Food Science of Animal Resources
Food Sci. Anim. Resour. 2025 January 45(1):223~242
DOI https://doi.org/10.5851/kosfa.2024.e129
pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

REVIEW

Cutting-Edge Technologies of Meat Analogs: A Review

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OPEN ACCESS

Received	November 2, 2024
Revised	December 2, 2024
Accepted	December 2, 2024

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Abstract This study was conducted to investigate the recent research trends of alternative protein foods being developed to replace traditional livestock foods and thus determine the current state of the technology and the potential for industrialization. The results of this study showed that the technology related to cultured meat has not yet reached industrialization. However, serum-free media development, technologies to improve culture efficiency, and technologies to improve taste and flavor are being researched. In addition, the research on improving the production efficiency of cultured meat is increasingly expanding from using muscle satellite cells obtained from animal muscles to research on cell lines or immortalized cell lines. Edible insect-derived proteins have a wide range of food applications, and researchers are actively working on utilizing their functional properties. Plant-derived protein materials are also being studied to improve the flavor and texture of plant-based meat products to make them more similar to traditional livestock foods, as well as to remove allergens. In conclusion, despite ongoing technological development, the industrialization of cultured meat is expected to take some time. There is a growing body of research on the types, functionalities, extraction, and texturizing technologies of plant-derived, mycoprotein, or insect-derived ingredients for formulating meat alternative products, and it is expected that improved products will continue to enter the market. Although animal product substitutes are not expected to significantly replace traditional livestock products, continuous improvement research will contribute to the expansion of the alternative protein food market.

Keywords meat alternatives, sustainable protein sources, cultured meat, plant-based meat, edible insect-based meat

Introduction

In recent years, alternative foods that can replace traditional livestock products have gained significant attention in the meat market due to their potential to reduce the economic cost of meat production and address various environmental issues. These meat alternatives are diverse and range from plant-based, insect-based, and mycoprotein-based to cultured meat. Whereas cultured meat is still in its infancy and a technology for its full-scale industrialization is yet to be realized, plant-based or insect-based meat alternatives imitating meat have already attained market scale (Yun et al., 2024). However, contrary to expectations, alternative protein foods have been unable to achieve the envisioned level of competitiveness, mainly due to their inferior taste and flavor compared to traditional livestock products (Lee et al., 2024a). Assuming these criteria can be fulfilled, it is still uncertain when and if price parity with traditional livestock products will be reached and whether the products can be produced in the volumes needed. However, the quest for better products, sources, and methods continues.

According to Research and Markets (2021), the worldwide market for cultured meat is anticipated to achieve $352.4 million in sales by 2028, in addition, Grand View Research (2021) predicts a global growth rate for cultured meat of 11.4% between 2022 and 2028, with the Asia-Pacific (APAC) region expected to experience the highest growth rate at 12.1%. In the development of cultured meat, it is important to establish stable cell proliferation and optimized culture conditions to improve production efficiency, and due to the limitations of proliferation through muscle satellite cells (MuSCs), research on cell immortalization as a strategy for stable cell proliferation is in full swing (Stout et al., 2024).

The plant-based meat market is expected to grow steadily, reaching a valuation of USD 9.42 billion in 2023, and the market is projected to expand at a compound annual growth rate (CAGR) of 8.55% from 2024 to 2029, eventually reaching approximately USD 15,570 million by 2029 (Statista, 2024). Plant-based protein products as substitutes for animal protein include plant-based meat, plant-based eggs, plant-based dairy products, and plant-based protein emulsion foods, which can be produced by technologies such as high-moisture extrusion, three-dimensional (3D) printing, and electrospinning (Xiao et al., 2023). New studies are continuously published on the types, forms, and functionalities of plant-derived proteins used in formulating plant-based protein products. Although there have been many negative opinions in recent years that alternative meats will not succeed in the market, the scientific literature indicates that active and widespread research on alternative meats continues; thus, it would be inadvisable to jump to any conclusions. Rather, this study aims to highlight and examine the various research studies on meat alternatives, including cultured meats, with a focus on recently published studies as it seeks to assess the level of current technology used and the potential for full-scale industrialization of meat alternatives.

Cutting-Edge Technologies in Cultured Meat

Technologies for improving sensorial characteristics of cultured meat

Current research on cultured meat primarily focuses on reconstructing muscle tissue *in vitro*; thus, the sensory attributes, such as taste, texture, and nutritional content of cultured meat, are often overlooked. The wide range and availability of meat alternatives in the market are significant for consumers, but they need to meet several criteria, including appearance, flavor (both aroma and taste), and texture. Many consumers place a high value on the taste and texture of meat, making these factors essential for consumer acceptance (Hoek et al., 2011). Actually, the taste and texture there are reported barriers to its acceptance by meat consumers. Trans-differentiation refers to the process of converting mature cells into a different cell type and can be utilized to convert muscle cells into adipocytes. Ma et al. (2024) established a protocol for the controlled trans-differentiation of chicken fibroblasts into myoblasts (Table 1). They confirmed the same results as previous studies that used treatments such as chicken serum medium and fatty acids to induce the conversion to adipocytes capable of fat storage (Lee et al., 2021). Furthermore, using immortalized cells derived from chicken, Ma et al. (2024) exploited the adipogenic/adipogenic

