 465 million tonnes by the year 2050 accompanied by a rise in annual greenhouse gas emissions to 19.7 gigatonne of carbondioxide, carbon equivalent (Steinfeld et al., 2006) . The animals themselves are mostly responsible for the emission of greenhouse gases (Williams et al., 2006) and therefore a reduction of the number of animals that could be achieved by in vitro meat production would result in an appreciable decline of greenhouse gas emission. There are many other reasons for promoting *in vitro* meat production including animal well fare, process monitoring, environmental considerations, efficiency of food production in terms of feedstock, decrease in intense land usage and greenhouse gas emissions (Stamp Dawkins and Bonney, 2008). Thus, continuing the production of meat by current methods is going to further aggravate the problems and *in vitro* meat production system seems to be an appealing alternative and is becoming increasingly justifiable in light of the sizable negative effects of current meat production system.

Advantages/need of *in vitro* meat

The first important advantage of producing cultured meat is better control over meat composition and quality by manipulating the flavor, fatty acid composition, fat content and ratio of saturated to poly-unsaturated fatty acids through composition of the culture medium or co-culturing with other cell types. Furthermore, health aspects of the meat can be enhanced by adding factors like certain types of vitamins to the culture medium which might have an advantageous effect on the health (van Eelen et al., 1999). Secondly due to strict quality control rules, such as Good Manufacturing Practice, that are impossible to be introduced in modern animal farms, slaughterhouses, or meat packing plants, the chance of meat contamination and incidence of food borne disease could be significantly reduced. In addition, the risks of exposure to pesticides, arsenic, dioxins, and hormones associated with conventional meat could also be significantly reduced. Third advantage may be the production of exotic cultured meats. In theory, cells from captive rare or endangered animals (or even cells from samples of extinct animals) could be used to produce exotic meats in cultures and thus a sustainable alternative to global trade of meats from rare and endangered animals will help in increasing wild populations of many species in many countries. Cultured meat also reduces animal use in the meat production system as theoretically a single farm animal may be used to produce the world's meat supply. Another advantage is the reduction in the amount of nutrients and energy needed for their growth and maintenance as the biological structures in addition to muscle tissue are not required to produce meat in an *in vitro* system. Furthermore, *in vitro* system significantly lowers time to grow the meat and takes several weeks instead of months for chickens and pigs and years for beef cattle before the meat can be harvested and thus, the amount of feed and labor required per kilogram of *in vitro* cultured meat is much lower. Another advantage of cultured meat is that the bioreactors for *in vitro* meat production, unlike farm animals, do not need extra space and can be stacked up in a fabric hall. Thus, nutritional costs for *in vitro* cultured meat will be significantly lower and the decrease in costs of resources, labor, and land may be compensated by the extra costs of a stricter hygiene regime, stricter control, computer management, etc. Need for other protein sources also demands production of cultured meat and because it is, unlike the other products, animal-derived and with respect to composition most like meat, it may be the preferred alternative. A definite market available for meat substitutes and a small market comprising the vegetarians who do not eat meat for ethical reasons also demand the production of *in vitro* meat. The proteins produced using plants and fungi are animal friendly, sustainable and have been used to make a variety of good chief products but they lack a good texture and taste and such products are no solution for the craving for meat. Further cultured meat will be safer than conventional meat and due to the non-sustainability of traditional meat production there is a huge market for this. The comparatively minimal land requirement of an *in vitro* meat production system allows meat production and processing to take place domestically in countries which would normally rely on imported meats. By bringing the stages of the meat production process closer together spatially and temporally, meat supply can be better determined by demand.

Culturing of *in vitro* meat

The idea of cultured meat for human consumption in a lab *ex vivo* is not a new concept but was predicted long back by Winston Churchill in the 1920s. Alexis Carrel in 1912 managed to keep a piece of chick heart muscle alive and beating in a Petri dish demonstrating that it was possible to keep muscle tissue alive outside the body, provided that it was nourished with suitable nutrients. It was Willem van Eelen of Netherlands who independently had the idea of using tissue culture for the generation of meat products in the early 1950s and in 1999 van Eelen's theoretical idea was patented. SymbioticA harvested muscle biopsies from frogs and kept these tissues alive and growing in culture dishes (Catts and Zurr, 2002). A study involving the use of muscle tissue from the common goldfish (*Carassius auratus*) cultured in Petri

dishes was published in 2002 (Benjaminson et al., 2002). Other research initiatives have also achieved keeping muscle tissue alive in a fungal medium, anticipating on the infection risk associated with serum based media (Benjaminson et al., 2002).

