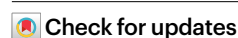


Stem cell-based strategies and challenges for production of cultivated meat

Received: 1 February 2023

Accepted: 5 September 2023

Published online: 16 October 2023



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Cultivated meat scale-up and industrial production will require multiple stable cell lines from different species to recreate the organoleptic and nutritional properties of meat from livestock. In this Review, we explore the potential of stem cells to create the major cellular components of cultivated meat. By using developments in the fields of tissue engineering and biomedicine, we explore the advantages and disadvantages of strategies involving primary adult and pluripotent stem cells for generating cell sources that can be grown at scale. These myogenic, adipogenic or extracellular matrix-producing adult stem cells as well as embryonic or inducible pluripotent stem cells are discussed for their proliferative and differentiation capacity, necessary for cultivated meat. We examine the challenges for industrial scale-up, including differentiation and culture protocols, as well as genetic modification options for stem cell immortalization and controlled differentiation. Finally, we discuss stem cell-related safety and regulatory challenges for bringing cultivated meat to the marketplace.

Skeletal muscle tissue contains a milieu of cells, but fundamental to meat are myofibres as the dominant component and rich in protein, adipocytes providing both flavour and tenderness¹, and fibroblasts creating the extracellular matrix (ECM) that contributes to texture². Both myofibres and adipocytes are post-mitotic, requiring progenitor or stem cells to expand cell numbers in culture. While early products have been developed with undifferentiated fibroblasts, in this Review, we focus on stem cells that have the potential to create the major cellular components of meat (Fig. 1).

Adult stem cells are the most readily available cells capable of expansion and are already tuned to proceed down a path of differentiation towards a given fate. For myofibres, the adult stem cells would be muscle stem cells (MuSCs)³. Adipogenic progenitors are present in various locations of the body, including adipose-derived stem cells (ADSCs) from fat⁴ or fibro-adipogenic progenitors (FAP) from muscle⁵. Alternatively, pluripotent stem cells (PSCs) can be differentiated into any cell type relevant for cultivated meat, offering a virtually infinite cellular source given their self-renewal capabilities⁶. In addition, genetic engineering approaches can endow stem cells with both prolific

expansion capacity and greater maturation. In this Review, we examine the advantages and disadvantages of each of these strategies as well as current efforts that will define the industrial challenges of bringing cultivated meat to the marketplace.

Primary adult stem cells

Mature skeletal muscle consists of adult stem cells that undergo expansion followed by differentiation. However, their capacity for growth is constrained to only 30–50 divisions by the Hayflick limit (that is, telomere shortening)⁷. Therefore, cultivated meat production would require the continual reseeded of production processes with adult stem cells.

Muscle stem cells and satellite cells

Skeletal muscle consists of myofibres, representing approximately 90% of muscle mass⁸, containing myofibrillar proteins that provide the bulk of nutrient protein⁹, including essential amino acids, iron and other minerals, and vitamins (A, E and B), as well as stores of fatty acids and glycogen. Stem cell-derived myofibres would most readily

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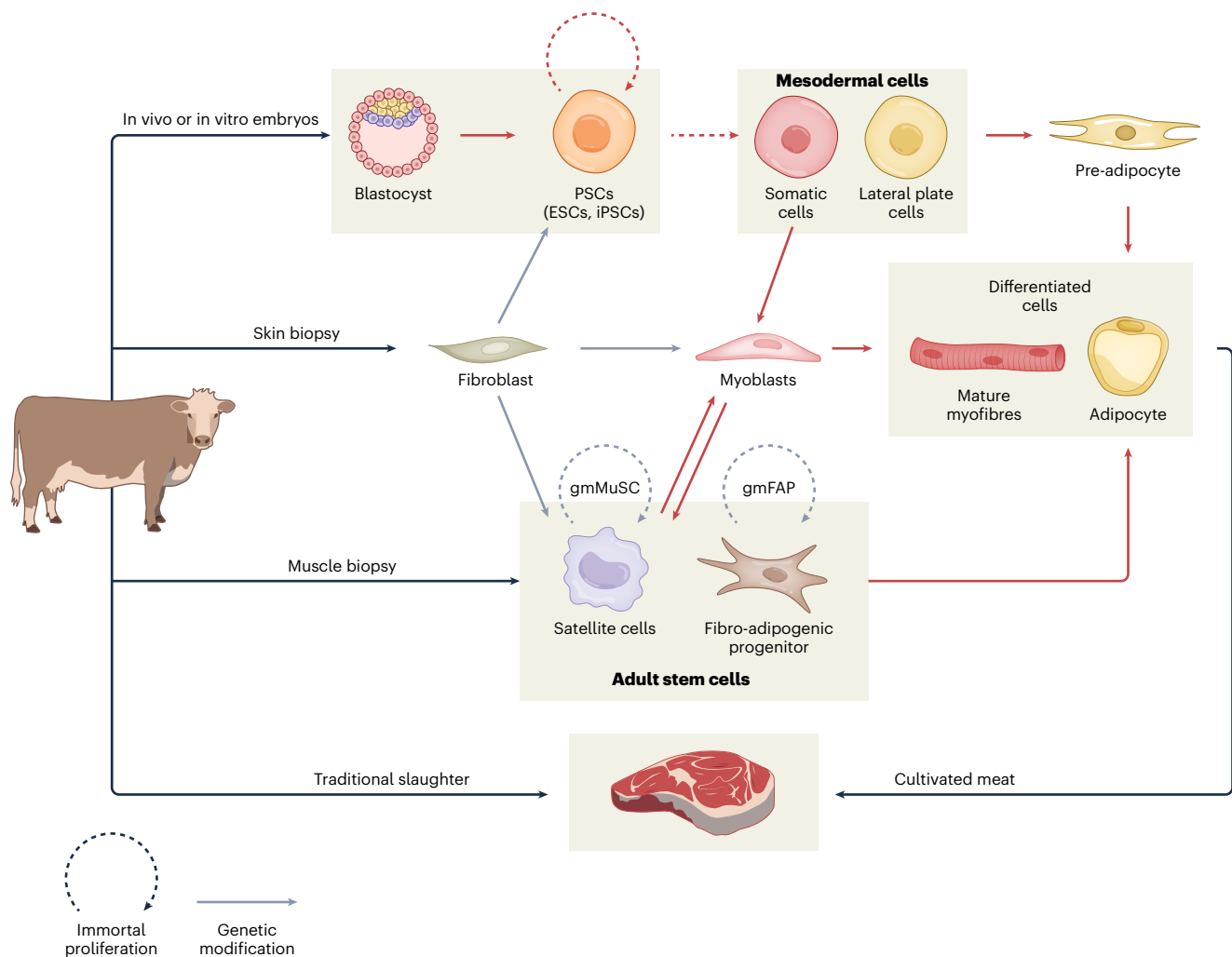


Fig. 1 | Cell sources and differentiation pathways necessary to produce cultivated meat. The obtention of myogenic and adipogenic cells from PSCs (either from embryos (ESCs) or from reprogrammed somatic cells that are induced (iPSCs)) requires more steps (indicated by the dashed arrow) than the differentiation of these cells from their progenitor satellite cells or fibro-adipogenic progenitor cells. Black arrows indicate human handling of cells.

