

In vitro meat production

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In this report, the possibility of using *in vitro* cell culturing techniques for the production of meat is investigated. There are several reasons for investing such a system. One application could be to reduce animal suffering, environmental pollution or land use associated with current meat production methods. Meat is an important nutritional and social factor for the human race and meat consumption in the world is expected to increase dramatically during the coming decades. Another application could be a continuous meat supply for long-term manned space missions in the far future. It is probably possible to produce meat *in vitro* by culturing loose myosatellite cells on a substrate. After differentiation muscle cells could be harvested and used as processed meat. The culturing of actual muscle tissue is also an option, as long as a nutrient- and oxygen perfusion system of some kind can be established. Detailed proposals exist for the former method, and experiments have been performed on the latter method. It is not known whether *in vitro* cultured meat would be well accepted by the consumer, but the initial reaction seems to be moderately negative. Especially genetic manipulation might hinder consumer acceptance. To establish a sustainable *in vitro* meat culturing system on an industrial scale, a great deal of research is still needed on the use of culture media.

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Disclaimer:

This report was written as a side-project during a stage in science journalism for a masters degree at the Wageningen University. The author is a trained biologist but has no particular affinity with the techniques described in this report. It is based solely on literature research, and neither detailed nor exhaustive. It must rather be seen as an introduction on and exploration of in vitro meat production.

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Abbreviations

BSE: *Bovine Spongiform Encephalopathy*: Mad cow disease, chronic degenerative disease affecting the central nervous system of cattle.

CELSS: *Controlled Ecological Life Support System*: A collection of organisms on board of a spaceship which recycle waste products and produce oxygen, water and food for the crew.

EDL: *Extensor Digitorum Longus*: Extensor muscle in the leg.

ES: *Embryonic Stem cell*: Embryonically derived stem cells (in mammals from the inner cell mass) which are pluripotent and have an unlimited division capacity.

IMPS: *IN VITRO Meat Production System*: A system for proliferating muscle cells or tissue for the purpose of creating meat.

NASA: *National Aeronautics and Space Administration*: The space administration of the USA.

PGA: *PolyGlycolic Acid*: A polymer commonly used to create scaffolds for tissue-engineering.

Genes

MRF4: Muscle Regulatory Factor 4

MYF5: Myogenic Factor 5

MYOD: Myoblast Determination Protein

MYOGENIN: Myogenin

1 Introduction

In this report the possibility of using *in vitro* cell or tissue culture for the production of edible animal muscle (meat) will be discussed. In this chapter, the concepts of such a system are introduced. First, in section 1.1, it will be explained what is meant by such a system. The following sections are a rationale for why such a system should be built; in section 1.2, aspects of human meat consumption are discussed, and in section 1.3, the problems associated with long-term manned space missions

are treated. In section 1.4, a brief overview on the structure, origin, and life cycle of muscle tissue is given.

In chapter 2, the technical possibilities and limitations of an *in vitro* meat production system (IMPS) system are discussed. Chapter 3 will deal with the social issues of this production process. The last chapter is the discussion.

This report is meant as an introduction to the field of *in vitro* meat production.; the mere *possibility* of an IMPS and its implications is investigated, and there are no practical recommendations or protocols to be found.

1.1 Description of an in vitro meat production system

It is possible to grow many cell types of both animal and plant origin without the organism of which it was derived. This is known as *in vitro* culturing. Culturing involves the extraction of cells from the organism and transferring them onto or into a suitable growth medium. This medium can contain nutrients, energy sources, growth factors, etc. depending on the goal of the cell culture. Cell (or tissue) culturing can be performed for several reasons, for example research on cellular processes or medicine production. In this report, the reason for a cell culturing system is the production of edible animal muscle, better known as meat. Such a system requires the proliferation of a small amount of muscle cells to a large muscle cell mass or tissue.

1.2 Meat consumption

1.2.1 Requirement of human body for animal proteins

The human species has evolved as an omnivore. To maintain the health of a human body, an adequate intake of food from both plant and animal origin is required [Nestle 1999]. Although there are strong indications that a high intake of meat increases the risk of chronic diseases [Nestle 1999], a certain intake is at least beneficial and probably required for the functioning of the human body. Animal products are especially important as a source of iron, protein, B-vitamins, and calcium [Voedingscentrum 2003]. Vegetarian diets can probably be perfectly

healthy, but these kinds of diets need to be composed with great care [Sanders 1999; Voedingscentrum 2003]. A lack of meat in these diets can be compensated by the ingestion of other animal products, like dairy products and eggs. Vegan diets on the other hand do not include any animal products at all. As a result, they contain low amounts of calcium, vitamin B₂, and certain proteins, which is hard to compensate for with natural food products of non-animal origin. Moreover, vitamin B₁₂ is lacking from a vegan diet altogether [Voedingscentrum 2003]. Vitamin B₁₂ (cobalamin) is required by bacteria, algae and animals, but not by higher plants. Therefore, it is completely absent in food sources derived from higher plants. In mammalian cells it is needed as a coenzyme for the enzymes methionine synthase and methylmalonyl-CoA mutase. All vitamin B₁₂ is ultimately derived from bacteria and algae, which are all but a few exceptions able to synthesize it. It enters the food chain of higher animals via large herbivores, where it is produced by the microflora in the gastro-internal tract. The microflora of omnivores and carnivores do not produce enough vitamin B₁₂ to fulfill the requirements, and even if it did it could not be absorbed by their intestine. This last observation also means that vitamin B₁₂ cannot be derived from a microorganismal food source by humans. A lack of vitamin B₁₂ leads to the conditions of megaloblastic anaemia¹ and subacute combined degeneration², and is lethal in the long term [Scott 1999; Fox 1996, table 19.4]. Hence a vegan diet is probably not possible without the intake of (chemically derived) food additions [Voedingscentrum 2003]. Data on the status of vitamin B₁₂ in vegan diets, even when it is artificially added is inconclusive [Sanders 1999].

Apart from the nutritional status, meat and other animal products play an important social role in the western society. In The Netherlands, the *per capita* meat consumption was 50.1 kg in 2001³ [De Productschappen Vee 2001]. In the period 1994/1995 this amount was for Germany and Switzerland respectively 61.6 and 57.7 kg. A sur-

vey in Switzerland showed that 24.8% of the respondents consumed meat or meat products at least once every day [Eichholzer and Bisig 2000]. The consumption of meat appears to be a strong social factor, as only a marginal group chooses to be vegetarian; 4.7% of the adults in Australia in 1995, 5.4% of the adults in Britain in 1997. This figure is probably even lower, as people who claim to be vegetarian often still consume meat from time to time [Lea and Worsley 2001]. From 1961 on there is a trend in both developed countries as in developing countries to eat more meat [Rosegrant et al. 1999], although meat consumption has stabilized in the last couple of years in the developed world⁴ [Nestle 1999]. Until 2020, meat demand is expected to increase highly in developing countries and slightly in developed countries [Rosegrant et al. 1999]. In an Australian survey, the nutritional value was mentioned as an important reason to eat meat, but also the factors that people like meat, the belief that “humans are meant to eat meat”, and the belief that vegetarian diets are boring. Furthermore, environment seems to be of great importance for the decision to eat meat, as people mentioned the fact that friends and family eat meat and that vegetarians are viewed as “weird” to be of consideration [Lea and Worsley 2001]. The western society is thus socially geared toward the consumption of meat.