Table 1. Current technologies in cultured meat

Types	Contents	References
Cell	- Chicken fibroblasts transformed into muscle cells using myogenic differentiation (MyoD) overexpression in a three-dimensional (3D) hydrogel scaffold, forming muscle fibers similar to native meat. - Achieved effective adipogenesis in two-dimensional (2D) and 3D cultures with chicken fibroblasts using a medium with 60 μg/mL insulin and 8 μg/mL fatty acids.	Ma et al. (2024)
	- Cultured muscle and fat layered in a 3:1 ratio on 2D hydrogel scaffolds to create beef-like cultured meat with enhanced sensory properties. - Low alginate (0.25%) hydrogel with low crosslinking (3 kPa) is ideal for adipocyte differentiation. - High alginate (2%) hydrogel with high crosslinking (11 kPa) is optimal for muscle cell differentiation.	Lee et al. (2024c)
	- Fermentation in cultured meat production offers natural food safety ingredients, enhancing taste, texture, nutrition, and shelf life. - Precision fermentation supports continuous synthesis of fetal bovine serum (FBS) replacement components, scaffolds, nutrients, and food additives.	Singh et al. (2022)
	- Frankfurt-style cultured meat sausages have similar hardness to commercial sausages and intermediate chewiness between processed turkey and raw chicken. - Cultured meat sausages have a higher Young's modulus than traditional sausages, indicating greater stiffness.	Paredes et al. (2022)
	- Cell growth rate and viability are higher at 37°C compared to 39°C in both C2C12 cells and Hanwoo muscle satellite cells (MuSCs). - C2C12 cells at 39°C show higher levels of myosin heavy chain (*MyHC*) and myoglobin (*MB*) gene - MuSCs also display increased *MyHC*, myogenic factor 6 (*MYF6*), and *MB* gene levels at 39°C. - Optimal culture efficiency for MuSCs involves proliferation at 37°C and differentiation at 39°C.	Oh et al. (2023)
	- Serum-free cultures (B27, AIM-V) effectively differentiate C2C12 cells, with increased glycerol-3-phosphate and uridine diphosphate *N*-acetylglucosamine as myotube maturation markers. - Lactate secretion reduced by about 50% in B27 and AIM-V media, showing less pH variation and better culture suitability than conventional media.	Jang et al. (2022)
	- Pronase isolates more porcine MuSCs compared to collagenase; combining pronase with Dispase II yields cells with good viability and muscle differentiation ability. - MuSCs isolated using pronase+Dispase II with 30-minute pre-plating are produced more efficiently than using fluorescence-activated cell sorting (FACS).	Li et al. (2022b)
	- C2C12 myoblasts in optimized serum-free media enter the logarithmic growth phase within 1 day and proliferate rapidly over 3 days, similar to serum-containing conditions. - Long-term passage in serum-free media maintains C2C12 proliferation rates akin to serum-supplemented media.	Dai et al. (2024)
	- Beefy-9 medium, supplemented with 800 μg/mL recombinant human albumin, effectively supports MuSC myogenesis and long-term culture maintenance. - MuSCs adhere better to flasks coated with 1.5 $\mu g/cm^2$ cleaved vitronectin than those coated with laminin fragment iMatrix-511.	Stout et al. (2022)
	- Recombinant bovine fibroblast growth factor 1 (rbFGF1) significantly enhances C2C12 myoblast proliferation by activating the ERK1/2 signaling pathway and increasing dynamin-related protein 1 phosphorylation, which governs mitochondrial fission. - rbFGF1 improves mitochondrial health by stabilizing the mitochondrial membrane potential and promoting fission, essential for cell proliferation and energy metabolism.	Liu et al. (2024)
	- Overexpressing FGF2 or RAS^{G12V} activates endogenous FGF2, restoring the effect of recombinant FGF and eliminating the need for exogenous FGF2, reducing culture media costs. - Modified cells maintain growth rates and myogenic characteristics, with slightly reduced myotube formation compared to those cultured with exogenous FGF2.	Stout et al. (2024)
	- Glucose extracted from *Chlorococcum littorale* or *Arthrospira platensis* and amino acids extracted from *Chlorella vulgaris* were shown to be excellent as medium additives for C2C12 mouse myoblast culture.	Okamoto et al. (2020)

Table 1. Current technologies in cultured meat (continued)

Types	Contents	References
	- Hydrophilic compounds derived by ultrasonic extraction of *Chlorococcum littorale* (CW) can be used as serum substitutes in mammalian cell proliferation. - The sample treated with 40% CW showed a proliferation rate similar to the control group in C2C12 cells.	Ghosh et al. (2024)
	- Treatment with 3,2′-dihydroxyflavone (10 μM) during the proliferation phase increases cell expansion by 34%, while quercetin (50 nM) during differentiation significantly boosts MyHC expression 4.73-fold compared to controls. - Flavonoid combination in optimized medium for cultured meat production expands the contractile area of cultured meat by 41.37%.	Guo et al. (2022)
	- Cytokine efficiency in cultured meat production is enhanced by simultaneous expression in *Saccharomyces cerevisiae*, with the CPK2B2 strain reaching the highest cytokine production at 1,845.67 μg/L. - DMEM with 5% FBS supplemented with 1 g/L CPK2B2 lysate increases porcine MuSC proliferation by 1.59-fold compared to DMEM with 5% FBS alone, without affecting differentiation potential.	Lei et al. (2023)
	- Gelatin and soymilk scaffold supports C2C12 cells with a 102.1% survival rate, increasing myosin expression 2.45 times, aiding muscle tissue formation. - For 3T3-L1 fat cells, the scaffold shows a 118.2% survival rate, with proliferator-activated receptor gamma (PPARγ) expression increasing 1.32 times, promoting fat accumulation.	Li et al. (2022a)
	- Aligned porous structures significantly enhance MuSC differentiation into muscle fibers, up-regulating myogenic genes and proteins, forming matured myotubes that mimic natural muscle tissue organization, and improving cultured meat texture and microstructure. - Aligned pore scaffolds improve mechanical properties, enhancing the texture of cell-cultured meat to resemble traditional meat in chewiness and resilience.	Chen et al. (2024)
	- The polyamide polyethylene double-layer laser welding device enables precise and stable welding and cutting. - Bovine mesenchymal stem cells cultured on food-grade rice puff scaffolds suggest cost-effective cultured meat production using a laser cutter.	Gome et al. (2024)
	- The glutenin-chitosan complex (G-CS) scaffold, fabricated through hydrothermal treatment, molecular assembly, and water annealing, features a regular hexagonal structure with small pore size, and increased compressive modulus due to chitosan and glutenin mixing. - The G-CS scaffold's microstructure enhances cell adhesion rate of porcine MuSCs and effectively promotes myotube fusion and proliferation.	Wu et al. (2024)
	- MuSCs from large yellow croaker show distinct morphologies in 2D vs. 3D systems, with enhanced adhesion and proliferation in 3D cultures using hydrogels and microcarriers. - MuSCs on microcarriers and hydrogels exhibit higher expression of adhesion-related genes (integrin β1, syndecan-4, vinculin) and myogenic markers (Pax7, Myod1) than 2D cultures; microcarriers induce slight spontaneous differentiation due to rapid proliferation.	Yin et al. (2024)
	- Compressive elastic moduli of crosslinked hydrogels can be adjusted by polymer concentration and crosslinking method; dual-crosslinked alginate hydrogels are stiffer and support muscle tissue well. - Dual-crosslinked alginate hydrogels are non-cytotoxic, maintaining high cell viability and adhesion, with arginyl-glycyl-aspartic acid–modified hydrogels supporting higher MuSC density. - C2C12 adhesion rate increases as visible light-crosslinked samples' elastic stiffness (49–88 kPa) aligns with muscle tissue elastic modulus (16–60 kPa).	Tahir and Floreani (2022)
	- Wet-spinning technology used alginate immersion in a zein coagulation bath with CaCl2 to produce zein-alginate (ZA) fibers. - Using a 30 G needle in fiber production significantly reduced ZA fiber diameter, resulting in a more aligned structure during cell culture.	Jeong et al. (2024)