Red arrows indicate in vivo development or reagent-mediated in vitro differentiation. Grey arrows indicate genetic modification. The circular dashed grey arrows indicate genetic modification to immortalize cell populations. gmMuSC, genetically modified muscle stem cell; gmFAP, genetically modified fibro-adipogenic progenitor.

replicate traditional meat, although the nutritional quality of these cells compared with traditional meat is still largely unknown¹⁰.

The MuSC or satellite cell is an adult stem cell resident on the periphery of muscle fibres and is responsible for creating new myonuclei in adult muscle. MuSCs are marked by the expression of transcription factor paired box 7 (PAX7) in a quiescent state (Fig. 2). Upon activation to myoblasts, myogenic regulatory factors control proliferation, eventual differentiation and fusion. Protocols for isolating and culturing MuSCs in mice and humans have long been established, with similar techniques being viable for species relevant to cultivated meat, including bovine¹¹, porcine¹² and chicken¹³ (Table 1). Cryopreservation techniques can preserve MuSC functionality, necessary for creating cell banks¹⁴. Isolating and seeding MuSCs from mature muscle for cultivated meat could involve substantial heterogeneity. MuSC expansion and differentiation properties may vary within species, for example, it has recently been reported that Belgian Blue and Limousin cattle breeds maintain differentiation capacity longer than other breeds¹⁵. Specific muscle types impact the number of MuSCs available, with psoas major and extensor carpi radialis providing the greatest number of MuSCs among nine porcine

muscles¹⁶. Donor animal age can detrimentally impact the percentage of MuSCs among mononuclear cells¹⁷ and the expansion capacity of MuSCs as they will have gone through additional cell cycles in vivo. In mammals, males typically have larger muscles and testosterone can regulate the number of MuSCs present in boars¹⁸. With these sources of variation in mind, further research is needed to determine the optimal animal sources for primary MuSCs.

Adipogenic stem cells and adipogenic precursors

Along with muscle protein, fat content is a critical component of meat quality¹⁹. While muscle cells can store fat, adipocytes form intramuscular fat, which makes up approximately 80% of the fat in meat²⁰ and correlates with the rating of taste, texture, juiciness and visual appearance of meat²¹. Cultivated meat products will need to emulate the palatability attributes of conventional meat fat to be competitive on the market and, in contrast to plant-based protein alternatives, cultivated meat can potentially render similar fat profiles to animal meat by using animal stem cells²² rather than mimicking animal-based fats. The direct addition of an exogenous fatty acid such as oleic acid via the culture media may enhance the palatability and

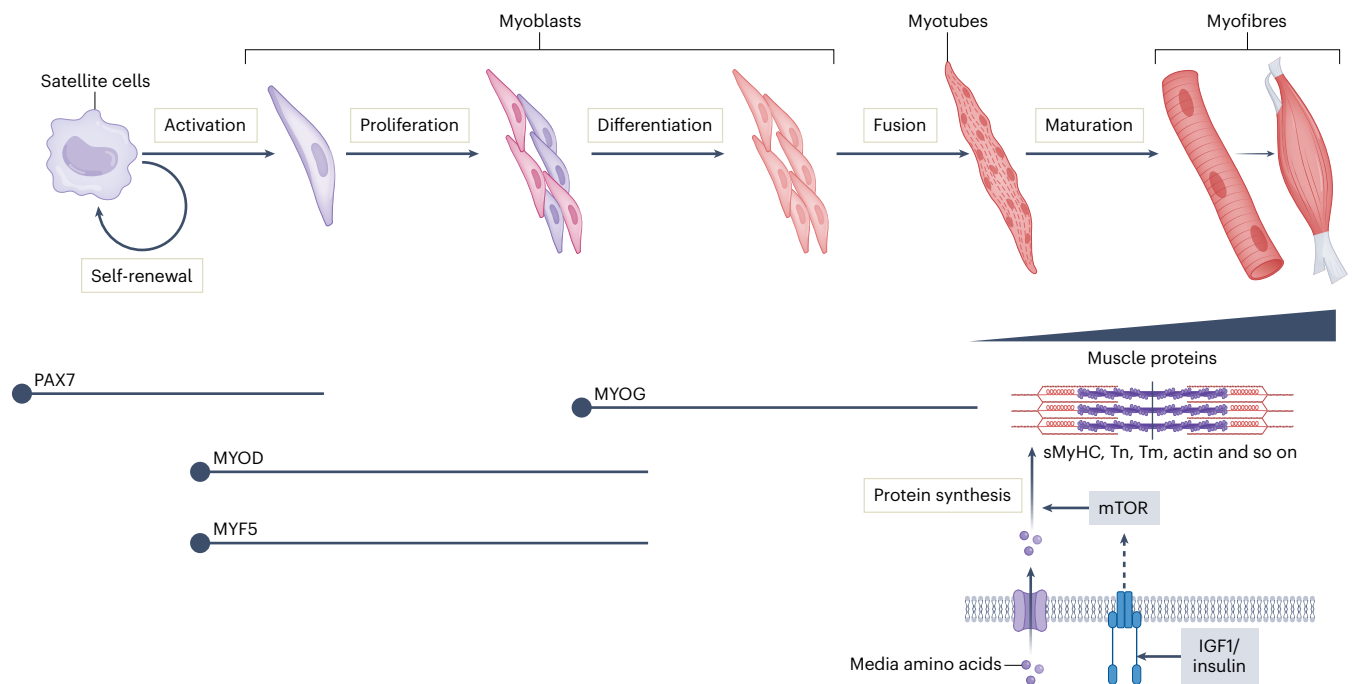


Fig. 2 | Brief summary of differentiation (myogenesis) and maturation (with protein synthesis) of myocytes starting with satellite cells (MuSCs). The canonical satellite cell marker is PAX7, but upon activation PAX7 wanes and early myogenic markers of myoblast determination protein 1 (MYOD) and myogenic factor 5 (MYF5) serve as transcription factors to promote myogenesis and proliferation. Later in differentiation, myogenin (MYOG) becomes a key transcription factor for terminal differentiation and fusion of myoblasts into