In addition to nutritional and social arguments, it is possible to speculate about two other reasons why the consumption of animal products is desired over a diet lacking animal products altogether. The first is that man has evolved as an omnivore, so its gastro-internal regulation and its physiology are optimized for the processing of an omnivorous diet. This could imply that some of the essential nutrients are digested best when it is consumed in such a form. The other reason is that the current scientific knowledge is incomplete on the exact dietary requirements. There might very well be unidentified factors in the food which are nevertheless essential for the functioning of the human body. Again, these are merely speculations.

¹This is a very typical form of anaemia in which megaloblasts are found in the bone marrow of the patient [Walker 1995].

²A condition in which motor and sensory nerve tracts degenerate. This is mainly manifested in the spinal cord, but can also occur in brain, eye, and peripheral nerves. The condition starts with demyelination but later on the entire nerve cell is destroyed [Walker 1995; Greene et al. 2003].

³This is the amount bought in the store by the consumer. If processing, cooking, and the pieces left on the plate are accounted for, 39.4 kg per person was actually consumed [De Productschappen Vee 2001].

⁴There is even a slight decline in the amount of meat consumed over the last years [De Productschappen Vee 2001; Nestle 1999; Lea and Worsley 2001; Solomon et al. 1997].

In conclusion, meat consumption is a very important nutritional factor in the human population, and, at least in the western society, a strong social factor. The meat demand of the world will continue to rise sharply over the next decades, mainly due to a rise in the meat demand of developing countries.

1.2.2 Problems associated with meat production

The production of meat has certain negative effects on animal welfare, land use and the environment. In this section the issues surrounding meat production are discussed.

Animal suffering To suit the meat demands of a modern western society, animals are intensively kept and production is optimized disregarding the well-being of the animals. Throughout history, domestic animals were able to adapt to the changing conditions, however since World War II the pace of change is increased to such a dramatic extent that this is no longer fully possible [Crok 2003]. Because of the high number of animals being used (120,000,000 each year just in The Netherlands) an efficient and cheap production system is required. This results in herding of animals in confined spaces in unfavorable conditions. The adaptability of the animal is not high enough to cope with this unnatural conditions, and high stress levels are observed, resulting in disease, abnormal behaviour and death [Crok 2003]. For example, pigs herded for meat consumption with intensive farming techniques live in concrete boxes of at most 1.0 m². Food provides the only distraction, but this is consumed quickly due to its high nutritional quality. This eventually leads to the biting of ears, legs and tails of other pigs [Hopster and Kranendonk 2003]. Some animal races like pigs and chickens domesticated for meat production have such a high growth rate for muscle tissue that the cardiovascular system is incapable of providing satisfactory amounts of oxygen to the animal body, leading to health problems and in chickens even to preliminary death [Crok 2003].

This raises serious ethical questions, and many people disagree with the current state of affairs. This is an addition to the older question whether it is right to kill an animal in order to eat it, regardless of the accommodation conditions. Large

groups of the western population oppose to the way in which meat is produced today, and some of them or looking for more animal-friendly alternatives like organic farming [Williams 2002] or refuse to eat meat altogether (as mentioned in the previous section).

Environmental and social issues The efficiency of meat production is unfavorable compared to plant-based food sources. In developed countries, it takes 2 kg of grains to produce 1 kg of chicken meat, 4 kg of grains to produce 1 kg of pig meat, and 7 kg grains per kg beef [Rosegrant et al. 1999]. It should be noted though, that some herbivores can cope with plant parts which are inedible to humans [Avery 1997]. It is unclear what the environmental effects of meat production exactly are, as the debate seems to be partly emotionally driven, but it is clear that it has a great impact on land, water and energy use, as well as on the emission of greenhouse gases and pollutants [Avery 1997; Solomon and Johnston 1997; Reay 2002; van Eelen et al. 1999]. In addition, there is the problem of antibiotics being used as growth promoters for animals kept in intensive farming. This use probably contributes to the emergence of multi-drug-resistant strains of pathogenic bacteria [Sanders 1999].

Another problem is that of animal disease epidemics, as recent outbreaks of bovine spongiform encephalopathy (BSE) in the UK and Europe [Johnson and Gibbs 1998; Sanders 1999] or the chicken flu in The Netherlands and Belgium illustrate. Both these animal diseases can potentially be dangerous for humans too. Consumption of certain tissues of cows contaminated with BSE can lead to a special form of Creutzfeld-Jacob disease [Johnson and Gibbs 1998]. While this risk is very low, a more serious threat is posed by the chicken flu, as this can lead to possible new influenza epidemics or even pandemics, which can kill millions of people [Webster 2002].

So intensive farming poses great problems in diverse areas, which many people oppose to. Yet the willingness to change diet in order to decrease these problems is very low.

1.3 Long-term manned space missions

A journey to Mars would take a time of 8 months (only to get there). Such a mission can only be ef-

ficient if a human crew would not only fly to Mars and back, but also stays there for a prolonged period. Some estimates for a manned round-trip to Mars are in the range of 4 years [Benjaminson et al. 2002]. More distant goals would take considerably longer; with the current rocket-technology a journey to Alpha Centauri, the closest solar system would take 70,000 years. At least for now, this is completely science-fiction. However, the Russian space agency is seriously considering a manned Mars mission within two decades [Knight 2002].

During such a mission, a human crew must remain both physically and psychically healthy, which requires the intake of high-quality food over the entire duration of the mission. Launch costs are currently estimated at \$4,600 per pound, which is over \$10,000 per kg [Crews 2003]. This renders the prospect of launching the necessary food in pre-processed form unattractive. Apart from the nutritional adequacy of the food, the pleasantness and variety is also an important factor. Astronauts often dislike the traditional space-foods and as a result, eat less of it than they normally would. It has been shown that with a low variety in food choice, people tend to dislike the food over time and start to eat less [Zandstra et al. 2000]. As for current space missions, supply and physiochemical regeneration (of water and oxygen) are the most cost-effective, but for longer periods and permanent bases, bioregeneration becomes more attractive [Drysdale et al. 2003]. This solution would be far more elegant, as it reduces the launch costs for long-duration missions, supplies the astronauts with fresh food, and extends or even removes the limit on duration of the mission. A controlled ecological life support system (CELSS) would not only provide food to the astronauts, but also deal with waste, and provide oxygen and water [Saha and Trumbo 1996; Benjaminson et al. 1998; Drysdale et al. 2003]. Several systems of recycling by means of micro-organisms, lower and higher plants [Saha and Trumbo 1996; Salisbury and Clark 1996; Knight 2001], fungi [Benjaminson et al. 1998], and combinations thereof are being investigated. As is discussed in section 1.2.1, a human crew would require at least some animal proteins or food-supplements, but the herding of animals on a spaceship would be extremely inefficient. For this reason, the national aeronautics and space administration (NASA) initiated the research on *in vitro* meat production. At the moment, think-

ing about IMPSES for long-termed space missions remains purely an academical exercise. However, one that is interesting enough (in my opinion) to speculate about.

1.4 Life cycle of muscle tissue

To understand the possibilities and limits of the culturing of vertebrate muscle tissue, a brief introduction on the structure, development, and growth of skeletal muscles in vertebrates will be given.

1.4.1 The anatomy of skeletal muscles

Skeletal muscles are built up of columns of muscle tissue (fascicles) embedded in fibrous connective tissue (the epymisium). Fascicles are surrounded by a sheath of connective tissue called the perimysium. Each fascicle is built up of several myofibers, which are the actual muscle cells. Myofibers are very long, multinucleate cells incapable of mitosis [Fox 1996, p. 309–310]. There are roughly two type of muscles; fast and slow. Fast muscles are specialized in short and fast actions, while slow muscles are specialized in prolonged and relatively slow actions [Fox 1996, p. 332].