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.

There are two similar untested detailed proposals based on emerging field of tissue engineering (Boland et al., 2003, Zandonella, 2003) for using cell culture for producing *in vitro* meat on scaffold-based techniques. One of the two proposals has been written by Vladimir Mironov for the NASA (Wolfson, 2002) while the other proposal has been written by Willem van Eelen who also holds a worldwide patent for this system (van Eelen et al., 1999). Catts and Zurr (2002) however, appear to have been the first to have actually produced meat by this method. Both of these systems work by growing myoblasts in suspension in a culture medium. Embryonic myoblasts or adult skeletal muscle satellite cells are proliferated, attached to a scaffold or carrier such as a collagen meshwork or microcarrier beads, and then perfused with a culture medium in a stationary or rotating bioreactor. By introducing a variety of environmental cues, these cells fuse into myotubes, which can then differentiate into myofibers (Kosnik et al., 2003). The resulting myofibers may then be harvested, cooked, and consumed as meat (Figures 1 and 2). Mironov proposal (Wolfson, 2002) uses a bioreactor in which cells are grown together with collagen spheres to provide a substrate onto which the myoblasts can attach and differentiate whereas van Eelen's proposal (van Eelen et al., 1999) uses a collagen meshwork and the culture medium is refreshed from time to time or percolated through the meshwork. Once differentiated into myofibers, the mixture of collagen and muscle cells can be harvested and used as meat. While these kinds of techniques work for producing ground processed (boneless) meats with soft consistency, they do not lend themselves to highly structured meats like steaks.

In order to produce highly structured meats, one would need self organizing/tissue culture techniques, creating structured muscle tissue as self-organizing constructs (Dennis and Kosnik, 2000) or proliferating existing muscle tissue *in vitro*. Benjaminson et al. (2002) cultured Gold fish (*Carassius auratus*) muscle explants. In this study muscle tissue cultured with crude cell extracts showed a limited increase in cell mass and the cultured muscle explants so obtained were washed, dipped in olive oil with spices, covered in breadcrumbs and fried. A test-panel judged these processed explants and agreed that the product was acceptable as food. Tissue culture techniques have the advantage that explants contain all the tissues which make up meat in the right proportions and closely mimics *in vivo* situation. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if separated for long periods by more than 0.5 mm from a nutrient supply (Dennis and Kosnik, 2000) . According to Vladimir Mironov entirely artificial muscle can be created with tissue engineering techniques by a branching network of edible porous polymer through which nutrients are perfused and myoblasts and other cell types can attach (Wolfson, 2002).

Cell sources for tissue engineered 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 self-renewal 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 self-renewal potential from pre-implantation embryos of farm animal species. Until now, true embryonic stem cell lines have only been generated from mouse, rhesus monkey, human and rat embryos (Talbot and Blomberg, 2008) but the social resistance to cultured meat obtained from mouse, rat or rhesus monkey will be considerable and will not result in a marketable product. Myosatellite cells isolated from different animal species have different benefits and limitations as a cell source and that isolated from different muscles have different capabilities to proliferate, differentiate, or be regulated by growth modifiers (Burton et al., 2000). Myosatellite cells have been isolated and characterized from the skeletal muscle tissue of cattle (Dodson et al., 1987), chicken (Yablonka-Reuveni, 1987), fish (Powell et al., 1989), lambs (Dodson et al., 1986), pigs (Blanton et al., 1999, Wilschut et al., 2008), and turkeys (McFarland et al., 1988). Porcine muscle progenitor cells have the potential for multilineage differentiation into adipogenic, osteogenic and

