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Commentary

In Vitro-Cultured Meat Production

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INTRODUCTION

Although meat has enjoyed sustained popularity as a foodstuff, consumers have expressed growing concern over some consequences of meat consumption and production. These include nutrition-related diseases, foodborne illnesses, resource use and pollution, and use of farm animals. Here we review the possibility of producing edible animal muscle (i.e., meat) *in vitro*, using tissue-engineering techniques. Such "cultured meat" could enjoy some health and environmental advantages over conventional meat, and the techniques required to produce it are not beyond imagination. To tissue engineers this subject is of interest as cultured meat production is an application of tissue-engineering principles whose technical challenges may be less formidable than those facing many clinical applications.

CULTURED MEAT PRODUCTION

Most edible animal meat is made of skeletal muscle tissue. The idea that skeletal muscle tissue-engineering techniques could be applied to produce edible meat dates back at least 70 years,[1] but has been seriously pursued by only three groups of researchers. Their efforts can be divided roughly into scaffold-based and self-organizing techniques.

In scaffold-based techniques, 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.[2] The resulting myofibers may then be harvested, cooked, and consumed as meat. van Eelen, van Kooten, and Westerhof hold a Dutch patent for this general approach to producing cultured meat.[3] However, Catts and Zurr appear to have been the first to have actually produced meat by this method.[4]

A scaffold-based technique may be appropriate for producing processed (ground, boneless) meats, such as hamburger or sausage. But it is not suitable for producing highly structured meats, such as steaks. To produce these, one would need a more ambitious approach, creating structured muscle tissue as self-organizing constructs[5] or proliferating existing muscle tissue *in vitro*.

The latter technique was employed by Benjaminson, Gilchriest, and Lorenz, the first researchers to have applied tissue-engineering techniques to meat production.[6] They placed skeletal muscle explants from goldfish (*Carassius auratus*) in diverse culture media for 7 days and observed an increase in surface area between 5.2 and 13.8%. When the explants were placed in a culture containing dissociated *Carassius* skeletal muscle cells, explant surface area increased by 79%.

Explants have the advantage of containing all the cells that make up muscle in their corresponding proportions, thus closely mimicking an *in vivo* structure. However, lack of blood circulation in these explants makes substantial growth impossible, as cells become necrotic if

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separated for long periods by more than 0.5 mm from a nutrient supply.[5]

Future efforts in culturing meat will have to address the limitations of current techniques through advances that make cultured cells, scaffolds, culture media, and growth factors edible and affordable.

CELLS

Skeletal muscle is a tissue consisting of several cell types. Skeletal muscle fibers are formed by the proliferation, differentiation, and fusion of embryonic myoblasts and, in the postnatal animal, satellite cells, to form large multinucleated syncytia.[7] Attempts to force skeletal muscle fibers to proliferate are typically counterproductive, as most myonuclei remain postmitotic.[2] Embryonic stem cells have the drawback that despite the high proliferation and differentiation potential, considerable effort must be applied to force them to differentiate and cell yields from harvests are low. Moreover, it is not clear whether embryonic stem cells forced to commit to a skeletal muscle lineage will have the proliferative characteristics of embryonic stem cells, or become indistinguishable from myoblasts. Thus the most practical cell source for cultured meat is probably embryonic myoblasts or postnatal/posthatch skeletal muscle cells called satellite cells.

Satellite cells with high proliferative potential have been isolated and characterized from the skeletal muscle of chickens, turkeys, pigs, lambs, and cattle.[8–12] In each case medium conditions have been established by these investigators to support the proliferation and differentiation of cells to form immature muscle fibers called myotubes in culture.

The simplest cultured meat system would likely use a single myogenic cell line from one of these animals, or a coculture with fat cells. After culture and harvest, cells might then be prepared for consumption as a processed meat. To replicate the taste and texture of unprocessed meats is a more ambitious goal, as vascular cells would be needed and fibroblasts for the production of connective tissue. Moreover, these would have to be properly organized in a three-dimensional structure. A proper growth factor milieu would be essential to direct the construction of a structured skeletal muscle tissue.

It is unclear how much cultured meat a single cell could yield. Cells in culture are believed to undergo a fixed number of doublings, called the Hayflick limit. The Hayflick limits of farm animal muscle progenitor cells have not been well established. It has been shown that satellite cells cloned from turkey breast muscle express telomerase.[13] This finding suggests that some domestic animal satellite cells may generate enough daughter cells to produce huge quantities of cultured meat. (For instance, back-of-the-envelope calculations suggest that a single parent cell with a Hayflick limit of 75 could theoretically satisfy the current annual global demand for meat.) For other species, it may be necessary to proliferate a sufficient number of stem cells in culture before differentiation into myoblasts—or to use cells transfected with the telomerase gene, that have higher Hayflick limits.

FIELDS

Mechanical, electromagnetic, gravitational, and fluid flow fields have been found to affect the proliferation and differentiation of myoblasts.[2,14] Powell and others found that repetitive stretch and relaxation equal to 10% of length, six times per hour, increased differentiation into myotubes.[15] Yuge and Kataoka seeded myoblasts with magnetic microparticles and induced differentiation by placing them in a magnetic field, without adding special growth factors or any conditioned medium.[16] Electrical stimulation also contributes to differentiation, as well as sarcomere formation within established myotubes.[2,14]

SCAFFOLDS

Myoblasts are attachment dependent, meaning that a substratum or scaffold must be provided for proliferation and differentiation to occur.[17] For cultured meat, a scaffold and its by-products must be edible and may be derived from nonanimal sources. A further challenge is to develop a scaffold that can mechanically stretch attached cells to stimulate differentiation. A flexible substratum is also necessary to prevent detachment of developing myotubes that will normally undergo spontaneous contraction.