1.4.2 Formation of skeletal muscle

Embryonically, all skeletal muscle tissue in vertebrates is derived from the mesoderm. At the somite stage, the somites split in the sclerotome and the dermamyotome. The dermamyotome then gives rise to the skin and the skeletal muscle [Buckingham 2001; Wolpert et al. 1998, p. 100–102]. Several transcription factors have been identified in the determination of muscle cell fate, the most important being Myogenic Factor 5 (MYF5) and Myoblast Determination Protein (MYOD). These are two (mutually inhibitory) transcription factors which induce the transcription of several muscle-specific proteins like action, myosin II, tropomyosin and creatine phosphate kinase. A requirement for the formation of actual muscle fibers is withdrawal from the cell cycle. As long as the muscle precursors are able to proliferate, they are unable to form muscle fibers and vice versa. After cell fate determination, Muscle Regulatory Factor 4 (MRF4) and Myogenin (MYOGENIN) become active in muscle differentiation [Hawke and Garry 2001; Wolpert et al. 1998,

p. 287–288]. It is proposed that there are embryonically two waves of myoblast proliferation. The first generation of myoblasts will differentiate into the slow myofiber type, while the myoblasts from the second generation will give rise to the fast myofiber type [Buckingham 2001].

After embryogenesis, little muscle cell proliferation takes place in higher animals. In postnatal muscle tissue, full-grown muscle fibers are accompanied with single-nucleated satellite cells which lie juxtaposed to the muscle fibers between the sarcolemma and the basal lamina [Hawke and Garry 2001]. These cells are the primary source for new nuclei in postnatal muscle tissue, although other sources can be used as well [Grounds et al. 2002]. Satellite cells are distinct in their appearance because of their small nuclear size and a high cytoplasm to nucleoplasm ratio [Hawke and Garry 2001]. By donating their DNA to existing muscle fibers or by fusing to form new fibers, they can facilitate muscle growth and regeneration [Burton et al. 2000; Buckingham 2001]. Normally, satellite cells are quiescent and nonproliferative, but upon stimulation by hypertrophy, increased exercise, atrophy or other forms of injury, they start to divide [Burton et al. 2000; Seale and Rudnicki 2000; Buckingham 2001; Hawke and Garry 2001; Grounds et al. 2002] and express myogenic markers [Hawke and Garry 2001].

1.4.3 Culturing of muscle tissue

It is possible to culture muscle fibers *in vitro*, however, they do not proliferate. As an alternative, satellite cells can be cultured. Typically neonatal individuals are selected for the isolation of myosatellite cells, because these cells are much more abundant in muscles of young animals than in muscles of older animals [Hawke and Garry 2001]. Isolation requires the mincing of complete muscles, followed by enzymatic treatment and separation of the satellite cells by differential centrifugation, preplating, Percoll gradients, or a combination thereof. Several additional techniques can be used as well [Burton et al. 2000]. On the removal of growth factors from the culture medium, these myoblasts fuse to form myofibers. After fusion, the myofibers start to contract randomly [Wolpert et al. 1998, p. 287].

1.4.4 Cell senescence

Cells in culture typically can live up to a limited number of generations [Renault et al. 2000; Wolpert et al. 1998, p. 439]. There are two subsequent stages involved in the dying of old cell cultures; senescence, the stage in which cells normally die, and crisis, which occurs when cells for some reason have survived senescence [Prowse and Greider 1995; Counter et al. 1998; Lustig 1999; O’Hare et al. 2001]. The shortening of telomeres is involved in these processes. Telomeres are highly conserved G-rich sequences at the end of the chromosomes which ensure chromosome integrity by stabilizing chromosome ends and inhibiting chromosome fusions and rearrangements [Prowse and Greider 1995; Wolpert et al. 1998]. They loose around 100 base pairs each mitotic cycle [Renault et al. 2000; Wolpert et al. 1998, p. 439]. Although it is unclear to what extent chromosome shortening plays a role in senescence, it is very likely that the main cause of crisis is that the telomeres become too short [Lustig 1999; O’Hare et al. 2001]. Telomere length correlates with the life span of many cell types both *in vitro* and *in vivo* [Prowse and Greider 1995; Renault et al. 2000].

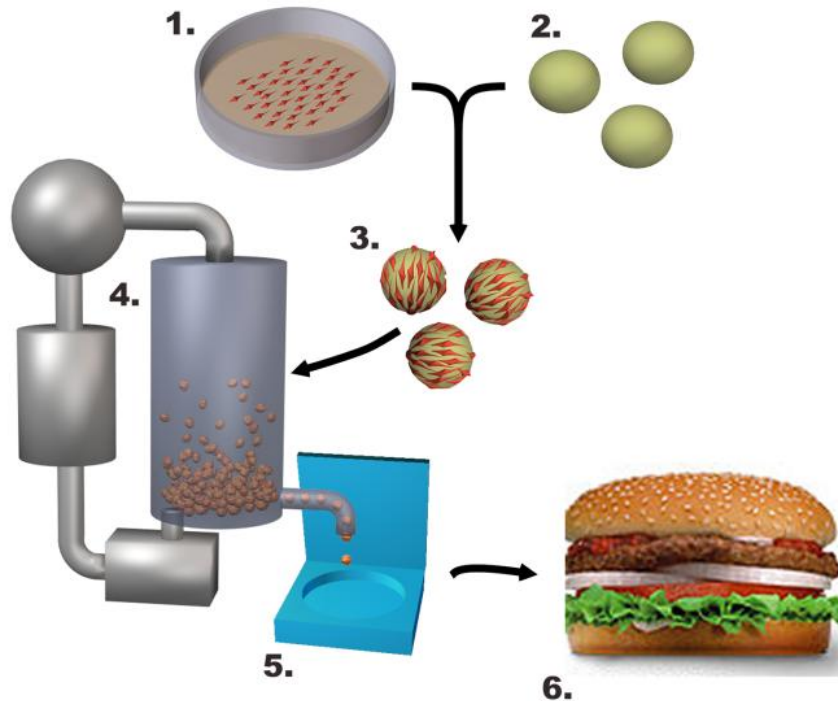
The proliferative capacity of satellite cells depends on the species, age, and disease state of the donor. This capacity decreases with the age of the donor, although the decrease for human satellite cells is significantly greater in the childhood years than at later ages. The satellite cells of newborn infant humans are capable of dividing 55–65 times, but at the age of 9 this number is reduced to ± 20 –30 divisions [Hawke and Garry 2001]. In addition, older satellite cells are less motile and form thinner and more fragile myotubes [Renault et al. 2000]. The number of satellite cells *in vivo* is also dependant on age. In neonate mouse muscles, $\pm 30\%$ of the muscle nuclei consist of satellite cells, whereas this number drops to $\pm 4\%$ in adults and $\pm 2\%$ in senile individuals [Hawke and Garry 2001].