Table 1. Current technologies in cultured meat (continued)

Types	Contents	References
	- Pre-processing methods such as ultrasound, microwave, and high-pressure treatment, along with controlling ink formulation using lipids or hydrophilic colloid and transglutaminase, were suggested to improve printability. - 3D printing can convert low-value meat by-products and trimmings into higher-value food products, addressing waste and sustainability issues in the meat industry.	Dong et al. (2023)
	- Gelatin/alginate/ε-poly-L-lysine (GAL) hydrogel, with 5% gelatin, 5% alginate, and ε-poly-L-lysine (4:1 molar ratio to alginate), shows excellent compressive strength, porosity, and shape fidelity, ideal for 3D printing. - GAL hydrogel supports porcine MuSC culture, achieving over 96.6% cell viability and stable MyoD differentiation marker expression, demonstrating successful cellular differentiation.	Wang et al. (2024a)
	- 100 μM L-ascorbic acid 2-phosphate (Asc-2P) effectively sustains muscle stem cell culture from neonatal pig tissues, increasing PAX7-positive cells compared to adult pigs. - Optimized polydimethylsiloxane mold, collagen solution, and porcine MuSCs form a 3D porous tissue network for cultured meat production; Asc-2P treatment enhances MyHC protein and MYOG expression with longer myotubes and stronger contractile force.	Zhu et al. (2022)
	- MuSCs experience adverse attachment and proliferation effects at 1, 10, and 50 μg/mL microplastic concentrations, with highest viability at 10 μg/mL; microplastic concentration minimally affects differentiation marker expression [MyoD1, MYOG, troponin T 3A (TNNT3A)].	Sun et al. (2024)
	- Co-culture of C2C12 myoblasts and 3T3-L1 adipocytes forms stacked cell sheets mimicking meat structures, a platform for alternative meat products; multilayer assembly contracts into stable constructs without extracellular matrix, with a 1:3 cell ratio crucial for replicating meat texture and flavor.	Shahin-Shamsabadi and Selvaganapathy (2022)
	- Porcine pre-embryonic epithelial stem cells (pgEpiSCs) differentiate into myogenic precursor cells via Wnt activation and transforming growth factor-β inhibition in serum-free medium. - Plant-based 3D scaffold using glucomannan, sodium alginate, and calcium ions supports over 95% adhesion with C2C12 cells and porcine MuSCs, with pgEpiSCs-muscle cells showing expanded myofiber morphology and producing cultured meat.	Zhu et al. (2023)

switch differentiation of chicken fibroblasts in a 3D culture system utilizing gelatin methacryloyl (GelMA) hydrogel to accumulate fat in muscle tissue without co-culture or mixing of fat. Using fish gelatin/alginate scaffolds, muscle and fat structures were assembled by regulating cell differentiation to produce cultured meat with a softer texture and higher elasticity with a flavor similar to traditional beef (Lee et al., 2024c).

Cultured meat production can be made more effective and efficient by using biomass and fermentation technologies with the aim of improving the texture and flavor of cultured meat (Attaran Dowom et al., 2019). In particular, precision fermentation has improved the organoleptic properties of cultured meat by using genetically engineered organisms to biosynthesize secretory bio-based heme (Jin et al., 2018) and by inserting targeted genes into specific yeast strains and fermenting them to produce fats with a molecular structure similar to that of conventional meat (Singh et al., 2022). Precision fermentation has also been used to produce bitter blockers to eliminate the bitterness associated with soy-based additives, and supernatants of cultures used to ferment cordyceps and several fungal strains (genetically engineered DH5 *Escherichia coli*) have been added to foods to reduce bitterness (Dunstan et al., 2020). In this way, biomass fermentation using fungi can not only help improve the flavor of cultured meat but also contribute to a sustainable industry by utilizing resources. Texture profile analysis and rheological methods were used to characterize mechanical properties of cultured meat (Paredes et al., 2022). The textural properties (hardness, cohesiveness, chewiness, resilience, springiness, and Young's modulus) of commercial sausage, turkey breast, chicken breast, and cultured meat were similar, and sausage cooked with cultured meat exhibited higher elastic modulus and shear modulus than other commercial meats, identifying some parameters that should be considered in the initial cultured meat manufacturing process.

Technologies for improving the production efficiency of cultured meat

Even though cultured meat is not yet commonly available in the market, consumer interest in trying it has been unexpectedly high, and its increasing popularity is promising. Cultured meat presents as feasible path for future development, however, further studies related with the mass production on consumer acceptance would be advantageous to secure success when the cultured meat is widely launched in the future. In the development of cultured meat, stable cell proliferation and optimized culture conditions are key to improving production efficiency (Table 1). As a strategy for stable cell proliferation, immortalization of cells is being investigated. Fibroblasts from several chicken breeds were naturally immortalized to form genetically stable cell lines, which grew at high densities despite the use of serum-free media and were able to produce high yields of cultured meat without genetic modification (Pasitka et al., 2023). Research on optimized culture conditions to improve production efficiency is also ongoing. When Hanwoo myosatellite cells and C2C12 cells were cultured at 37°C and 39°C to compare their proliferation and differentiation efficiency, it was found that culturing at 39°C, which significantly increased the gene expression levels of *myosin heavy chain* (*MyHC*), *myogenic factor 6* (*MYF6*), *myogenin* (*MYOG*), and *myoglobin* (*MB*), could increase the production efficiency of cultured meat (Oh et al., 2023). In addition, the use of pronase is more efficient than collagenase for the efficient isolation of porcine muscle stem cells, specifically the use of pronase and Dispase II, which promotes higher attachment and proliferation rates of muscle stem cells (Li et al., 2022a).