myotubes. Muscle proteins, predominantly contractile proteins such as skeletal muscle myosin heavy chain (sMyHC), troponin (Tn), tropomyosin (Tm) and actin, are expressed during maturation into a myofibre. Amino acids from the media undergo protein synthesis to make muscle proteins, which is controlled by media insulin-like growth factor 1 (IGF1) or insulin activating mammalian target of rapamycin (mTOR) signalling for protein synthesis.

health benefits of cultivated fat (Table 2). Moreover, a considerable percentage of the polyunsaturated fatty acids from the phospholipid bilayers of cultivated fat may offer an adequate supply of the essential polyunsaturated fatty acids required for the maintenance of brain and immune system health²³.

Mature adipocytes can be derived from several sources, including mesenchymal stem cells (MSCs), FAPs or ADSCs⁴. FAPs and ADSCs have similar stem cell potential to MSCs, while differing in their anatomical location. Specifically, MSCs are located in bone marrow, while FAPs reside in the perimysium and ADSCs reside in adipose tissue. MSCs are among the most widely studied stem cells, partially due to their capacity to undergo adipogenesis²⁴, and are typically isolated by incubating bone marrow on culture flasks or dishes and expanding the resulting adherent cells²⁵. By contrast to MSCs, both FAPs and ADSCs are isolated via enzymatic (commonly type II collagenase) digestion of muscle or fat tissue and subsequent centrifugation. The cell pellet retrieved after centrifugation is identified as the stromal vascular fraction comprising preadipocytes (FAPs and ADSCs) and somatic cells such as immunocytes and pericytes, and the purity of the preadipocytes is enhanced by subsequent expansion or fluorescence-activated cell sorting (FACS) using cell surface markers of adipogenic precursors (for example, Lin⁺, Sca⁺, CD31⁺, CD34⁺, PDGF- α ⁺, CD29⁺ and CD117⁺)^{23,26}.

The adipogenic potential of preadipocytes can be assessed by monitoring changes in transcription factor expression and cell cycle progression. From the multipotent state of MSCs, FAPs and ADSCs, the transcription factor Zfp423 plays a critical role in early differentiation to preadipocytes²⁷. In FAPs from bovine muscle, Zfp423 has been shown to mark highly adipogenic FAPs with overexpression leading to a dramatic increase in adipogenic differentiation²⁸. Following the transition to the preadipogenic state, the nuclear hormone receptor peroxisome proliferator activating receptor gamma

(PPAR- γ) is an essential regulator of adipogenesis²⁹. PPAR- γ interacts with the transcription factors of the CCAAT/enhancer-binding protein (C/EBP) family to activate the adipogenic transcriptional programme³⁰ (Fig. 3). The expression of the C/EBP family is higher in the extensively marbled Wagyu steers than in the less marbled Holstein breed³¹. The mature adipocyte maintains PPAR- γ expression, which is often used as an adipogenic marker along with the lipogenic genes such as fatty acid synthase (FAS), fatty acid binding protein 4 (FABP4) and perilipin³² (Fig. 3). While cells undergoing adipogenesis often proceed through the cell cycle, once they are mature, the adipocytes enter growth arrest³³.

Prudent resource use could support sustainable cultivated meat production. Unlike MSCs, FAPs can be isolated from muscle tissue alongside MuSCs, and ADSCs could be sourced tissues that are highly accessible and usually discarded in traditional meat production. Furthermore, while most research into ADSCs has been directed towards regenerative medicine³⁴, they could be well suited to cultivated meat as porcine ADSCs have shown multipotency as well as strong adipogenic potential³⁵. However, the limited expansion capacity of adult primary stem cells compared with cell lines remains a drawback. De-differentiation of mature adipocytes is another option for sourcing adipogenic progenitors as they can be isolated by attaching mature adipocytes on the ceiling of full media-loaded culture flasks using their buoyancy and incubated for 1–2 weeks³⁶. De-differentiated adipocytes readily re-differentiate into multiple cell fates such as adipocytes, osteoblasts, myocytes and chondrocytes, although they are less plastic than other stem cells such as MSCs or embryonic stem cells³⁷. Compared with adipogenic precursors from the stromal vascular fraction, de-differentiated mature adipocytes typically exhibit higher homogeneity and adipogenic potential in vitro^{37,38}.

Table 1 | Methods for muscle differentiation using different cell types and animal models