Cytodex-3 microcarrier beads have been used as scaffolds in rotary bioreactors. However, these beads have no stretching potential. One approach to mechanically stretch myoblasts would be to use edible, stimulus-sensitive porous microspheres made from cellulose, alginate, chitosan, or collagen that undergo, at minimum, a 10% change in surface area after small changes in temperature or pH. Once myoblasts attach to the spheres, they could be stretched periodically. It is not clear how the variation in pH or temperature, or the differential mechanical stresses that bead curvature imposes on cells, would affect cell proliferation, adhesion, and growth.

Theoretically, giant sheets of muscle tissue could be cultured on thin membranes or arrays of narrowly spaced fibers. The sheets could be mechanically conditioned by minimal stretch to induce development of aligned myotubes. The membranes or fibers could be extracted from the meat (e.g., a thermoresponsive polymer could

be used, and the muscle biofilm separated from the substrate with a change of temperature). Or they could be made from edible material. The freed sheets could then be rolled up to a substantial thickness and processed.* Developing a scaffold for unprocessed meats presents greater technical challenges, because of the need for vascularization. It may be possible to build a branching network from an edible, elastic, and porous material, through which nutrients are perfused. Myoblasts and other cell types can then attach to this network. Approaches to creating such a network for the purpose of tissue engineering have been proposed 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 a three-dimensional network.[18] However, this approach does not lend itself to mass production.

Alternatively, one could attempt to create a highly structured meat without a scaffold. Benjaminson, Gilchriest, and Lorenz proposed solving the vascularization problem through controlled angiogenesis of explants.[6]

CULTURE MEDIA AND GROWTH FACTORS

To enjoy many of the potential advantages over conventional meat production, cultured meat would need to employ an affordable medium system. Such a medium must contain the necessary nutritional components and be presented in a form freely available to myoblasts and accompanying cells, as no digestive system is involved. Improvements in the composition of commercially available cell culture media have enhanced our ability to successfully culture many types of animal cells.

McFarland and others developed a serum-free medium that supported the proliferation of turkey satellite cells in culture.[8] Kosnik, Dennis, and Vandenburgh refer to serum-free media developed by Allen *et al*., Dollenmeier *et al*., and Ham *et al*.[2] Benjaminson and others succeeded in using a serum-free medium made from maitake mushroom extract that achieved higher rates of growth than fetal bovine serum.[6] And it has been shown that lipids such as sphingosine 1-phosphate can replace serum in supporting the growth and differentiation of embryonic tissue explants (W.S. Argraves, Medical University of South Carolina, Charleston, SC; personal communication, May 22, 2004).

In addition to supplying proper nutrition to growing muscle cells in culture, it is necessary to provide an appropriate array of growth factors. Growth factors are synthesized and released by muscle cells themselves and, in tissues, are also provided by other cell types locally (paracrine effects) and nonlocally (endocrine effects). The liver is the primary source of circulating insulin-like growth factor I. Appropriate coculture systems may be developed such that liver cells (hepatocytes) provide growth factors necessary for cultured muscle (meat) production. Typically, investigators initiate differentiation and fusion of myoblasts by lowering the levels of mitogenic growth factors. The proliferating cells then commence synthesis of insulin-like growth factor II, which leads to differentiation and formation of multinucleated myotubes.[19] So, the successful system must be capable of changing the growth factor composition of the medium.

BIOREACTORS

The importance of bioreactor design to tissue engineering has been discussed elsewhere.[20,21] Cultured meat production is likely to require the development of new bioreactors that maintain low shear and uniform perfusion at large volumes. Much skeletal muscle tissue engineering research has employed NASA rotating bioreactors. Their chief advantages are that cells are in near-continuous suspension, fluid shear is minimal, and suspension is possible for tissue assemblies up to 1 cm. These bioreactors can sustain biomass concentrations up to 10^8 cells/mL. Research-size rotating bioreactors (10 to 250 mL) have been scaled up to 3 L and, theoretically, scale-up to industrial sizes should not affect the physics of the system. Industrial scales are already available for low-shear particle-based biofilm reactors, allowing biomass concentrations as high as 30 kg/m^3.[22]

CONCLUSIONS

Relative to conventional meat, cultured meat could offer a number of benefits. With cultured meat, the ratio of saturated to polyunsaturated fatty acids could be better controlled; the incidence of foodborne disease could be significantly reduced; and resources could be used more efficiently, as biological structures required for locomotion and reproduction would not have to be grown or supported. Whether or not cultured meat is economically practical is a different question. A number of tissue engineers have speculated on its prospects.[23]

Cultured meat, at least of the scaffold-based variety, appears technically feasible. However, significant challenges remain before it could be produced economically. Most of these challenges are common to skeletal muscle tissue engineering, generally. The environmental cues needed to promote myofiber development are not well

* We owe this suggestion to an anonymous reviewer.

understood. And it is not clear which, among them, are essential to cultured meat production. Still, it is safe to assume that the level of functionality needed for most clinical applications of muscle tissue engineering exceeds that needed to produce cultured meat with nutritional and aesthetic properties sufficiently similar to those of conventional meat. Thus, cultured meat should present fewer technical challenges than functional engineered muscle. Future research is likely to be most fruitful if focused on developing scaffold-based techniques appropriate for processed meat products, and affordable, nonserum media needed to support them.

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