To reduce production costs and address the stability issues of cultured meat, research is being conducted on the development of serum-free media and various additives for the successful development of cultured meat. It has been confirmed that efficient production of high-quality cultured meat is possible by checking the metabolism of muscle cells (Jang et al., 2022). Non-essential amino acids, pyruvate reduction, and transamination showed significant differences in serum and serum-free medium (B27, AIM-V) culture conditions, and the metabolic profile confirmed the predominance of glycolytic and oxidative metabolism in C2C12 myotubes cultured in serum and B27 medium. Dai et al. (2024) selected 19 components and developed an optimized serum-free medium by combining each component through a Plackett–Burman design. Through this approach, they obtained a system capable of long-term culture and mass production of C2C12 myoblasts. Beefy-9, a serum-free medium for long-term culture of bovine satellite cells (BSCs), was developed by adding recombinant human albumin to promote BSC growth and maintain myogenicity of the cells and was shown to be suitable for mass production at an economical cost (Stout et al., 2022).

Fibroblast growth factor 1 (FGF1) signaling plays an important role in muscle stem cell proliferation. It is known to contribute to the proliferation of satellite cells and maintain their proliferation rate. Its effectiveness as an additive is excellent, but its high cost is a barrier to cultured meat production. Liu et al. (2024) developed a method to efficiently produce soluble, bioactive recombinant bovine FGF1 (rbFGF1) protein in *E. coli*. rbFGF1 promoted mitochondrial division and proliferation of MuSCs under serum-free medium conditions. The study showed that rbFGF1 promoted the proliferation of C2C12 cells, increased the mitochondrial membrane potential, and induced cell proliferation through extracellular signal-regulated kinases1/2 (ERK1/2) signaling, making it an effective additive in serum-free medium conditions. FGF2, widely used in cultured meat production, is an expensive growth promoter that increases the cost of serum-free media for related cells, including MuSCs. To lower the cost, one study developed immortalized BSCs (iBSCs) that can grow without external growth factors by overexpressing FGF2 and mutant Ras^{G12V} (Stout et al., 2024). These immortalized cells were shown to be able to proliferate for multiple generations in FGF2-free medium and retain their originality despite reduced root canal formation, confirming that this is an effective cell engineering technique for reducing cultured meat production.

To investigate the feasibility of utilizing extracted components derived from microalgae as growth promoters in serum-free media conditions, an extract from the microalga *Chlorococcum littorale* was obtained by sonication. The addition of the extract to mammalian cell lines (C2C12 cell lines, 3T3 cell lines, and CHO cells) in place of fetal bovine serum (FBS) resulted in high proliferation rates (Ghosh et al., 2024). Glucose extracted from *C. littorale* or *Arthrospira platensis* and many amino acids extracted from *Chlorella vulgaris* have been shown to be excellent as media additives for C2C12 mouse myoblast cell cultures (Okamoto et al., 2020). Furthermore, the acid hydrolysis method for extracting the growth-promoting factors from the algae is regarded as simple, economical, and environmentally friendly, with applications in cultured meat production as well as various other fields such as regenerative medicine and gene/cell therapy (Okamoto et al., 2020). Porcine-derived muscle stem cells tend to lose their stemness during cultured meat processing. Therefore, to promote the proliferation and differentiation of the cells, various flavonoids (quercetin, icariin, 3,2′-dihydroxyflavone) were added to the culture media (Guo et al., 2022). While 3,2′-dihydroxyflavone stood out for its role in maintaining stem cell competence, quercetin was superior to the other flavonoids in inducing differentiation and upregulating the expression of MyHC.

Cytokines play an important role in promoting rapid cell proliferation. However, their application in cultured meat production has been challenging due to the high cost of commercial cytokines as well as potential food safety issues (Laulund et al., 2017). Lei et al. (2023) established a system to obtain a recombinant strain CPK2B2 co-expressing four cytokines (including long-chain human insulin growth factor-1, platelet-derived growth factor-BB, basic FGF, and epidermal growth factor) through the Cre-loxP system in *Saccharomyces cerevisiae*, with a yield of 18.35 mg/L. Subsequent to cell lysis and filter sterilization, the CPK2B2 lysate was used to stimulate porcine muscle stem cell proliferation. Overall, the study afforded a simple and cost-saving approach for cultured meat production and efficient cytokine production.

Technologies for organizational development in cultured meat

Scaffolds are often used to maintain the morphology of existing cultures and promote the development of muscle, fat, and connective tissue. Consequently, there is substantial research focused on scaffold formation methods and scaffold materials. To accurately recreate the extracellular matrix (ECM), which regulates the dynamic behavior of cells in tissues, provides structural support, and transmits information between cells, cell scaffold structures must be 3D and contain interconnected networks embedded in viscoelastic materials. Various biomaterials have been designed as *in vitro* supports that reproduce the ECM to support cell viability, growth, and migration (Tahir and Floreani, 2022). However, the main issue with current scaffolds for cultured meat is striking a balance between the need for food-grade materials and the cell adhesion, proliferation, and differentiation capabilities. Edible proteins have been used as scaffolds to overcome these limitations. Seah et al. (2022) suggested that scaffold technologies for cultured meat should focus on biocompatibility, biodegradability, pore size, strength of the scaffold material, structure, and fabrication techniques. Rabbit skeletal myoblasts (RbSkMCs) cultured on gelatin fibers produced from co-spinning gelatin and microbial transglutaminase (a food-grade crosslinker) formed 3D tissues with visible cytoskeletal (F-actin) networks throughout the tissue (MacQueen et al., 2019). Jiang et al. (2013) demonstrated that electrospun zein nanofibers can be used for cell adhesion and cell growth of fibroblasts. In another example, scaffolds synthesized from gelatin and soymilk expressed myosin in C2C12 cells and upregulate the expression of PPARγ, an adipogenic transcription factor, in 3T3-L1 cells (Li et al., 2022a). It was found that the bioactive isoflavones such as daidzein, genistein, and glycitein from soymilk induced muscle formation, suggesting that abundant integrin binding sites of gelatin help to improve cell adhesion and migration and induce differentiation signaling, which could be useful for cultured meat production (Li et al., 2022a).