Animal and cell type	Differentiation media	Proliferation media	Reference
Bovine satellite cells	DMEM-F12, 0.5 mg ml ⁻¹ BSA, 0.1 nM dexamethasone, 100 µg ml ⁻¹ transferrin, 0.5 µg ml ⁻¹ linoleic acid, 1 µM insulin, 1 µM cytosine arabinoside	DMEM-F12, 10% FBS, 1 µM insulin	11
Porcine satellite cells	DMEM, 10% FBS, DMEM, 2% HS	High-glucose DMEM, 20% FBS, 100 U ml ⁻¹ penicillin, 100 µg ml ⁻¹ streptomycin	12
Avian satellite cells	MEM, 10% HS, 5% embryo extract, 10 ⁵ U l ⁻¹ penicillin and streptomycin, 2.5 mg l ⁻¹ amphotericin B, 5.0 mg l ⁻¹ gentamicin	MEM, 25% HS	13
Bovine satellite cells	DMEM (1 g l ⁻¹ glucose), 2% FBS	DMEM-F12, 1% ITS-X, 1% L-glutamine, 5 mg ml ⁻¹ human serum albumin, 50 µg ml ⁻¹ L-ascorbic acid, 36 ng ml ⁻¹ hydrocortisone, 20 ng ml ⁻¹ human interleukin-6, 1 µg ml ⁻¹ alpha linolenic acid, 10 ng ml ⁻¹ FGF2, 10 ng ml ⁻¹ VEGF, 100 ng ml ⁻¹ IGF1, 5 ng ml ⁻¹ HGF, 10 ng ml ⁻¹ PDGF-BB	47
Bovine satellite cells	Neurobasal and L15 basal media (1:1), 1% antibiotic-antimycotic, 10 ng ml ⁻¹ IGF1, 100 ng ml ⁻¹ EGF	DMEM-F12, 200 µg ml ⁻¹ 2-phospho-L-ascorbic acid, 20 µg ml ⁻¹ insulin, 20 µg ml ⁻¹ transferrin, 20 µg ml ⁻¹ sodium selenite, 40 ng ml ⁻¹ FGF2, 0.1 ng ml ⁻¹ neuregulin, 0.1 ng ml ⁻¹ TGFβ3, 6.4 mg ml ⁻¹ rAlbumin	154
Bovine satellite cells	DMEM, 2% FBS	F10 medium, 20% FBS, 5 ng ml ⁻¹ bFGF, 1% penicillin-streptomycin; additional p38i and DMSO	45
Bovine satellite cells	DMEM, 5% FBS, 1 mM sodium pyruvate, 1× antibiotics 0.1 mg ml ⁻¹ gentamicin, 100 U ml ⁻¹ penicillin and 0.1 mg ml ⁻¹ streptomycin, 2.5 µg ml ⁻¹ amphotericin B	DMEM with 10% FBS, 10% HS, 1 mM sodium pyruvate and 1× antibiotics: 0.1 mg ml ⁻¹ gentamicin, 100 U ml ⁻¹ penicillin and 0.1 mg ml ⁻¹ streptomycin, 2.5 µg ml ⁻¹ amphotericin B	155
Porcine satellite cells	DMEM, 2% HS or 0.4% Ultrosor G serum substitute	F10 medium, 15% FBS, 5 ng ml ⁻¹ FGF, 1% penicillin-streptomycin	16
Porcine satellite cells	DMEM containing 2% HS, 1% penicillin-streptomycin	F10 medium containing 20% FBS, 5 ng ml ⁻¹ bFGF, 1% penicillin-streptomycin; where indicated 100 µM L-ascorbic acid 2-phosphate	156
Murine satellite cells	DMEM, 2% HS	F10-DMEM (50:50), 15% FBS, 2.5 ng ml ⁻¹ bFGF	157
Human satellite cells	High-glucose DMEM, 20% FBS, 1% penicillin-streptomycin, 10 µM rho associated protein kinase inhibitor	High-glucose DMEM, 30% FBS	158
Avian, bovine and porcine satellite cells	Avian: high-glucose DMEM, 10% HS, 4% chick embryo extract, 1% penicillin-streptomycin Bovine: high-glucose DMEM supplemented with 20% FCS and 1% penicillin-streptomycin Porcine: DMEM containing 0.4% Ultrosor G serum substitute, 1% penicillin-streptomycin	Avian: high-glucose DMEM, 10% HS, 4% chick embryo extract, 1% penicillin-streptomycin Bovine: high-glucose DMEM supplemented with 20% FCS and 1% penicillin-streptomycin Porcine: SkBM-2, SkBM-2 SingleQuots kit	159
Porcine and bovine embryonic stem cells	DMEM-F12, 1% ITS, 1% non-essential amino acids, 0.2% penicillin-streptomycin, 3 µM CHIR99021, 0.5 µM LDN193189 (20 ng ml ⁻¹ FGF) DMEM-F12, 1% ITS, 1% non-essential amino acids, 0.2% penicillin-streptomycin, 15% KSR, 0.5 µM LDN193189, 0.1 mM 2-mercaptoethanol, 10 ng ml ⁻¹ HGF, 2 ng ml ⁻¹ IGF1, 20 ng ml ⁻¹ FGF	DMEM-F12, 1% ITS, 1% non-essential amino acids, 0.2% penicillin-streptomycin, 15% KSR, 0.5 µM LDN193189, 0.1 mM 2-mercaptoethanol, 10 ng ml ⁻¹ HGF, 2 ng ml ⁻¹ IGF1 DMEM-F12, 1% ITS, 1% N2 supplement, 0.2% penicillin-streptomycin, 1% L-glutamine	57
Human and murine embryonic stem cells	Human: E6 medium: 543 µg ml ⁻¹ NaHCO ₃ , 64 µg ml ⁻¹ ascorbic acid, 19.4 µg ml ⁻¹ insulin, 10.7 µg ml ⁻¹ transferrin, 0.014 µg ml ⁻¹ sodium selenite, 50 µg ml ⁻¹ gentamicin, (E8 only: 100 ng ml ⁻¹ FGF2, 2 ng ml ⁻¹ TGFβ1), 0.1% CHIR99021 or BMP4 and INHBA, 20 µM Forskolin and 10 ng ml ⁻¹ FGF2, 0.5 or 10 µM CHIR99021 Mouse: 75% IMDM, 25% DMEM-F12, 1% B27 supplement (without retinoic acid) supplement, 1% penicillin-streptomycin, 0.5% BSA, 0.5% N2 supplement, 0.45 mM monothioglycerol, 50 µg ml ⁻¹ ascorbic acid, 5 ng ml ⁻¹ VEGF, 0.1% CHIR99021 or BMP4 and INHBA	Human: DMEM-F12, 1% N2 supplement, 1% ITS, 5 µg ml ⁻¹ gentamicin Mouse: DMEM-F12, 1% N2 supplement, 1% penicillin-streptomycin	88

DMEM, Dulbecco's modified Eagle medium; MEM, Eagle's minimal medium; SkMB-2, skeletal muscle basal medium-2; IMDM, Iscove's modified Dulbecco's medium; HS, horse serum; BSA, bovine serum albumin; FCS, fetal calf serum; FBS, fetal bovine serum; DMSO, dimethylsulfoxide; ITS-X, insulin-transferrin-selenium-ethanolamine; ITS, insulin-transferrin-selenium; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; PDGF-BB, platelet-derived growth factor two B subunits; TGFβ(1/3), transforming growth factor beta; bFGF, basic fibroblast growth factor; INHBA, inhibin beta A.