2 Implementation of the system

2.1 Perfusion of the growing muscle tissue

The ultimate goal of an IMPS is to proliferate animal muscle tissue. However, to grow actual muscle tissue in culture is problematic because of the absence

Figure 1: The Mironov proposal for myoblast culturing



In a proposal from Vladimir Mironov, myoblasts are allowed to grow onto collagen spheres in a bioreactor. After growth and differentiation, these spheres can be harvested and used for processed foods like sausages or hamburgers [Wolfson 2002]. Image kindly provided by Vladimir Mironov.

of blood circulation. In a normal skeletal muscle, blood is forced through the veins by means of blood pressure [Fox 1996], thereby constantly providing nutrients and oxygen to the muscle tissue while removing waste products. To keep muscle cells alive, the maximum distance to the blood supply may be at most $200 \mu\text{m}$ [Wolfson 2002].

It has been demonstrated that it is possible to grow small muscle-like organs, termed myoids, *de novo* from co-cultures of myoblasts and fibroblasts. These organs are able to contract both spontaneously and by electrical stimulation, albeit with only a fraction of the force observed in control muscles. This is probably caused by a lack of innervation [Dennis and Kosnik 2000; Dennis et al. 2001; Kosnik et al. 2001]. The diameter of myoids is limited by lack of perfusion to 1 mm at most [Kosnik et al. 2001]. Perfusion is probably the biggest problem to overcome in designing an IMPS.

2.2 Approaches; cell vs. tissue culture

There are different design approaches for an IMPS, all of which are designed to overcome the diffusion barrier. They can roughly be divided in cell culture and tissue culture.

2.2.1 Cell culture

There are currently two detailed proposals for using cell culture for creating meat, similar in nature. They borrow from the emerging field of tissue engineering [Boland et al. 2003; Zandonella 2003]. This research discipline aims at creating tissues and organs *de novo* for use in organ transplantations. The common approach for organ creation is by using a scaffold made of polyglycolic acid (PGA) which is seeded with loose cells. The structure is placed in

a bioreactor which provides nutrients and oxygen. The cells then grow on this scaffold to create the desired organ. Before transplantation, the scaffold is removed [Boland et al. 2003; Zandonella 2003]. Vladimir Mironov has written a detailed proposal for the NASA to create an IMPS [Wolfson 2002], while Willem van Eelen holds a worldwide patent for a similar system [van Eelen et al. 1999; De Telegraaf 2001]. Neither system has been tested.

Both systems work by growing myoblasts in suspension in a culture medium. In the Mironov proposal, the cells are grown in a bioreactor, together with collagen spheres to provide a substrate onto which the myoblasts can attach and differentiate (see figure 1). In the van Eelen proposal, a collagen meshwork is used. In this proposal, 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. Instead of collagen, other edible proteins or artificial substrates can be used. An alternative, mentioned in the van Eelen proposal is to use two-dimensional monolayers of muscle cells, which are sandwiched onto each other after harvesting.

Although these cell culture techniques will provide muscle protein, it will not be structured as meat. However, it can likely be used adequately in processed meat products like sausages, hamburgers, etc.

2.2.2 Tissue culture

Tissue culturing aims to create meat which is structured as such. This can be done by creating muscle tissue *de novo*, like in tissue engineering, or by proliferating existing muscle tissue.

The latter approach is employed by Morris Benjaminson. Gold fish (*Carassius auratus*) muscle explants were placed in diverse culture media and allowed to grow for one week. Depending on the medium, the explants had grown a small amount (fetal bovine serum: 13.8%, fishmeal extract: 7.1%, shiitake extract: 4.8%, maitake extract: 15.6%) [Benjaminson et al. 2002]. Although these initial results are encouraging, a lack of blood circulation in these explants can impose problems for extended periods of growth. In another experiment by Morris Benjaminson, chicken muscles could be kept alive in a petri dish for at most two months

before they became necrotic [Wolfson 2002]. His team plans on solving this problem in the future by controlled angiogenesis [Benjaminson et al. 2002].

Explants have the advantages that they contain all the tissues which make up meat in the right proportions and that the *in vivo* situation is closely mimicked, that is, all the mitogenetic substances are present *in vitro*. However, the control over the production process is limited. Vladimir Mironov proposes to create an entirely artificial muscle with tissue engineering techniques. According to his idea, a branching network of edible porous polymer is to be created, through which nutrients are perfused. Myoblasts and other cell types can then attach to this network [Wolfson 2002]. The method used for creating the artificial capillaries is not mentioned in detail, but it has to be elastic to a certain degree (see section 2.3). Successes in creating such a network for the purpose of tissue-engineering have been made by creating a cast onto which a collagen solution or a biocompatible polymer is spread. After solidification, the original material is dissolved, leaving a branched network of microchannels behind, which can be stacked onto each other to form an three-dimensional network [Technology Review 2003; Zandonella 2003]. It is possible to co-culture the myoblast with other cell types in order to create a more realistic muscle structure, like the myoids mentioned above. These small organs are organised in much the same way as real muscles [Dennis and Kosnik 2000; Dennis et al. 2001; Kosnik et al. 2001].

2.3 Atrophy and exercise

A potential problem of *in vitro* cultured meat is that of atrophy or muscle wasting. Atrophy is described as a loss of muscle mass caused by a lack of use, denervation, or one of a variety of diseases [Charge et al. 2002; Ohira et al. 2002; Greene et al. 2003] due to a reduction of cell size [Fox 1996, p. 71]. Atrophy caused by reduced neuromuscular activity is often accompanied by a reduction of the cytoplasmic volume per myonucleus (the myonuclear domain) and an increase in myofibers expressing a fast phenotype (based on their composition of the myosin heavy chain). This type of atrophy is usually much more pronounced for slow than for fast muscles [Ohira et al. 2002]. Atrophy due to decreased neuromuscular activity is also manifested in developing animals as a retardation of growth [Ohira et al. 2002].

For an IMPS, the danger of atrophy is also present, especially because of growth retardation. It is not clear whether the absence of innervation poses a problem. For urodelian amphibians it has been shown that, if a limb is denervated very early in the development, the limb develops and regenerates normally [Wolpert et al. 1998, pp. 407-408] (see also section 2.6). On the other hand, the low contractile force of myoids is contributed to their chronic denervation [Dennis et al. 2001]. Exercise in elderly humans has been shown to prevent mass and function loss [Charge et al. 2002]. In rats, exercise induced by electrical stimulation can cure the muscle mass loss induced by denervation almost completely [Dennis et al. 2003]. It might be possible that mechanical or electrical stimulation can promote growth and structure of the cultured meat. As a matter of fact, newly formed myotubes in culture start to contract spontaneously [Wolpert et al. 1998, p. 287]. This can also be observed in myoids, which contract spontaneously at approximately 1 Hz. once formed [Dennis and Kosnik 2000]. So exercise by electrical stimulation might be a viable solution to overcome atrophy in an IMPS.

It is as yet not clear to what extent a need for exercise exists in *in vitro* cultured meat. It might very well be that in a simple cell culturing system as described in section 2.2.1 this need is absent but that for the development of a functionally structured muscle as described in section 2.2.2 exercise is an essential element.