Various studies have been published on porous supports that provide space for cell attachment and proliferation. An edible scaffold was developed using various food-grade materials (proanthocyanidins, dialdehyde chitosan, collagen) and various proportions of yeast protein. The developed scaffold had a porous structure and was enriched with tripeptides, which provided space for cell attachment and proliferation, promoting growth and differentiation. In addition, the cultured meat produced through this method showed a level of springiness and chewiness similar to that of traditional meat, which had a positive effect on improving texture (Wang et al., 2024b). Gome et al. (2024) cultured immortalized bovine mesenchymal stem cells (bMSCs) using a macrofluidic single-use bioreactor fabricated with a plant-based scaffold and polyamide polyethylene (PAPE) film as a leak-proof, strongly sealed form of container. The porous structure of rice puffs used as the plant-based scaffold resulted in improved cell attachment and proliferation, and high water absorption was confirmed, indicating that it can be utilized for economical and efficient cultured meat production. Glutenin-chitosan 3D porous supports with pore diameters of 18 to 67 μm and compression moduli of 16.09 to 60.35 kPa effectively promoted cell growth and muscle differentiation and were found to be effective in enhancing the texture and mouthfeel of porcine-derived cultured meat by increasing multiple myofibril fusion with cytoskeleton expansion and tissue maturation (Wu et al., 2024). In addition, customized supports were also developed for fish cultured meat to analyze biological differences. Satellite cells obtained from the large yellow croaker were cultured in two-dimensional (2D) and 3D cell culture systems. The results showed that cell adhesion receptors and myogenic markers [paired box 7 (Pax7), myogenic differentiation 1 (MyoD1), desmin] were more highly expressed in 3D cultures using hydrogel/microcarrier, which was favorable for the production of fish cultured meat (Yin et al., 2024).

Alginate, which is often used in scaffolds, is derived from brown algae and is known to be sustainable, readily available, and an ideal material for making hydrogel supports (Kang et al., 2021). One study synthesized methacrylated alginate (AlgMA) through a dual crosslinking system to form covalent bonds and arginyl-glycyl-aspartic acid (RGD) conjugates (AlgMA-RGD) to develop hydrogels with tune bale mechanical properties (Tahir and Floreani, 2022). The hydrogel scaffolds showed excellent effects on the viability and attachment of MuSCs. Jeong et al. (2024) developed a technique for culturing and aligning muscle cells using zein-alginate fibers made by coating zein protein on alginate fibers. The fibers were produced using a wet spinning technique that takes advantage of the fact that when zein solution encounters hydrophilic alginate hydrogels, its solubility decreases, and it coagulates on the alginate surface. The fibers had excellent cell affinity, biodegradability, and a high strain rate of more than 75%, which promoted the maturation of muscle cells and the formation of aligned myotubes. This economical and simple production of fiber scaffolds using plant-based materials can eliminate toxic chemicals as a limiting factor and is conducive to cell stacking and structuring, suggesting the potential for mass production of cultured meat.

Depending on the material and combination of inks, 3D printing can affect the texture and various rheological properties, which can significantly impact quality. 3D printing could provide solutions for the critical issues of cultured meat, utilizing by-products and solving sustainable industrial and food contamination problems (Dong et al., 2023). A 3D printable scaffold based on gelatin/alginate/ε-poly-L-lysine hydrogel was developed to provide a platform for cultured meat production by confirming the cell attachment, increased proliferation rate, and maintenance of the differentiation capacity of C2C12 mouse skeletal myoblasts and porcine muscle stem cells (Wang et al., 2024a). In addition, a polydimethylsiloxane mold fabricated by 3D printing technology was used to form a 3D skeletal muscle tissue network using porcine muscle stem cells (Zhu et al., 2022). The results showed improved texture and increased amino acid content, suggesting an effective working process for cultured meat production. Another study generated a meat-like cell sheet structure without 3D constructs, bioprinting technology, or ECM components for cell attachment. The meat-like tissues with meat-like texture could be produced by combining myoblasts (C2C12) and adipose progenitor cells (3T3-L1) in an optimal ratio (Shahin-Shamsabadi and Selvaganapathy, 2022).

Research is also being conducted on the aspects of cultured meat safety and health protection. Atlantic mackerel (*Scomber scombrus*) skeletal muscle cells were used to determine the effects of microplastic exposure on the production efficiency of cultured seafood (Sun et al., 2024). The results showed that microplastic concentration significantly affected cell adhesion and proliferation but not differentiation. There have also been studies that have used a combination of different techniques to produce cultured meat. Porcine pre-gastrulation epiblast stem cells were used to develop a cell line capable of long-term culture and genetic stability, and a differentiation system was established with serum-free medium to successfully differentiate muscle cells. Edible scaffolds with porous and homogeneous structures were utilized to produce cultured meat through ionic crosslinking of various formulations of konjac glucomannan, sodium alginate, and calcium ions (Zhu et al., 2023).

Cutting-edge technologies for meat alternatives obtained from edible insect

The market for edible insects is forecasted to grow to $17.9 billion by 2033, with a CAGR of 28.6% from 2024 to 2033. In terms of volume, it is anticipated to reach 4.7 million tons by 2033, growing at a CAGR of 36.3% over the same period (Meticulous Research, 2023). Insect protein is one of the emergent protein sources expected to enter the sustainable food market and is a highly efficient protein source. Insect farming is an environmentally friendly way of raising animals, with less water and land requirements than farmed animals and less pollution compared with the pollution issues associated with livestock farming. Insect-derived ingredients are mainly composed of proteins derived from insect muscles, fat bodies, and the cuticle layer that covers the epidermis (Lamsal et al., 2019). Insect-based protein ingredients are often made by grinding whole insects and processing them into powder for various food or feed applications, with insect protein content varying from 13% to 77% by building (Lamsal et al., 2019). Edible grasshoppers in Mexico exhibited a protein content of 44% to 77% and a fat content of 4% to 34%, which fulfills essential amino acid requirements (Blásquez et al., 2012; Paul et al., 2016). Defatted, alkaline, and ultrasound-assisted extraction methods were found to increase the amount of protein extracted from edible mealworm (*Schistocerca gregaria*) and honeybee (*Apis mellifera*) by 57.5% and 55.2%, respectively (Mishyna et al., 2019a; Table 2). Furthermore, the extracted proteins exhibited high foaming and emulsification stability, and the proteins obtained from honeybees, in particular, exhibited high thermocoagulability, indicating that insect-based proteins have the potential as food feeds or dietary supplements (Mishyna et al., 2019a). Similarly, Queiroz et al. (2023) found that techniques such as autoclaving, sonication, pulsed electric fields, and ohmic heating improved various functional properties of insect proteins, including solubility, emulsification, and foam-forming ability. Another study investigated the temperature- and pH-dependent aggregation and gelation of edible bee larval proteins (Mishyna et al., 2019b). The highest aggregation rates (73.7% and 68.4%, respectively) were found at pH 5 and 7 at 85°C, and the protein properties were changed by heating, with minimum gelling concentrations of 5% at pH 7% and 11% at pH 3. Therefore, the potential of using edible insects as gelling agents was confirmed. Lee et al. (2024b) confirmed that *Tenebrio molitor* larvae ethanol treatment could change the structure of proteins to improve their technical and functional properties. They found that the protein molecular weight decreased, the α-helix structure decreased, and the β-sheet structure increased with increasing ethanol treatment concentration. In addition, ethanol treatment increased protein surface hydrophobicity and foaming ability, and the best antioxidant activity was found at