ECM-producing cells

Connective tissue or ECM makes up approximately 10% of muscle dry mass but varies considerably by muscle and species⁹. The collagen-rich ECM provides dietary protein and establishes muscle stiffness and meat tenderness³⁹. Fibroblasts are primarily responsible for secreting and organizing the ECM in muscle⁴⁰ and have a short doubling time in vitro. In muscle, fibroblasts may also be called FAPs⁴¹ and can extend to a broader category of MSCs as well⁴². FAPs can be isolated from skeletal

muscle, so can be acquired in conjunction with MuSCs¹⁴, but there are many other sources of fibroblast cells. For example, skin contains abundant fibroblasts with bovine dermal fibroblasts typically taken at embryonic stages⁴³. Thus, fibroblasts may be an initial choice of cell type for cultivated meat for their ease of use, but they are unable to create muscle. As cultivated meat products advance to mimic muscle fibroblast-like cells can both enhance myogenesis of other cells and dictate meat tenderness.

Table 2 | Methods for adipose tissue differentiation using different cell types and animal models

Animal and cell type	Differentiation media and duration	Maturation media and duration	Reference
Bovine de-differentiated fat cells	DMEM supplemented with 0.25% FBS, 25 µM dexamethasone, 0.5 mM IBMX, 5 µg ml ⁻¹ insulin, volatile fatty acid test treatments	DMEM supplemented with 2.5% FBS and volatile fatty acid test treatments	37
Bovine adipose- derived stem cells	DMEM with 10% FBS, 1% AB-AM, free fatty acid treatments (concentration not mentioned)	No specific maturation stage	108
Bovine adipose- derived stem cells	DMEM with 5% FCS, 2× AB-AM, 2.5 µg ml ⁻¹ insulin, 0.5 mM IBMX, 0.25 µM dexamethasone, 5 µM troglitazone, 10 mM acetate, 2 days	DMEM, 5% FCS, 2× AB-AM, 2.5 µg ml ⁻¹ insulin, 5 µM troglitazone, 10 mM acetate with fatty acid treatments, 4 days	160
Bovine adipose- derived stem cells	DMEM with 5% FBS, antimicrobials, 0.5 mM IBMX, 0.25 µM dexamethasone, 2.5 µg ml ⁻¹ insulin, 5 µM troglitazone, 2 days	DMEM with 5% FBS, 2.5 µg ml ⁻¹ insulin, 5 µM troglitazone, 6 days	161
Porcine de-differentiated fat cells	DMEM-F12 supplemented with 2% or 20% FBS, 0 or 500 µg ml ⁻¹ intralipid, 100 µg ml ⁻¹ AB-AM, 0.5 µM dexamethasone, 0.5 mM IBMX, 5 µM rosiglitazone, 2 mM (1X) GlutaMAX, 20 µM biotin, 10 µM calcium D-pantothenate	DMEM-F12 supplemented with 2% or 20% FBS, 0 or 500 µg ml ⁻¹ intralipid, 100 µg ml ⁻¹ Primocin, 0.5 µM dexamethasone, 5 µM rosiglitazone, 2 mM (1X) GlutaMAX, 20 µM biotin, 10 µM calcium D-pantothenate	162
Porcine adipose- derived stem cells	DMEM with 10% FBS, 0.5 mM IBMX, 1 µM dexamethasone, 5 µg ml ⁻¹ insulin, 2 days	DMEM with 10% FBS, 5 µg ml ⁻¹ insulin, 2 days; DMEM with 10% FBS, 4–6 days	163
Porcine adipose- derived stem cells	DMEM with antibiotics, 850 nM insulin, 10 nM dexamethasone, 2 nM triiodothyronine, 1, 3 or 6 days	No specific maturation stage	164
Murine 3T3-L1 cell line	High-glucose DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, 10 µg ml ⁻¹ insulin, 0.1 µM dexamethasone, 0.5 mM IBMX, 2 days	High-glucose DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, 10 µg ml ⁻¹ insulin with fatty acid treatments, 5 days	165
Murine FACS-isolated preadipocytes	DMEM with 10% FBS, 10 ng ml ⁻¹ bFGF, 1 µg ml ⁻¹ insulin, 0.25 µg ml ⁻¹ dexamethasone, 0.5 mM IBMX, 3 days	DMEM with 10% FBS, 9 days	166
Human adipose- derived stem cells	DMEM with 250 nM dexamethasone, 0.5 mM IBMX, 2 µM rosiglitazone, 10 µg ml ⁻¹ insulin, day 0–3, day 5–7, day 8–10	DMEM with 10 µg ml ⁻¹ insulin, day 3–5, day 7–8	167
Human adipose- derived stem cells	DMEM-F12 (1:1) with 100 U ml ⁻¹ penicillin, 100 mg ml ⁻¹ streptomycin, 66 nM insulin, 100 nM dexamethasone, 0.5 mM IBMX, 0.1 mg ml ⁻¹ pioglitazone, 1 nM triiodo-L-thyronine, 10 mg ml ⁻¹ human transferrin, 5 days	DMEM-F12 (1:1) with 100 U ml ⁻¹ penicillin, 100 mg ml ⁻¹ streptomycin, 66 nM insulin, 100 nM dexamethasone, 1 nM triiodo-L-thyronine, 10 mg ml ⁻¹ human transferrin, 9 days	168

IBMX, 3-isobutyl-1-methylxanthine; AB-AM, antibiotics-antimycotics.

Industrial use of adult stem cells

Using adult stem cells requires dependence on traditional agriculture as a source of these cells. However, the theoretical yield from stem cells from meat is many factors larger than the original meat, for example, a 500 mg bovine muscle biopsy could yield approximately 5,000 kg of cultivated beef⁴⁴. This estimation assumed 35 doublings, a factor that exponentially impacts the yield. However, primary bovine MuSCs typically decrease in differentiation efficiency after approximately 13 doublings; by roughly 25 doublings, differentiation efficiency has decreased to 20% (ref. 45), which equates to approximately 1 kg if only differentiated cells are used. However, if culture conditions were modified to approach the upper end of the Hayflick limit, that is, 45 doublings were achieved while maintaining efficient differentiation, the yield would approach 5,000,000 kg. For example, inhibition of the p38 pathway substantially enhances differentiation at 25 doublings⁴⁵. However, cell utility after so many passages can result in the expansion of impurities. For example, in a three-dimensional culture system, fibroblasts and FAPs proliferated more rapidly than MuSCs in co-culture, with the myogenic cell population increasing from >60% at day 1 to approximately 25% by day 16 and <5% by day 50 (ref. 46). Achieving approximately 40 doublings while maintaining efficient differentiation would make adult stem cells highly viable for cultivated meat.