2.4 Manipulation of meat quality

There are different requirements to the composition, taste, and tenderness of meat. Eating quality is characterized by the tenderness, juiciness and flavour of the meat [Wood et al. 1999], of which tenderness is the most important [Solomon et al. 1997; Wood et al. 1999]. The tenderness is determined by the protein structure of the myofibrils in the meat, the fat content, and the interplay between the two. Rapid freezing after slaughtering of the animal causes the muscles to contract, increasing the force needed to shear the muscles dramatically. However, if the carcass is suspended in a way that muscles are stretched or if the internal energy stores are depleted with high-voltage electrical stimulation before freezing, tenderness is retained. Fat tissue protects the carcass from rapid

freezing, thus enhances tenderness. Furthermore, during ageing of the meat, the muscle structure is fragmented by proteolytic enzymes, which also enhances meat tenderness. Fat in the muscle itself forces the myofibers apart and even dilutes fibrous protein, thereby reducing the force required to shear the muscle proteins [Wood et al. 1999]. Optionally, meat tenderness can be improved artificially by applying mechanical stress or chemical treatment after slaughtering [Solomon et al. 1997]. The taste of meat too is influenced by its composition and processing. Stress prior to slaughtering reduces the glycogen content, thereby intensifying the abnormal flavors. Ageing has a positive effect on meat flavor by increasing the amount of peptides and amino acids. In addition, the structure of carbohydrates, which depends on the species, age, and feed of the animal, has a marked influence on the flavor of the meat [Wood et al. 1999].

Another issue is the health value of the meat. Usually meat has a high content of saturated fatty acids and a low content of poly-unsaturated fatty acids. The former group has been implicated in the risk of heart disease, while the latter group has a beneficial effect on the blood cholesterol content [Wood et al. 1999; Voedingscentrum 2003]. The fatty acid content in meat can be altered by altering the diet of the animal [Wood et al. 1999].

This information is interesting for enhancing the quality of *in vitro* cultured meat. By manipulating the composition of the culture medium, the flavor and fatty acid composition of the cultured meat can be influenced. Moreover, health aspects of the meat can be enhanced by adding factors to the culture medium which might have an advantageous effect on the health, like certain types of vitamins. This is also mentioned in the patent description of the van Eelen system [van Eelen et al. 1999]. Co-culturing with other cell types might further enhance the meat quality, for example co-culturing with adipocytes (and using the right culture medium) can increase the fat content. Furthermore, some of the post-processing techniques which are mentioned above can be used on the *in vitro* cultured meat.

2.5 Longevity

In section 1.4.4 it was mentioned that cells in culture typically can live up to a fixed number of di-

visions. If a cell were able to divide 60 times, it could give rise to $2^{60} \simeq 1.15^{18}$ nuclei. For mice it was shown that *in vivo* there are approximately 5 nuclei for each 100 μm of muscle fiber in the soleus and approximately 6 nuclei per 100 μm of muscle fiber of the extensor digitorum longus (EDL) [Bruusgaard et al. 2003]. The diameter of muscle fibers is approximately 50 μm , so the cross-sectional area is $\pi \times 0.0005^2 = 7.85 \times 10^{-9} \text{ m}^2$, thus for 1 m^3 of muscle fiber, 1.27×10^8 m myotube is required, which comes down to 7.00×10^{13} nuclei per m^3 muscle tissue. Given these parameters, $1.64 \times 10^4 \text{ m}^3$ meat could theoretically be produced from a single satellite cell. Data on the ratio between volume and weight of meat could not be obtained, but given the fact that meat consists for 75% of water [Solomon et al. 1997], and no particularly light or heavy components are found in muscle tissue, it can be assumed that this ratio will not differ extremely from 1. So somewhere in the range of 200 people in the western world can be satisfied in their meat demands during one year with one donor satellite cell. Clearly, this is an extremely optimistic estimate which assumes the maximal possible number of cell divisions before differentiation⁵ and no loss of cells. Furthermore, parameters might vary considerably between animal species. Unfortunately, data for a more realistic estimate could not be obtained. However, if only a fraction of this amount of meat produced by an IMPS is feasible, it shows an enormous growth potential nonetheless.

Still, senescence eventually imposes a challenge for an IMPS based on satellite cells. There are several approaches to overcome this problem:

1. Start fresh cell culture when needed
2. immortalize cell culture
3. Use an embryonic stem cell (ES) culture

Approach 1 is the most obvious. From time to time fresh satellite cells are extracted from animal donors and used to start new cell cultures. The extraction procedure can be performed without harming the animal [van Eelen et al. 1999], although a more common practice is that the animal is slaughtered [Burton et al. 2000].

Approach 2 consists of the modification of the cells in culture such that both senescence and crisis

can be overcome. This at least involves the ectopic expression of the gene for the telomerase enzyme [Alberts et al. 1994, p. 904], for example by transfecting the cell line with a construct of the telomerase gene with a constitutive promoter. The telomerase enzyme is a ribonuclein protein, which provides an RNA template of the G-rich box of the telomeres, and elongates it in the 5'-to-3' direction [Prowse and Greider 1995; Alberts et al. 1994, p. 364]. As is explained in section 1.4.4, it is not known to what extent chromosome shortening plays a role in senescence of cell cultures. So while ectopic expression of the telomerase gene is sufficient to overcome crisis, additional expression of an oncogene may be required to overcome senescence [Prowse and Greider 1995; Counter et al. 1998; Lustig 1999; O'Hare et al. 2001]. A big drawback of removing the division limit from a cell culture, is that this method falls within the domain of genetic modification, which might severely hinder consumer acceptance (see section 3.2.1).

Approach 3 is to use immortal cell cultures from the beginning on. An embryonic stem cell is pluripotent and apparently has an unlimited capability for division [Burdon et al. 2002]. A cell culture of ESes derived from a single donor can therefore be theoretically propagated unlimited and be used for meat production without senescence. The big drawback of this method is that, while satellite cells are already primed to become muscle cells, embryonic stem cells have to differentiate to muscle cells before they can be used [Mironov, personal communication].

2.6 Difference between lower and higher animals

It has been known for long that vertebrates on a lower taxonomic scale have a greater regeneration potential than higher animals. Especially urodelian amphibians have received much attention. For example, newts can regrow lost limbs, retina, lens, jaw, tail, dorsal crest, and ventricular and atrial cardiac muscle [Grounds et al. 2002; Wolpert et al. 1998, p. 405]. In contrast to higher animals, differentiated cells from urodelian amphibians can re-enter the cell cycle, dedifferentiate, and redifferentiate again to the desired cell types [Wolpert et al.

⁵Which raises viability problems on its own, see section 1.4.4.

1998, pp. 406-407]. Furthermore, in fish, hyperplasia plays an important role in muscle repair. In this type of growth new muscle fibers are formed as opposed to hypertrophy, in which only existing muscle fibers grow by merging with myoblasts. Hyperplasia plays no significant role after embryonic development in higher animals [Benjaminson et al. 2002; Fox 1996, p. 71].

It makes sense to choose species which are capable of hyperplasia if existing muscles need to grow in mass. It is for this reason that a fish species was chosen for Benjaminson's pilot study instead of a higher animal. However, for consumption it is desirable to produce mammal or avian muscle tissue, as this is, next to fish, most common to consume in the western world. With a cell culture as described in section 2.2.1, hyperplasia is more or less mimicked. Therefore, there does not seem to be any reason why, if newly muscle cells are formed *in vitro*, no meat from higher animals could be produced.

2.6.1 Long-term manned space missions

It is not unthinkable that *in vitro* meat production in space is less efficient than on earth or even impossible. Striking is the atrophy which occurs in astronauts even after a few days in space [Ohira et al. 2002; Fox 1996, p. 71]. However, atrophy can be reduced by exercise (see in section 2.3). On the other hand, it has been shown both in microstats and on board of space flights that microgravity has a stimulating effect on cell proliferation, at least in lower vertebrates. Therefore regeneration after injury is faster in these animals in a microgravity environment than in normal gravity [Grigoryan et al. 2002]. This effect, which lasts for some time after return to a normal gravity situation, is non-specific, that is, it has an influence both on cells involved and cells not involved in regeneration. It is not known what the cause is for this enhanced cell proliferation. It could be that it is an effect of changes in the metabolism of the animal, which disturb the hormonal and calcium regulation, which in turn has an effect on the cells. On the other hand, it could be that there is a direct effect, for example through means of heat shock proteins. If this effect is a cellular response, it could mean that meat production on board of a spaceship can be even better than in normal gravity [Grigoryan et al. 2002].