Table 2. Current technologies in meat alternatives obtained from edible insect and plant

Types	Contents	References
Edible insect	- Protein content enhancement in edible grasshopper and honey bee brood is enhanced through defatting, alkaline treatment, and ultrasound-assisted extraction. - Extracted proteins from grasshopper and honey bee brood show high foaming capacity and emulsification stability. - Proteins from honey bee brood exhibit high thermal coagulation properties.	Mishyna et al. (2019a)
	- Aggregation and gelation of honey bee larvae proteins depend on temperature and pH, with maximum aggregation at 85°C at pH 5 and 7. - At pH 3, disulfide bonds play a lesser role, whereas at pH 5 and 7, exposed hydrophobic domains contribute to aggregation. - The pH impact on gel's rheological and textural properties is less pronounced due to system complexity involving proteins and polysaccharides.	Mishyna et al. (2019b)
	- Adding *Alphitobius diaperinus* insect protein to burgers decreases pH, monounsaturated, and polyunsaturated fatty acids, while increasing total lipids and saturated fatty acids. - Burgers with 10% insect protein have the best flavor, but those with 5% insect protein achieve the highest overall acceptability. - Insect protein minimally affects the physical properties of plant-based meat substitutes and enhances nutritional value.	Krawczyk et al. (2024)
	- A biscuit with locust meal powder provides sufficient daily protein for children aged 12–24 months, meeting 24%–38% of the recommended dietary allowance. - Adding 5% locust powder raises energy, protein, fat, and moisture contents but reduces carbohydrate and ash contents compared to controls.	Dewi et al. (2020)
	- Increasing temperature to 140°C and 160°C or reducing water flow rate from 10 to 9 mL/min enhances the tensile strength of the mixture. - A soybean protein isolate-cricket meal mixture with 30% low-fat cricket flour achieves the best anisotropic and fibrous structure under extrusion conditions of 10 mL/min WFR and 160°C.	Kiiru et al. (2020)
	- Tribo-electrostatic separation produces higher protein and carbohydrate yields than air-based fractionation methods for legume or plant protein fractions. - Wet protein extraction techniques are improving to enhance protein quality, yield, and stability without reducing solubility. - Additional methods like microwave, ultrasound, pulsed electric field, and high hydrostatic pressure enable high protein yield beyond traditional dry and wet extraction.	Thakur et al. (2024)
Plant	- Heat treatment and high-pressure technologies are not energy and cost-efficient, thus not aligning with sustainable development goals. - Chemical modifications like glycation are evolving to align with food safety regulations and the trend towards 'Clean-label' ingredients. - Biological methods, including enzymes and fermentation, are environmentally friendly and low-energy, promoting the advancement of technologies for enhancing plant protein quality.	Nasrabadi et al. (2021)
	- Significant advancements in plant protein sources, including soy, legumes, grains, and seaweed, enhance meat and fish analogues. - like chemical, physical, and biological modifications improve plant protein properties, while additional ingredients influence texture and quality. - Evolving technologies such as extrusion, shear cell technology, and three-dimensional (3D) printing contribute to plant-based products mimicking meat and fish textures and tastes.	Nowacka et al. (2023)
	- Typical physical modification minimally impacts plant protein structures but enhances functional properties by altering secondary and tertiary structures. - Chemical modification provides benefits like short reaction time, low cost, minimal equipment needs, and significant modification effects. - Enzyme modifications, including fermentation and germination, enhance processing, nutritional properties, and bioavailability of plant proteins.	Xiao et al. (2023)
	- Adding 30% banana flower and jackfruit results in no significant differences in chewiness and flavor compared to the control group. - All treatment groups exhibit high overall acceptability and have higher fiber and protein contents than the control group.	Keerthana Priya et al. (2022)

Table 2. Current technologies in meat alternatives obtained from edible insect and plant (continued)

Types	Contents	References
	- Increasing oat fiber concentrate (OFC) concentration reduces mechanical properties and pore space in fibrous meat analogs, allowing for 30%–50% OFC addition. - Fiber structure alignment changes with increased long cooling die temperature (LCDT), while β-glucan extractability and viscosity remain preserved at low LCDT.	Ramos Diaz et al. (2022)
	- Developing biopolymer composites with meat-like textures involved coacervation and heat-induced gelation of gellan gum and potato protein blends, with electrical properties influenced by solution pH and polymer ratio, leading to gels with varied microstructures and textures.	Hu et al. (2024)

20% ethanol treatment (Lee et al., 2024b).