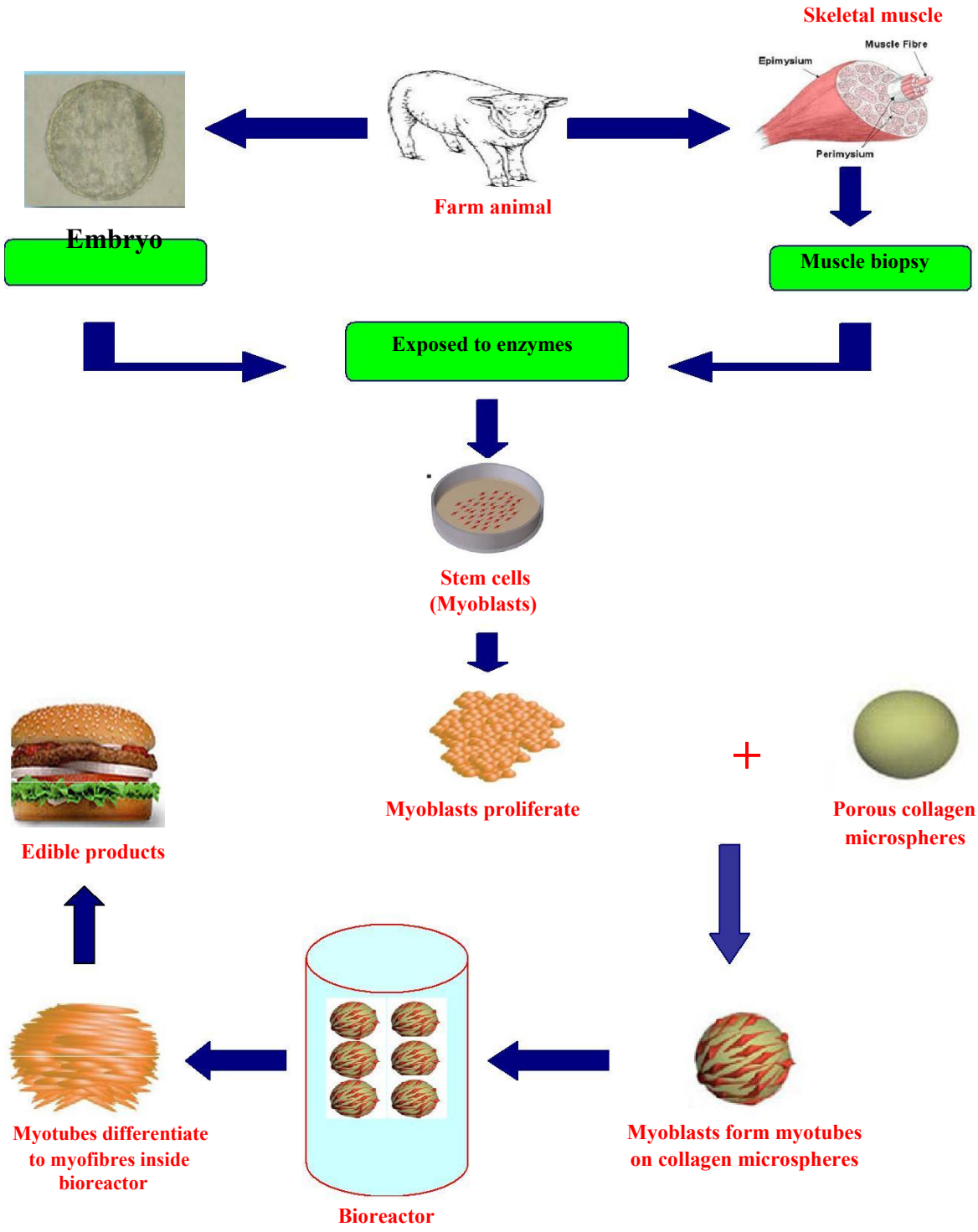

Figure1. Scaffold-based cultured meat production.

chondrogenic lineages, which may play a role in the development of co-cultures (Wilschut et al., 2008).

Adult stem cells from farm animal species can alternatively be used and myosatellite cells are one example of an adult stem-cell type with multilineage potential (Asakura et al., 2001). Adult stem cells have been isolated from several different adult tissues (Wagers and Weissman, 2004) but their *in vitro* proliferation capacity is not unlimited and can proliferate *in vitro* for several months at most. These cells also have the