Another difficulty with space travel is the in-

creased radiation by high-energy charged particles. For astronauts, there are two major hazards; short events of relatively high radiation levels can destroy cell pools of sensitive tissue, and prolonged exposure to increased radiation can damage brain tissue, reproductive organs, and other tissues, and induce cancer [Schimmerling et al. 2003]. For an IMPS, the risks are considerably lower, but nevertheless increased radiation, both high doses in a short period and relatively low doses over an extended period, may degrade the quality of the cell culture. Due to the mass of the shielding, spacecrafts or space-bases cannot be shielded in such a way that radiation levels are as low as on earth [Schimmerling et al. 2003]. Fortunately, to protect the food production system, only a small part of the ship has to be shielded, so this will probably not pose a problem.

3 Social issues

3.1 Potential financial and environmental advantages

It is hard to come up with concrete numbers on the economical and environmental costs of an IMPS without detailed data. However, it is possible to identify several aspects which show savings in such a system.

1. An animal kept for meat production possesses, in addition to muscle tissue, organs which are required for successful living and reproduction. These organs include bones, respiratory system, digestive system, appendages, skin, brains, etc. This kind of organs do not have to be grown to produce meat in an IMPS, which reduces the amount of nutrients and energy needed for growth and maintenance.
2. The time it takes to grow the meat chunks is probably significantly lower than with traditional meat production. It will rather take several weeks instead of months (for chickens) or years (for pigs and cows) before the meat can be harvested. This means that the time that tissue has to be maintained is much smaller than with traditional farming methods. As a result, the amount of feed and labor required per kg of *in vitro* cultured meat

is much lower than with traditionally farmed meat.

3. The space used for producing *in vitro* cultured meat can be used more efficiently than with traditional farming. Live animals require a larger space than their own volume. Moreover, for a proper animal treatment, a significant larger space than their own volume and access to fresh air and sunlight is required, for example by meadows. In contrast, bioreactors for *in vitro* meat production do not need extra space and can be stacked up in a fabric hall.

For these reasons, the nutritional costs for *in vitro* cultured meat will be significantly lower than for traditionally cultured meat. It is yet unclear what the financial advantages are. It might very well be that the decrease in costs of resources, labor, and land described above is compensated by the extra costs of a stricter hygiene regime, stricter control, computer management, etc. Moreover, prices for agricultural products are greatly influenced by political processes [Rosegrant et al. 1999]. However, the team of Willem van Eelen anticipates that the eventual product is less costly to produce than traditionally cultured meat⁶.

3.2 Consumer acceptance

The consumption of meat by humans is as old as the human species, and perhaps even older, as is implied by the fact that most hominid primates consume significant amounts of meat [Nestle 1999]. However, meat was always obtained by killing an animal. The *in vitro* culturing of meat is a completely novel technique, and cultured meat can be regarded as a novel product. Therefore, it is likely that it is not accepted by the consumer right away, and perhaps never will be. At least the fact that the form of the meat will likely not resemble actual muscles poses no problem for consumer acceptance, as there is a market for boneless and skinless, thus convenient pieces of meat [Elsner et al. 1998]. Because of the novelty of the food, there are no published studies on consumer acceptance. So for an estimate on the acceptability of

this food source for the consumer, I will mention the general responses found on two internet forums. Note that these are not representative and can at best give an indication of consumer acceptance. The forums investigated were Slashdot (Lab-Grown Steak <http://science.slashdot.org/article.pl?sid=02/12/31/1425214&mode=thread&tid=134>) and De Duurzaamlijst (Kunstvlees <http://www.ddh.nl/pipermail/duurzaamlijst/2001/thread.html#40>). The first forum is an American/Worldwide forum visited in general by people interested in science and technology, the latter is a Dutch list for people interested in developments in sustainability.

The reactions toward the possibility of this novel technique seem to be mostly negative. There are several arguments mentioned:

1. The technique is unnatural
2. It alters the intrinsic value of the animal
3. Genetically engineered, Frankensteinfood

The opponents can mostly be placed in one of two groups; people who feel that it is natural to kill animals for consumption and “green” people, that is, people who are concerned with animal welfare and fair distribution of resources in the world. Strangely enough, genetic manipulation is mentioned several times by the “green” opponents and by individuals not falling into either group concerned about their safety. However, in the original articles which are discussed in these forums, no mention at all was made of genetic engineering. Therefore, I suspect that argument 3 has to do with fear of the unknown (see section 3.2.1). As for argument 1, this is mentioned by both groups of opponents of this technique. Argument 2 is mentioned by people falling in the “green” group. They feel that an animal should be treated with respect, and turning them literally into meat plants (no pun intended) seems disrespectful to life in general.

3.2.1 Genetic manipulation

Genetic manipulation for food and medicine production is a complicated issue for consumer acceptance. The general public has too little knowledge

⁶In fact, they plan on using the profit for the provision of clean drinking water in developing countries [van Eelen et al. 1999].

on the techniques to make an educated decision, and the fear of risks is greater than confidence in the benefits [Danner 1997; Moseley 1999]. In Europe a great mistrust of genetically engineered food exists and in the USA the trust is declining after an initial period of acceptance [Moseley 1999; Finucane 2002; Kalaitzandonakes and Bijman 2003]. For acceptance of bio-engineered vaccines, Kurt Danner provided six guidelines which are [Danner 1997]:

1. Adherence to principles of safety.
2. Establishment of analytical and control methods.
3. Well functioning regulatory and reporting systems.
4. Demonstration of usefulness and economic benefits.
5. Open communication.
6. Correct and prudent wording.

These guidelines can as well be applied to food production, although the emphasis on safety may be less pronounced than for medical use. However, it is of vital importance that the general public is well informed, and that the obvious benefits are shown, like lack of animal suffering. Otherwise consumers do not have an incentive to switch from trusted products to a novel product. Moreover, it has to be made clear what this kind of *in vitro* cultured meat actually is, as the fear of the general public is mostly a fear of the unknown; lack of knowledge prevents the average consumer from making a well-formed opinion about the risks of the product [Danner 1997; Finucane 2002]. In addition, there seems to be a great mistrust of large companies whose ultimate goal is to make money [Danner 1997; Finucane 2002]. Therefore, like guideline 3 suggests, it is of vital importance that an independent organization (i.e. government) evaluates the new food and advises the public about it. Still, history has shown that even in such cases the consumer is very awkward towards genetically engineered food. Therefore, it would be preferable from an acceptance perspective not to use genetic modification for *in vitro* meat production.