The development and evaluation of edible insect-based food products are being actively explored. The addition of insect protein (*Alphitobius diaperinus*) was shown to decrease the pH, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) while increasing total lipids and saturated fatty acids (SFA) in burgers, with the highest sensory acceptability score at 5% insect protein (Krawczyk et al., 2024). A study on the preparation of baby biscuits using wood grasshopper flour (*Melanoplus cinereus*) found that the addition of 5% insect powder provided the most appropriate energy and nutrient content and was the most preferred in terms of taste, aroma, and texture (Dewi et al., 2020). In addition, the developed biscuits also provided high-quality protein and were found to meet Indonesian supplementary food regulations. The effect of insect addition, extrusion temperature, and moisture content on the texture properties of a meat substitute prepared from cricket flour and soy protein isolate was determined using a high-moisture extrusion cooking technique (Kiiru et al., 2020). The tensile strength increased with increasing extrusion temperature but decreased with increasing cricket flour addition. In particular, meat alternatives with the best properties and texture were obtained at an extrusion temperature of 160°C and 30% low-fat cricket flour addition, and moisture content was found to play an important role in the structural integrity and texture formation of meat alternatives (Kiiru et al., 2020). Totally, Queiroz et al. (2023) reported ultrasound processing, high hydrostatic pressure, pulsed electric fields collectively provide innovative solution for improving the textural and flavor profiles of insect-based protein products, making them more appealing as meat alternative ingredients. Insect protein is a highly sought-after resource due to its high protein and oil content. However, it has very low consumer acceptance, so many researchers have been adding insects in powder form to existing foods or processing them as a way to address this issue. Furthermore, to maximize the potential of insects as an alternative food resource, it is believed that research should focus on demonstrating the functional properties (water and fat absorption capacity, emulsification, foaming, gelation, rheological properties) and various food applications of insect protein.

Cutting-edge technologies for meat alternatives obtained from mycoprotein

Based on market analysis, the US mycoprotein industry is expected to achieve a value of $523.4 million by the end of 2030 (Khan et al., 2024). In comparison, China’s market potential is anticipated to reach approximately $238.6 million by 2030 (Derbyshire and Delange, 2021). Additionally, this figure is expected to exceed $1.1 billion by 2030. Mycoprotein contains high-quality protein, 25% fiber, high amounts of MUFA and PUFA, low SFA, a wide range of essential amino acids, and produces less greenhouse gas emissions than plant and animal proteins (Ahmad et al., 2022; Khan et al., 2024). It also contains similar levels of protein utilization to milk-derived protein and has been shown to be beneficial for muscle protein production in children (Monteyne et al., 2020). Mycoprotein is primarily produced through a fermentation process, although it can also be produced from various agricultural wastes (Zhang et al., 2023). It is currently manufactured by Marlow Foods under the brand name Quorn by culturing *Fusarium venenatum* in a specific environment and mixing it with egg whites, colors, and flavoring compounds to create a meat-like texture, which is sold in the United States, United Kingdom, France, Europe, and Singapore (Finnigan et al., 2019; Khan et al., 2024). Fermentation methods for the mass production of mycoprotein are classified solid-state fermentation (SSF) and submerged fermentation (SmF; Ahmad et al., 2022; Majumder et al., 2024). SSF has been utilized for centuries in the production of traditional fermented foods like soy sauce, tempeh, miso, koji, red fermented rice, and tapai (Lizardi-Jiménez and Hernández-Martínez, 2017). Mycoprotein produced via SSF, which operates under low moisture conditions and uses solid substrates, is nutritionally superior, with a higher protein digestibility-corrected amino acid score than chicken or beef, effectively addressing the limitations of plant-based proteins (Cerda et al., 2019; Kim and Kim, 2012). SmF involves the growth of microorganisms in a liquid medium with high water content (over 95%), utilizing carbon, nitrogen, and micronutrients for cultivation (Majumder et al., 2024). This method, which supports both anaerobic and partially anaerobic processes, has become a preferred technique in the production of bioactive mushroom compounds due to its ease of management and rapid control of growth conditions (Majumder et al., 2024). The SmF process for mycoprotein production includes microbial growth in the liquid medium, followed by incubation, centrifugation, washing, and filtration to isolate the protein-rich biomass (Reihani and Khosravi-Darani, 2019). While SmF is widely used in large-scale industrial enzyme production due to its efficient handling and monitoring capabilities, it is also associated with significant challenges, including high costs, lower productivity, and the complexity of the required medium (Ahmad et al., 2022; Manan and Webb, 2017).

Pavis et al. (2024) found that a dietary invention with mycoprotein-containing foods significantly reduced total cholesterol, low-density lipoprotein cholesterol, and non-high-density lipoprotein cholesterol concentrations in adults with overweight, improving cardiometabolic health by reducing hypercholesterolemia. *Neurospora crassa* mycoprotein, traditionally used as a fermented food mainly in Indonesia and China, has been safely used as animal feed (Liu et al., 2016). This nutritious material is rich in complete protein (45 g/100 g), dietary fiber (35 g/100 g), potassium, and iron with no toxicity or allergenicity, confirming its potential as an alternative material for meat production (Bartholomai et al., 2022). Nuggets prepared using mycoprotein were found to have 57.9%, 24.1%, 13.2%, and 2.1% moisture, protein, fat, and ash content, respectively, and exhibited similar characteristics to chicken nuggets in terms of texture, color, and physicochemical properties, and with 33% lower cooking loss (Hashempour-Baltork et al., 2023).

Cutting-edge technologies for meat alternatives obtained from plant-based materials

The main sources of plant protein are grains (wheat, corn, oat, rice, rye), legumes (pea, red bean, chickpea, lentil, faba bean), seed oils (sunflower, rapeseed, flaxseed, hemp seed, cotton seed, sesame seed, pumpkin seed), nuts (almond, pistachio, cashew, walnut, peanut), and others (quinoa, buckwheat, chia seed, amaranth, potato; Munialo, 2024). Alternative proteins, such as plant-derived proteins, have the potential to contribute to reducing the environmental impact of animal agriculture. While plants produce much lower greenhouse gas emissions and require fewer resources than animals, structural changes to water-soluble proteins during extraction or drying can affect the protein's function and bioactivity (Munialo, 2024). Plant-based proteins are also often associated with a lack or imbalance of essential amino acids, reduced flavor and texture compared to animal proteins, and potential allergenicity, which can be a major barrier to consumers choosing plant-based protein products. Therefore, there is a need to address taste, nutritional value, and allergenicity to create meat alternatives using plant-based proteins.