Many companies are pursuing adult stem cell culture for cultivated meat production. Mosa Meat primarily targets adult primary stem cells and has contributed to the literature, particularly on bovine cells^{23,44,47}. Many companies have been less active in publishing their work but advertise the use of stem cells free from genetic modification. Steakholder Foods uses three-dimensional bioprinting to create

structured steak-like products from primary bovine cells. Aquatic species have garnered substantial interest due to their attributes, including tolerance of hypoxia and low-temperature culture conditions^{48,49}. BlueNalu has filed a patent application concerning methods to enhance primary adipocyte viability and differentiation while also increasing lipid uptake⁵⁰. Mission Barns is developing cultivated fat, particularly porcine, from primary preadipocytes initially as a food additive⁵¹. Companies are targeting seafood using stem cells from adult salmon (Wildtype Foods)⁵² or shrimp, crab and lobster (Shiok Meats)⁵³. Notably, in November 2022, Opo Bio announced the commercial availability of primary bovine MuSCs to support research in the area, with fibroblasts and preadipocytes available soon⁵⁴. Providing established adult stem cell lines can focus resources towards overcoming the limitations of adult stem cells in scale-up.

Pluripotent stem cells

PSCs are cells that possess the capacity to indefinitely self-renew while having the ability to differentiate into most cell types of an organism. Embryonic stem cells (ESCs) can be derived from the inner cell mass of blastocysts and stably cultured in defined media. PSCs have been established for longest with mouse⁵⁵ and human⁵⁶ PSCs studied in most detail. Very recently, the field of PSC research in agricultural species has undergone substantial advances, greatly expanding the possibilities for cellular agriculture. In 2018, bovine ESCs were derived and cultured⁶, followed by others that were collected at different embryonic stages and furthermore, feeder-free culture conditions were also established^{57,58}. Stable ESCs have been reported for pigs^{57,59} and sheep⁶⁰. PSCs have also been generated from somatic cells by cellular reprogramming and activation of critical genes that promote pluripotency, namely,

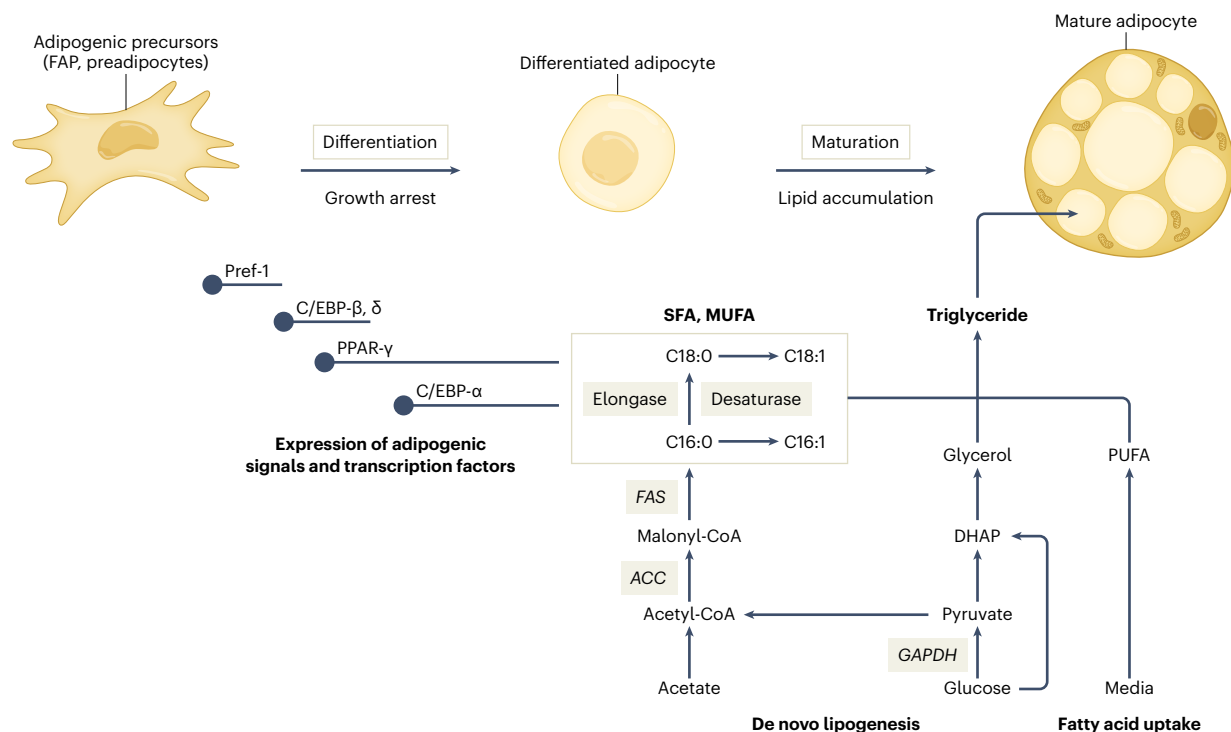


Fig. 3 | Brief summary of differentiation (adipogenesis) and maturation (lipogenesis) starting with adipogenic precursor cells. At the beginning of differentiation, the expression of preadipocyte factor 1 (Pref-1) decreases and the expression of adipogenic signals such as C/EBP-β and C/EBP-δ and transcription factors such as PPAR-γ and C/EBP-α increases (left part of the figure). During maturation, elevated transcription factors stimulate the expression of glycolytic

and lipogenic genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS), fatty acid elongase, Δ9 desaturase and fatty acid binding protein (FABP) to accumulate saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) from de novo lipogenesis and fatty acid uptake.

Oct3/4, *Sox2*, *Klf4* and *c-Myc* (known as the Yamanaka factors)⁶¹, and are termed induced PSCs (iPSCs). These have received considerable attention in the fields of human research due to their potential use in cell-based therapies and regenerative medicine⁶². Induced PSCs have been described in cattle⁶³ and pigs⁶⁴.

Compared with multipotent adult stem cells such as satellite cells or FAPS, PSCs have two main advantages for the development of cultivated meat technologies. First, they are a single cell source with the potential to generate the three main components of meat: muscle, adipose and connective tissue. Second, the unlimited self-renewal of PSCs could allow for the creation of cell banks and eventually eliminate the need for animal tissue biopsies as the source material.