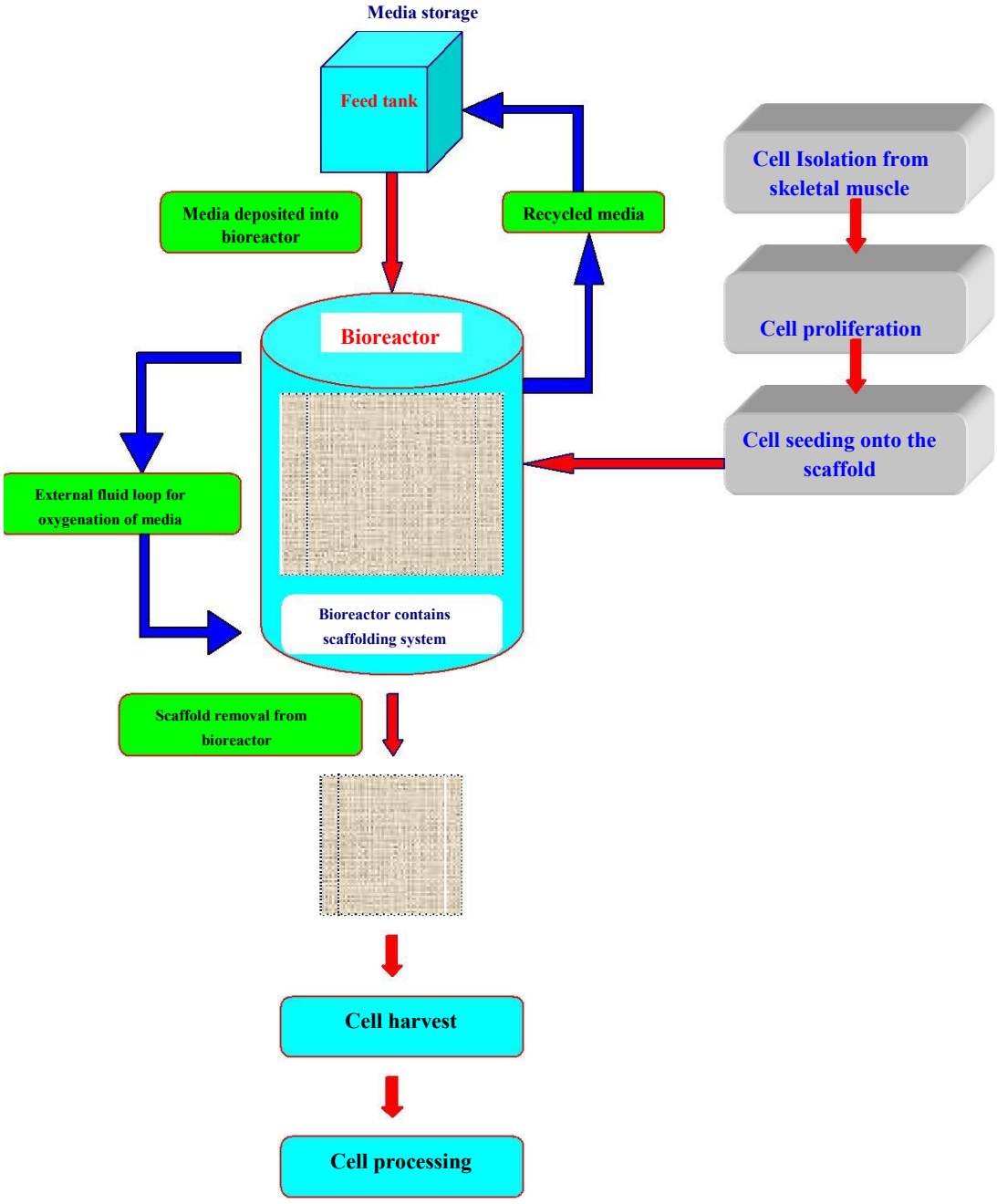

Figure 2. Possible *in vitro* meat production scheme.

capacity to differentiate into skeletal muscle cells, although not very efficiently but for now, these are the most promising cell type for use in the production of cultured meat. However, adult stem cells are prone to malignant transformation in long-term culture (Lazennec and Jorgensen, 2008) that is the greatest matter of debate.

A rare population of multipotent cells found in adipose tissue known as adipose tissue-derived adult stem cells (ADSCs) is another relevant cell type for *in vitro* meat production (Gimble et al., 2007) which can be obtained from subcutaneous fat and subsequently trans-differentiated to myogenic, osteogenic, chondrogenic or adipogenic cell lineages (Kim et al., 2006). It has been observed that adipose tissue-derived adult stem cells immortalize at high frequency and undergo spontaneous transformation in long-term (4 to 5 months) culturing (Rubio et al., 2005), while evidence of adult stem cells remaining untransformed have also been reported (Bernardo et al., 2007). To minimize the risk of

spontaneous transformation, re-harvesting of adult stem cells may be necessary in an *in vitro* meat production system and as such obtaining ADSCs from subcutaneous fat is far less invasive than collection of myosatellite cells from muscle tissue. Matsumoto et al. (2007) reported that mature adipocytes can be dedifferentiated *in vitro* into a multipotent preadipocyte cell line known as dedifferentiated fat (DFAT) cells, reversion of a terminally differentiated cell into a multipotent cell type. These DFAT cells are capable of being transdifferentiated into skeletal myocytes (Kazama et al., 2008) and appear to be an attractive alternative to the use of stem cells. This process known as “ceiling culture method” certainly seems achievable on an industrial scale but Rizzino (2007) has put forth the argument that many of the claims of transdifferentiation, dedifferentiation and multipotency of once terminally differentiated cells may be due to abnormal processes resulting in cellular look-alikes.