In order to utilize plant proteins as a substitute for animal proteins, it is important to select a suitable extraction method based on the protein matrix. Protein extraction methods include dry, wet, enzymatic, sub- and supercritical water extraction, and reverse micelles extraction (Thakur et al., 2024). Dry methods utilize air and are energy efficient; however, they are known to be unsuitable for certain feedstocks due to their lipid content, which can lead to fractionation or cluster formation of impurities (Banjac et al., 2017). Wet methods can utilize different types of extraction solvents, and functional properties such as gelation, foaming, and emulsification can be applied to the extraction method. Methods that utilize enzymes to degrade proteins and cell walls/membranes require long processing times, high costs, and high energy consumption but can minimize environmental impact and yield high-quality samples (Gouseti et al., 2023; Sari et al., 2013). Subcritical-supercritical extraction does not require organic solvents and is a sustainable extraction method, with protein content increasing with increasing temperature (Knez et al., 2018). Finally, reverse extraction can be significantly affected by pH changes, electrostatic interactions, concentration and nature of the target protein, ionic strength, and reverse micelles composition (Zhao et al., 2018).

Physical, chemical, and biological methods can be used to improve the functionality of vegetable proteins to increase their utilization or incorporation into various products, especially by enhancing their taste, nutritional, and functional properties (Nasrabadi et al., 2021; Table 2). Nowacka et al. (2023) identified various chemical (glycosylation, deamidation, phosphorylation, acylation), physical (pulsed electric fields, ultrasound, high hydrostatic pressure, dynamic high-pressure treatment, cold plasma), and biological (fermentation, enzymatic modification) methods that are used to enhance the functional properties of proteins. They suggested that meat- and fish-like products can be produced by appropriately combining different production techniques and materials (Nowacka et al., 2023).

A growing body of studies is exploring the utilization of plant-derived proteins to develop meat substitutes by identifying their functional properties and analyzing their structure and texture. Plant-based protein products as substitutes for animal protein include plant-based meat, plant-based eggs, plant-based dairy products, and plant-based protein emulsion foods, which can be manufactured by techniques such as high-moisture extrusion, 3D printing, and electrospinning (Xiao et al., 2023). In a previous study, pea and rice were selected as low-allergenic materials analyzed for their functionalities in comparison to wheat and soybeans (Zhao et al., 2020). It was found that although the functionality of pea protein is similar to that of soybean protein and superior to rice protein, the excellent water absorption and emulsification properties of rice protein make it suitable as an alternative protein material. To evaluate the potential of banana floret (*Musa paradisiaca*) and jackfruit (*Artocarpus heterophyllus* Lam.) as meat substitutes, they were incorporated into a vegan sausage (Keerthana Priya et al., 2022). The developed vegan sausage was rich in fiber and protein. It showed good hardness, adhesion, chewability, and elasticity, improving the texture properties and overall palatability, which was similar to commercial chicken sausage. Oat fiber concentrate and pea protein isolate were processed using a high-moisture extrusion method to prepare fibrous meat substitutes with 33% dietary fiber content (Ramos Diaz et al., 2022). An evaluation of the mechanical and physicochemical properties of the meat substitutes showed that the structure became softer with increasing dietary fiber, the high-cooling temperature strengthened the fiber structure, and the extractability and viscosity of β-glucan were well maintained using a human digestion model. In another example, a biopolymer composite with a meat-like texture was fabricated by complex aggregation of gellan gum, a polysaccharide, and potato protein, and the electrical characterization, microstructure analysis, dynamic shear flow, and texture of the composite were analyzed (Hu et al., 2024). At pH 4, excessive air bubbles formed after heating, resulting in a spongy gel with relatively low hardness. In contrast, at pH 6, a fibrous structure with the highest hardness and elasticity was formed after heating. Additionally, in the high-moisture extrusion process, disulfide bonds significantly contribute to the fibrous texture of meat analogs, largely due to the important role of wheat gluten (Chiang et al., 2019). By employing a Shear Cell or a lab-scale Couette Cell, highly anisotropic fibrous samples can be produced under processing conditions. Couette cell is preferred for its scalability and potential for future continuous operation, which facilitates the production with uniform thickness, while the Shear Cell is more suitable for laboratory-scale applications (Krintiras et al., 2016). Therefore, it was suggested that composite aggregation and thermal coagulation will help in the formation of plant-based products with meat-like texture and structure.

Plant-based protein products are important not only for vegetarians but also for those who want to eat a low-fat, high-fiber diet to reduce the risk of disease and decrease their intake of calories. A study by Meixner et al. (2024) examined where plant-based protein products provide the highest value utility in each food market and how much Generation Z consumers are willing to pay for them. The most important attributes of plant-based protein products were origin, price, and vegan, in that order, with domestic and European products being more positively valued than third-country imports. To increase consumer awareness of plant-based protein products, the first step is to create a favorable product. The selection of optimal plant-derived ingredients and processing technologies that can improve the functional properties of high-quality proteins is crucial. It will contribute significantly to the growth of environmentally friendly, nutritionally rich, sustainable meat substitutes.

Conclusion

Various alternative protein foods are being developed to replace traditional animal products. The current state-of-the-art technologies for producing alternative protein foods and the potential for industrialization of meat substitutes are summarized as bellows;

- Technologies related to cultured meat, such as serum-free media development, continue to improve. Technologies for improving culture efficiency are being researched, along with technologies for improving taste and flavor, but production efficiency has not yet reached industrialization.
- The transition from using primary muscle stem cells to cell lines or immortalized cell lines holds promise for improving production efficiency. There are few commercialized products of cultured meat despite the ongoing development of technology, and industrialization is expected to take some time. Despite the hurdles, the continuous technological improvements suggest a promising future for cultured meat.
- Future challenges include enhancing the texture to match traditional meat products and overcoming cultural resistance to insect consumption. Continued innovation in processing technologies could lead to broader application in diverse food products, thereby expanding consumer acceptance.
- Plant-derived protein products are also being researched to improve flavor and texture to be comparable with traditional animal products and to eliminate allergens.
- Ongoing research aims to address these issues to make plant-based products more appealing to consumers. The market potential for plant-based proteins is significant, with research efforts increasingly yielding tangible results.

In conclusion, while the tangible market formation for these alternative protein technologies has not met initial expectations, the diverse array of ongoing research activities and technological innovations suggests a high potential for gradual market expansion. Continued interdisciplinary collaboration and innovation are essential to overcome the existing challenges and fully realize the potential of these alternative protein sources.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the High Value-added Food Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (322008-5). This research was also supported by the Chung-Ang University Graduated Research Scholarship in 2023.

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Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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