ESC availability in agriculturally relevant species

ESCs were first derived from mouse embryos (mESCs) in 1981⁵⁵ and from human embryos (hESCs) in 1998⁵⁶. Although embryos of different species share similarities in the early stages of development and all ESCs are derived from the inner cell mass of a blastocyst-stage embryo, the pluripotency state of mESCs is different from that of hESCs. Human ESCs resemble the morphology and molecular signature of post-implantation epiblast-derived mouse stem cells (EpiSCs)⁶⁵. EpiSCs and hPSCs, although still pluripotent, are considered 'primed' for differentiation, whereas mESCs are classified as being in a 'naïve' state of pluripotency. Naïve cells can contribute to chimaeric animals when injected into a blastocyst, while primed ESCs are more susceptible to differentiation, which may be an advantage when attempting the differentiation of specific cell types^{6,66}. Naïve and primed ESCs require distinct signalling pathways that must be active for maintaining pluripotency and cell renewal. This is of critical relevance when devising adequate culture conditions for the maintenance, proliferation and targeted differentiation of ESCs.

The establishment of domestic animal ESCs has been challenging despite the year-long efforts of scientists around the world. The derivation of stable bovine ESCs (bESCs) was first described in 2018⁶; the cells were derived from pre-implantation blastocysts using conditions suitable for both hESCs and mouse EpiSCs. More recently, a simplified, serum-free culture system for bESCs allowed for feeder-free cultures (that is, without the need of culturing in the presence of mouse fibroblasts⁵⁸). Bovine ESCs have been cultured for more than 40 passages (equivalent to approximately 120 doublings with an average doubling time of 36 h), maintaining pluripotency, consistent self-renewal and a stable karyotype.

The establishment of ESCs from sheep, pig and other agricultural species has been described, but they have been less studied so far. Vilarino et al. described the establishment and maintenance of sheep ESCs for over 40 passages with a stable karyotype and morphology⁶⁰. Although the doubling time was not provided in their report, it is estimated to be similar to that of bESCs. The establishment of stable porcine ESCs in 2019⁶⁷ were followed by improvements to culture conditions to reach long-term viable cell maintenance⁵⁹. Porcine ESCs have been established from embryonic discs—a pre-gastrulation embryonic stage that is a few days further along the developmental path than the blastocyst⁵⁷. These embryonic disc ESCs sustained stable self-renewal, expression of pluripotency markers and readily originated different cell lineages upon stimulation.

iPSC methods of induction

The other route to generating PSCs is to induce pluripotency in a somatic cell. Induction of pluripotency was first achieved in mouse embryonic fibroblasts using a retroviral transduction to insert *Oct3/4*, *Sox2*, *Klf4* and *c-Myc*, the core transcription factors responsible for the maintenance of pluripotency⁶¹. Following the success in promoting

pluripotency in murine cells, the induction protocol based on the expression of these four transcription factors was applied to human cells with similar results⁶⁸. In addition to retroviral transduction, there are other methods of genetic modification, including the use of small molecules⁶⁹, lentiviral induction⁷⁰, adenoviral induction⁷¹, plasmid induction⁷², transposon-mediated reprogramming through the piggyBac system⁷³ and the direct use of proteins to reprogramme cells⁷⁴. Each method has benefits and drawbacks, for example, the transfection approach may lead to unwanted mutations and potential gene disruption resulting from the transfection insert, issues that could be circumvented using the clustered regularly interspaced short palindromic repeats (CRISPR) approach, which may be less prone to causing off-target effects⁷⁵. Bovine iPSCs (biPSCs) have been achieved through lentiviral transduction⁷⁶, transposon reprogramming⁷⁷ and somatic cell nuclear transfer⁷⁸. In two independent reports, biPSCs obtained via lentiviral transduction were described as being in a naïve state of pluripotency due to successful chimaeric contributions to blastocyst-stage embryos⁶³. Similar iPSCs have been induced from porcine cells (piPSC) through lentiviral transduction⁶⁴, non-integrated vectors⁷⁹ and small-molecule induction⁸⁰. The profile of piPSCs was initially thought to resemble that of hESCs⁸¹, but there is evidence that piPSCs can show both mESC and hESC characteristics under their respective culture conditions⁸².

Somatic cells can be used to generate iPSCs without the use of embryos and have the benefit of being genetic clones with the defined genomics of the original somatic cell rather than the unpredictable result of the fusion of maternal and paternal genomes. Although both cell types show relatively similar gene expression⁸³, iPSCs are subject to disruption of genome integrity if genetic modification is used for induction^{84,85}. This can result in copy number variations creating a genetically mosaic cell population⁸⁴. Trisomies can arise from human PSC induction in both by reprogramming molecules and retroviral transduction⁸⁵. In addition, iPSCs can retain epigenetic methylation profiles that push them towards differentiating back to their original somatic fate⁸⁶. In the context of transgenic cell lines, researchers have explored methods for the removal or silencing of the reprogramming factors following successful induction; however, this has often resulted in the cells losing their pluripotent characteristics. A report on biPSCs published in 2021⁶³ described spontaneous silencing of the transgenes' ten passages after biPSC induction from MSCs. As methods for the stabilization of pluripotency continue to evolve, iPSCs are likely to gain more importance as a potential source of cultivated meat due to easier availability than ESCs.

Differentiation of PSCs

Myogenic differentiation protocols and efficiency. PSC differentiation towards the myogenic lineage has been achieved in cells from mice and humans^{87,88}, and more recently in cells from pigs and cows^{57,89}. To generate myogenic cells from PSCs, the physiological process of differentiation and myogenesis that would happen within the embryo must be recreated *in vitro*.

Stepwise directed differentiation is widely used to achieve the differentiation of mouse and human PSCs. The first step recreates the formation of the primitive streak in the early embryo, which relies on the gradients of Wnt and bone morphogenic factor (BMP) proteins. Wnt signalling is the driving force behind the elongation of the primitive streak and promotes the expression of presomitic mesoderm markers such as Brachyury (T), T-box transcription factor 6 (TBX6) and mesogenin 1 (MSGN1)^{90,91}. Modulation of Wnt signalling activation, together with the inhibition of BMP signalling (the counter gradient to Wnt), ensures that PSCs are directed towards the paraxial mesoderm fate and away from the lateral plate mesoderm^{87,92}. Hepatocyte growth factor (HGF) and insulin-like growth factor 1 (IGF1) are additional growth factors used in myogenic directed differentiation. During embryonic development, HGF functions as a signal for the migration of myoblasts in the somite, aiding their development⁹³. IGF1 is a growth hormone

known for its role in metabolic regulation⁹⁴ and particularly for its anabolic effect in muscle⁹⁵. Moreover, IGF1 has an additive effect with Wnt to promote myogenesis and activate myocyte fusion⁹⁶. Furthermore, the combination of HGF and IGF1 with Wnt and fibroblast growth factor (FGF2) has proven to be a successful differentiating cocktail that produces cells positive for the myogenic marker PAX7⁸⁷.