4 Discussion

From the previous sections, it became clear that it is feasible to culture meat *in vitro*. Plans for such a system are in development [van Eelen et al. 1999; De Telegraaf 2001, Westerhof, personal communication]. To what extent does such a system solve the problems described in section 1.2.2? The number of animals which need to be herded is of course greatly reduced with this approach. However, depending on whether or not immortal cell lines are used, cells need to be obtained from donor animals from time to time. And even with immortalized cell cultures, it is still imaginable that degradation of the lineage occurs due to cumulation of genetic damage after time. Although cells can be obtained without killing the donor animal, it is unlikely that this will happen unless there are clear economic incentives to do so. It is probably easier, and thus cheaper, to kill the animal and process its muscles as whole. There is also no reason for optimism about the way in which the donor animals are kept. Some people argue that meat derived from well-treated animals has a higher quality. However, donor animals would be kept exclusively for their cells, so that reason would no longer be valid. Moreover, the donor-animals should be raised under sterile conditions [van Eelen et al. 1999], which might further restrict their environment. So the number of animals kept and the environmental costs would be greatly reduced by *in vitro* meat production, but there is no reason to assume that the amount of suffering for these donor animals will be diminished. However, if the system can be made animal-friendly, it can dramatically leverage consumer acceptance, thus providing an economic incentive. Emphasizing the advantages for animals and the environment could maybe be the main selling point for *in vitro* cultured meat. Giving an impression of being “natural”, for example by using an inhibitory bacterial strain instead of artificial conservatives [Bredholt et al. 2001], might further help overcome consumer resistance. The *in vitro* cultured meat might also evolve towards a niche market, like a sort of meat replacement which can be consumed by vegetarians. Ironically, the strongest opposition against the idea of *in vitro* cultured meat seems to come from the group of vegetarians. Furthermore, it seems that genetic manipulation is a great hindrance to broad acceptance of *in vitro* cultured meat. This is even

more so because the meat is already unnatural, thus awkward. Of course, when a production system for a space journey is considered, this is less of a problem. An interesting possibility of an IMPS is that it can be used not only to produce meat from commonly kept animals but also from rare and exotic species, like whales or kangaroos [van Eelen et al. 1999], or even a mixture of two or more species.

There are still some unresolved issues regarding IMPSes. To begin, it is not clear whether an IMPS system can solve the antibiotics problem described in section 1.2.2. There is no immune system whatsoever, so the cell cultures are very susceptible to infections. Therefore, the culture vessels have to be kept sterile and have to be sterilized from time to time, and perhaps inhibitors of microorganisms such as antibiotics have to be used. On the other hand, such a system is completely closed, so much more control can be exerted over the substances entering and leaving the system as would be the case with traditional farming. This implies amongst others, that even if antibiotics would be used, its leakage toward the environment can probably be prevented. In addition, because the system is closed, the spread of animal diseases would probably be inhibited; both spread of infection into the factory and out of the factory are harder than is the case with animals living in a (semi-)open environment. And again, because of better monitoring and control, the risk of diseases can greatly be reduced.

Another concern is what the source of the nutrients to culture the cell mass will be. In the van Eelen proposal, a complete artificial culture medium composed of separate ingredients is used. These would probably have to be extracted from natural sources in an industrial setting. A more elegant solution would be to use a product based on microorganismal, fungal, or plant sources directly or after pre-processing, as in proposals for a CELSS. However, such a medium should contain the necessary components in well-balanced quantities, and they should be presented in a form accessible to the myoblasts (and accompanying cells), as no digestive tract is involved. In fact, the role of digestive tract is taken over by the pre-processing plant. An important factor to consider is that vitamin B₁₂ can only be produced by microorganisms (see section 1.2.1) and cannot be taken up in this form by eukaryotic cells. Clearly, research on the issue of culture medium for industrial-scale meat production

has a long way to go.

Furthermore the source of secondary ingredients, i.e. those substances which are not directly involved in cell growth, should be considered. These include collagen and polymers (for protein spheres, scaffolds, and artificial blood vessels). Collagen sources derived from animals directly would defeat the purpose of the IMPS. Recombinant collagen sources are available, but these suffer from a bad consumer acceptance.

In conclusion, *in vitro* cultured meat holds great promises as an alternative to traditionally produced meat, if consumer resistance can be overcome. Open communication and the (non-rational) refusal of genetic recombination techniques seem to be essential for this to happen. However, a great body of research has to be performed before this kind of meat can be produced on an industrial scale. Clearly, if *in vitro* cultured meat will ever become commonplace, it will not be in the immediate future.

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References

- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1994). *Molecular Biology of the Cell*. Garland Publishing, 3 edition.
- Avery, A. (1997). Red meat production can be part of an environmentally sound future. *J Am Diet Assoc*, 97(11):1249–50.
- Benjaminson, M., Gilchrist, J., and Lorenz, M. (2002). In vitro edible muscle protein production system (MPPS): stage 1, fish. *Acta Astronaut*, 51(12):879–89.
- Benjaminson, M., Lehrer, S., and Macklin, D. (1998). Bioconversion systems for food and water on long term space missions. *Acta Astronaut*, 43(3-6):329–48.
- Boland, T., Mironov, V., Gutowska, A., Roth, E., and Markwald, R. (2003). Cell and organ printing 2: Fusion of cell aggregates in three-dimensional gels. *Anat Rec*, 272A(2):497–502.

- Bredholt, S., Nesbakken, T., and Holck, A. (2001). Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. *Int J Food Microbiol*, 66(3):191–6.
- Bruusgaard, J., Liestol, K., Ekmark, M., Kollstad, K., and Gundersen, K. (2003). Number and spatial distribution of nuclei in muscle fibres of normal mice studied in vivo. *J Physiol*.
- Buckingham, M. (2001). Skeletal muscle formation in vertebrates. *Curr Opin Genet Dev*, 11(4):440–8.
- Burdon, T., Smith, A., and Savatier, P. (2002). Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends in Cell Biology*, 12(9):432–8.
- Burton, N., Vierck, J., Krabbenhoft, L., Bryne, K., and Dodson, M. (2000). Methods for animal satellite cell culture under a variety of conditions. *Methods Cell Sci*, 22(1):51–61.
- Charge, S., Brack, A., and Hughes, S. (2002). Aging-related satellite cell differentiation defect occurs prematurely after Ski-induced muscle hypertrophy. *Am J Physiol Cell Physiol*, 283(4):C1228–41.
- Counter, C., Hahn, W., Wei, W., Caddle, S., Beijersbergen, R., Lansdorp, P., Sedivy, J., and Weinberg, R. (1998). Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. *Proc Natl Acad Sci U S A*, 95(25):14723–8.
- Crews, R. (2003). Space Launch Costs. *Distant Star*. http://www.distant-star.com/issue13/april_2003_space_launch_costs.html.
- Crok, M. (2003). Bij de beesten af. *Natuur & Techniek*, 71(4):46–49.
- Danner, K. (1997). Acceptability of bio-engineered vaccines. *Comp Immunol Microbiol Infect Dis*, 20(1):3–12.
- De Productschappen Vee, V. e. E. P. (2001). Vee, Vlees en Eieren in Nederland: cijfers en informatie over de sectoren in het jaar 2001. http://www.veevleesei.nl/publicaties/2002_nl.pdf.
- De Telegraaf (2001). Vlees zonder been uit reageerbuis. *De Financiële Telegraaf*. <http://kranten.telegraaf.nl/krant/archief/20010920/teksten/fin.vlees.westerhof.willem.html>.
- Dennis, R., Dow, D., and Faulkner, J. (2003). An implantable device for stimulation of denervated muscles in rats. *Med Eng Phys*, 25(3):239–53.
- Dennis, R. and Kosnik, 2nd, P. (2000). Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. *In Vitro Cell Dev Biol Anim*, 36(5):327–35.
- Dennis, R., Kosnik, 2nd, P., Gilbert, M., and Faulkner, J. (2001). Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines. *Am J Physiol Cell Physiol*, 280(2):C288–95.
- Drysdale, A., Ewert, M., and Hanford, A. (2003). Life support approaches for Mars missions. *Adv Space Res*, 31(1):51–61.
- Eichholzer, M. and Bisig, B. (2000). Daily consumption of (red) meat or meat products in Switzerland: results of the 1992/93 Swiss Health Survey. *Eur J Clin Nutr*, 54(2):136–42.
- Elsner, R., McWatters, K., and Resurreccion, A. (1998). Consumer acceptance of stir-fry and kabobs from dark chicken meat and their packaging. *Poult Sci*, 77(8):1241–52.
- Finucane, M. (2002). Mad cows, mad corn and mad communities: the role of socio-cultural factors in the perceived risk of genetically-modified food. *Proc Nutr Soc*, 61(1):31–7.
- Fox, S. I. (1996). *Human Physiology*. Wim C. Brown Publishers.
- Greene, A., Juhn, G., Hart, J., and Peckham, C., editors (2003). *MEDLINEplus Medical Encyclopedia*. <http://www.nlm.nih.gov/medlineplus/encyclopedia.html>.
- Grigoryan, E., Mitashov, V., and Anton, H. (2002). Urodelean amphibians in studies on microgravity: effects upon organ and tissue regeneration. *Adv Space Res*, 30(4):757–64.