Co-culturing

Myoblasts 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 vascularization 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 m, a vasculature needs to be created (Jain et al., 2005).

Scaffolds

A substratum or scaffold must be provided for proliferation and differentiation of myoblasts as they are anchorage- dependent cells (Stoker et al., 1968). 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) and its by-products must be edible and natural and may be derived from non-animal sources, though inedible scaffold materials cannot be disregarded. Scaffolds based on new biomaterials may be developed that may offer additional characteristics, such as fulfilling the requirement of contraction for proliferation and differentiation (De Deyne, 2000). Thus, challenge is to develop a scaffold that can mechanically stretch attached cells to stimulate differentiation and a flexible substratum to prevent detachment of developing myotubes that will normally undergo spontaneous contraction.

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

Bioreactors

Commercial production of *in vitro* meat based products requires large bioreactors for large-scale culturing for the generation of sufficient number of muscle cells. Development of new bioreactors that will maintain low shear and uniform perfusion at large volumes is required. 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 three-dimensional culture submerged in the medium in a perpetual free fall state (Carrier et al., 1999) which improves diffusion with high mass transfer rates at minimal levels of shear stress, producing three dimensional tissues with structures very similar to those *in vivo* (Martin et al., 2004). Direct perfusion bioreactors

appear more appropriate for scaffold based myocyte cultivation and flow medium through a porous scaffold with gas exchange taking place in an external fluid loop (Carrier et al., 2002). Besides offering high mass transfer they also offer significant shear stress, so determining an appropriate flow rate is essential (Martin et al., 2004). Direct perfusion bioreactors are also used for high-density, uniform myocyte cell seeding (Radisic et al., 2003). Another method of increasing medium perfusion is by vascularizing the tissue being grown. Levenberg et al., (2005) had induced endothelial vessel networks in skeletal muscle tissue constructs by using a co-culture of myoblasts, embryonic fibroblasts and endothelial cells co-seeded onto a highly porous biodegradable scaffold. Research size rotating bioreactors have been scaled up to three liters and, theoretically, scale up to industrial sizes should not affect the physics of the system.

As cell viability and density positively correlate with the oxygen gradient in statically grown tissue cultures, it is necessary to have adequate oxygen perfusion during 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. Modified versions of hemoglobin or artificially produced perfluorochemicals (PFCs) that are chemically inert are used as oxygen carriers (Lowe, 2006) but their bovine or human source makes them an unfit candidate and alternatively, human hemoglobin has been produced by genetically modified plants (Dieryck et al., 1997) and microorganisms (Zuckerman et al., 1998).

Culture media and growth factors

To enjoy its potential advantages over conventional meat production, *in vitro* meat would need an affordable medium system containing the necessary nutritional components available in free form to myoblasts and accompanying cells. Myoblast culturing usually takes place in animal sera, a costly media that does not lend itself well to consumer acceptance or large-scale use. Animal sera are from adult, newborn or fetal source, with fetal bovine serum being the standard supplement for cell culture media (Coecke et al., 2005). Because of its *in vivo* source, it can have a large number of constituents in highly variable composition and potentially introduce pathogenic agents (Shah, 1999). The harvest of fetal bovine serum also raises ethical concern and for the generation of an animal- free protein product, the addition of fetal calf serum to the cells would not be an option and it is therefore essential to develop a serum-free culture medium. Commercially available serum replacements and serum -free culture media offer some more realistic options for culturing mammalian cells *in vitro*. Serum-free media reduce operating costs and process variability while lessening the potential source of infectious agents (Froud, 1999).