- Grounds, M., White, J., Rosenthal, N., and Bogoyevitch, M. (2002). The role of stem cells in skeletal and cardiac muscle repair. *J Histochem Cytochem*, 50(5):589–610.
- Hawke, T. and Garry, D. (2001). Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol*, 91(2):534–51.
- Hopster, H. and Kranendonk, G. (2003). Krijgen gestreste varkensmoeders probleembiggen? *Natuur & Techniek*, 71(4):50–3.
- Johnson, R. and Gibbs, Jr, C. (1998). Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies. *N Engl J Med*, 339(27):1994–2004.
- Kalaitzandonakes, N. and Bijman, J. (2003). Who is driving biotechnology acceptance? *Nat Biotechnol*, 21(4):366–9.
- Knight, W. (2001). Space toilet key to conquering final frontier. *NewScientist.com news service*. <http://www.newscientist.com/news/news.jsp?id=ns99991093>.
- Knight, W. (2002). Russia proposes manned Mars mission by 2015. *NewScientist.com news service*. <http://www.newscientist.com/news/news.jsp?id=ns99992511>.
- Kosnik, P., Faulkner, J., and Dennis, R. (2001). Functional development of engineered skeletal muscle from adult and neonatal rats. *Tissue Eng*, 7(5):573–84.
- Lea, E. and Worsley, A. (2001). Influences on meat consumption in Australia. *Appetite*, 36(2):127–36.
- Lustig, A. (1999). Crisis intervention: the role of telomerase. *Proc Natl Acad Sci U S A*, 96(7):3339–41.
- Moseley, B. (1999). The safety and social acceptance of novel foods. *Int J Food Microbiol*, 50(1-2):25–31.
- Nestle, M. (1999). Animal v. plant foods in human diets and health: is the historical record unequivocal? *Proceedings of the Nutrition Society*, 58:211–8.
- O’Hare, M., Bond, J., Clarke, C., Takeuchi, Y., Atherton, A., Berry, C., Moody, J., Silver, A., Davies, D., Alsop, A., Neville, A., and Jat, P. (2001). Conditional immortalization of freshly isolated human mammary fibroblasts and endothelial cells. *Proc Natl Acad Sci U S A*, 98(2):646–51.
- Ohira, Y., Yoshinaga, T., Nomura, T., Kawano, F., Ishihara, A., Nonaka, I., Roy, R., and Edger-ton, V. (2002). Gravitational unloading effects on muscle fiber size, phenotype and myonuclear number. *Adv Space Res*, 30(4):777–81.
- Prowse, K. and Greider, C. (1995). Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc Natl Acad Sci U S A*, 92(11):4818–22.
- Reay, D. (2002). Intensive farming, US-style, is not sustainable worldwide. *Nature*, 417(6884):15.
- Renault, V., Piron-Hamelin, G., Forestier, C., Di-Donna, S., Decary, S., Hentati, F., Saillant, G., Butler-Browne, G., and Mouly, V. (2000). Skeletal muscle regeneration and the mitotic clock. *Exp Gerontol*, 35(6-7):711–9.
- Rosegrant, M., Leach, N., and Gerpacio, R. (1999). Alternative futures for world cereal and meat consumption. *Proc Nutr Soc*, 58(2):219–34.
- Saha, P. and Trumbo, P. (1996). The nutritional adequacy of a limited vegan diet for a Controlled Ecological Life-Support System. *Adv Space Res*, 18(4-5):63–72.
- Salisbury, F. and Clark, M. (1996). Suggestions for crops grown in controlled ecological life-support systems, based on attractive vegetarian diets. *Adv Space Res*, 18(4-5):33–9.
- Sanders, T. (1999). The nutritional adequacy of plant-based diets. *Proc Nutr Soc*, 58(2):265–9.
- Schimmerling, W., Cucinotta, F., and Wilson, J. (2003). Radiation risk and human space exploration. *Adv Space Res*, 31(1):27–34.
- Scott, J. M. (1999). Folate and vitamin B12. *Proceedings of the Nutrition Society*, 58:441–8.
- Seale, P. and Rudnicki, M. (2000). A new look at the origin, function, and ”stem-cell” status of muscle satellite cells. *Dev Biol*, 218(2):115–24.

- Solomon, M., Long, J., and Eastridge, J. (1997). The hydrodyne: a new process to improve beef tenderness. *J Anim Sci*, 75(6):1534–7.
- Solomon, R. and Johnston, C. (1997). Production of red meat should be curbed in order to conserve natural resources. *J Am Diet Assoc*, 97(11):1249.
- Technology Review (2003). Fractals Support Growing Organs. *Technology Research News*. http://www.technologyreview.com/articles/rnb_072203.asp.
- van Eelen, W. F., van Kooten, W. J., and Westerhof, W. (1999). WO9931222: Industrial scale production of meat from in vitro cell cultures. Patent Description. <http://12.espacenet.com/espacenet/viewer?PN=W09931222>.
- Voedingscentrum (2003). <http://www.voedingscentrum.nl/>.
- Walker, P. M. B., editor (1995). *The Wordsworth Dictionary of Science & Technology*. Wordsworth Editions.
- Webster, R. (2002). The importance of animal influenza for human disease. *Vaccine*, 20 Suppl 2:S16–20.
- Williams, C. (2002). Nutritional quality of organic food: shades of grey or shades of green? *Proc Nutr Soc*, 61(1):19–24.
- Wolfson, W. (2002). Raising the steaks. *New Scientist*, pages 60–3.
- Wolpert, L., Beddington, R., Brockes, J., Jessel, T., Lawrence, P., and Meyerowitz, E. (1998). *Principles of Development*. Current Biology Oxford, 1 edition.
- Wood, J., Enser, M., Fisher, A., Nute, G., Richardson, R., and Sheard, P. (1999). Manipulating meat quality and composition. *Proc Nutr Soc*, 58(2):363–70.
- Zandonella, C. (2003). Tissue engineering: The beat goes on. *Nature*, 421(6926):884–6.
- Zandstra, E., de Graaf, C., and van Trijp, H. (2000). Effects of variety and repeated in-home consumption on product acceptance. *Appetite*, 35(2):113–9.