Serum-free media have been developed to support *in vitro* myosatellite cell cultures from the turkey (McFarland et al., 1991), sheep (Dodson and Mathison, 1988) and pig (Doumit et al., 1993). Variations among different serum-free media outline the fact that satellite cells from different species have different requirements and respond differentially to certain additives (Dodson et al., 1996). Thus, an appropriate array of growth factors is also required to growing muscle cells in culture in addition to proper nutrition and these growth factors are synthesized and released by muscle cells themselves and, in tissues, are also provided by other cell types locally (paracrine effects) and non-locally (endocrine effects). The myosatellite cells of different species respond differentially to the same regulatory factors (Burton et al., 2000) and as such extrinsic regulatory factors must be specific to the chosen cell type and species. Furthermore, formulation may be required to change over the course of the culturing process from proliferation period to the differentiation and maturation period, requiring different set of factors. A multitude of regulatory factors have been identified as being capable of inducing myosatellite cell proliferation (Cheng et al., 2006), and the regulation of meat animal-derived myosatellite cells by hormones, polypeptide growth factors and extracellular matrix proteins has also been investigated (Dodson et al., 1996, Doumit et al., 1993). Purified growth factors or hormones may be supplemented into the media from an external source such as transgenic bacterial, plant or animal species which produce recombinant proteins (Houdebine, 2009). Alternatively, a sort of synthetic paracrine signalling system can be arranged so that co-cultured cell types can secrete growth factors, which can promote cell growth and proliferation in neighbouring cells. Appropriate co-culture systems like hepatocytes may be developed to provide growth factors necessary for cultured muscle production that provide insulin-like growth factors which stimulate myoblast proliferation and differentiation (Cen et al., 2008), as well as myosatellite cell proliferation in several meat-animal species *in vitro* (Dodson et al., 1996).

Atrophy and exercise

One of the potential problems associated with cultured meat is that of atrophy or muscle wasting due to a reduction of cell size (Fox, 1996) caused by lack of use, denervation, or one of a variety of diseases (Charge et al., 2002, Ohira et al., 2002). Regular contraction is a necessity for skeletal muscle and promotes differentiation and healthy myofiber morphology while preventing atrophy. Muscle *in vivo* is innervated, allowing for regular,

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

Electrical stimulation

Neuronal activity can be mimicked by applying appropriate electrical stimuli *in vitro* cultures (Bach et al., 2004) and has proven to be pivotal in the development of mature muscle fibers (Wilson and Harris, 1993). It has been shown that induction of contractile activity promoted the differentiation of myotubes in culture by myosin heavy chain expression of different isoforms and sarcomere development (Fujita et al., 2007, Naumann and Pette, 1994). Electrical stimulation can provide a non invasive and accurate tool to assess the functionality of engineered muscle constructs (Dennis et al., 2009). Functional muscle constructs will exert a force due to active contractions of the muscle cells by generating a homogeneous electrical field inside the bioreactor but so far, these forces generated by engineered muscle constructs only reach 2 to 8% of those generated by skeletal muscles of adult rodents (Dennis et al., 2001). Thus, functional properties of tissue engineered muscle constructs are still unsatisfactory at this moment.

Mechanical stimulation

Mechanotransduction is the process through which cells react to mechanical stimuli and is a complex mechanism (Burkholder, 2007; Hinz, 2006) that is another important biophysical stimulus in myogenesis (Vandenburgh and Karlisch, 1989). It is mainly by means of the family of integrin receptors that cells attach to the insoluble meshwork of extracellular matrix proteins (Juliano and Haskill, 1993) transmitting the applied force to the cytoskeleton. The resulting series of events shows parallels to growth factor receptor signaling pathways, which ultimately lead to changes in cell behavior, such as proliferation and differentiation (Burkholder, 2007). Muscle growth and maturation is affected by different mechanical stimulation regimes and the application of static mechanical stretch to myoblasts *in vitro* results in a facilitated alignment and fusion of myotubes, and also results in hypertrophy of the myotubes (Vandenburgh and Karlisch, 1989). Furthermore, cyclic strain activates quiescent satellite cells (Tatsumi et al., 2001) and increases proliferation of myoblasts (Kook et al., 2008). Thus all these results indicate that mechanical stimulation protocols affect both proliferation and differentiation of muscle cells and different parameters that presumably influence the outcome of the given stimulus are percentage of applied stretch, frequency of the stimulus and timing in the differentiation process.

Food processing technology

Depending on the starting material utilized, new food processing technologies need to be developed to make *in vitro* meat based products attractive.

CONCLUSION

Being a sustainable, humane and safer system, cultured meat holds great promises as an alternative to traditionally livestock flesh provided consumer resistance can be overcome. *In vitro* meat production system can alleviate the ill consequences associated with current meat production methods by reducing the number of livestock animals, incidence of food borne disease, pollution level, emission of green house gases besides reducing the animal suffering significantly. Since crucial knowledge is still lacking on the biology and technology, it may be concluded that commercial production of cultured meat is as yet not possible and the focus must be on filling these gaps in knowledge.

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