Fetal bovine serum (FBS) is commonly used for cell culture and differentiation as it supplies the cells with a variety of growth factors and nutrients. ESC differentiation towards the myogenic lineage has been achieved by changing the FBS concentration in human and mouse cell cultures^{97,98}. However, FBS is a batch-specific and undefined culture component⁹⁹ with the added complication of potential ethical and environmental concerns surrounding its use in cultivated meat applications. A promising lead in the search for a replacement for FBS came from Messmer et al., who identified potential molecules provided in FBS by transcriptomic profiling of bovine satellite cells during myogenic differentiation and effectively replaced some of these molecules in a targeted manner¹⁰⁰. Alternatively, a common serum substitute is knockout serum replacement (KSR), composed of a variety of vitamins, proteins, amino acids, antioxidants and trace elements. KSR has variable efficiencies in different species, and although it was not effective in maintaining bESCs⁵⁸, KSR has been used as a component in differentiation media applied to human, mouse and cow PSCs^{57,87,89}.

Adipogenic differentiation protocols and efficiency. Most protocols for adipocyte differentiation of ESCs begin by forming embryoid bodies, followed by monolayer cell culture in the presence of specific growth factors for terminal differentiation^{101,102}. Retinoic acid added in the early stages of embryoid body culture promotes the differentiation of preadipocytes containing lipid droplets¹⁰². Retinoid X receptor activation upregulates the master adipocyte regulator PPAR. Once preadipocyte differentiation has been achieved, adipogenic precursors are mostly cultured with an adipogenic cocktail that includes dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), insulin and thiazolidinediones, although some studies have excluded IBMX or thiazolidinediones (Table 2). Dexamethasone upregulates PPAR- γ and C/EBP- α (ref. 103). IBMX is a xanthine derivative that inhibits phosphodiesterase to raise intracellular cyclic AMP and activate protein kinase A, promoting PPAR- γ expression¹⁰⁴. Insulin facilitates glucose transport for intracellular lipid synthesis¹⁰⁵, while thiazolidinediones bind to and activate PPAR- γ to enhance the expression of adipogenic and lipogenic genes¹⁰⁶. Although the chemical dose varies by study and species, this protocol has consistently achieved adipogenic differentiation of primary adipocytes and PSCs from humans, mouse and cattle (Table 2). Differentiation of adipose tissue is induced by BMP signalling¹⁰⁷. The use of serum for adipogenic differentiation is variable, being used in some studies¹⁰⁷ or replaced with KSR in serum-free systems^{101,102}.

Current adipogenic inducers are non-food grade due to their toxicity and steroidal nature¹⁶, creating the need to test food-sourced bioactive substances that could replace these inducers. Most candidates are lipids due to their binding affinity to PPAR- γ . Mehta et al. reported that differentiation media with free fatty acid supplements improved the adipogenesis of bovine ADSCs¹⁰⁸. Recently, lauric acid (C12:0) alone or in combination with palmitic acid (C16:0) strongly stimulated the activation of PPAR- γ in bovine hepatocytes *in vitro*¹⁰⁹. In the cultivated meat industry, it will be critical not only to find such bioactive molecules but also to investigate appropriate doses and effective combinations. Application of artificial intelligence models may be useful to reduce resource and labour demand¹¹⁰.

Genetic modification of stem cells

Genetic modification, which can occur spontaneously or by applying genome editing tools, enables traits to be passed down through generations of cells. Genetic modification in cultivated meat can enhance expansion or differentiation, yet has many more potential applications.

Immortalization of primary cell lines. Primary cell lines have limited capacity for expansion⁷. The spontaneous mutations from genetic drift that occur in vitro can result in the immortalization of cells derived from adult tissue, as in the murine myogenic cell line C2C12 (ref. 111). Genetic drift can be accelerated by non-lethal stress to increase the frequency of mutations, with ultraviolet irradiation being the most common technique¹¹². Osmotic stresses have induced mutations resulting in immortalized tilapia cell lines¹¹³. However, spontaneous mutations can have unpredictable consequences and require extensive analysis. For example, uncontrolled proliferation of myoblasts from mutations causing rhabdomyosarcoma block differentiation¹¹⁴. Cell source species may also regulate the probability of acquiring favourable mutations, with larger animals typically tolerating fewer mutations that could be selected. For example, elephants have multiple copies of the p53 tumour suppressor gene to enhance fidelity in DNA synthesis¹¹⁵. Recently, spontaneous immortalization of a chicken fibroblast line produced cells with a capacity to form a high-density suspended cell culture¹¹⁶ that were not myogenic but were capable of adipogenesis. Furthermore, MuSCs isolated from mackerel have shown spontaneous immortalization along with the capacity to undergo both myogenesis and adipogenesis¹¹⁷. The immortalization of cells for cultivated meat could greatly enhance their utility¹¹⁸, but in many cases targeted genome modifications may be necessary.

As the key limit for primary cell proliferation is telomere shortening, forced overexpression of the telomerase gene to extend telomeres is commonly used for immortalization¹¹⁹. However, relying on only telomerase expression may be insufficient for immortalization. For example, human myoblasts require both overexpression of both telomerase and cell cycle regulator cyclin-dependent kinase 4 (CDK4) to produce immortalized cells¹²⁰ and maintained transcriptional pathways and myogenic capabilities of primary myoblasts despite the genetic manipulation¹²¹. Human FAPs have successfully been immortalized using the same strategy¹⁴. Overexpression of human telomerase is commonly used across species, but in avian species the native chicken telomerase has been more effective in immortalization¹²². Immortalized cell lines in agriculturally relevant species will be fundamental for improving product consistency and further removing the need for animal inputs into cultivated meat. A recent report indicated that the immortalization of bovine MuSCs using telomerase reverse transcriptase (TERT) and CDK4 is feasible, albeit with reduced differentiation¹²³. However, issues of genetic drift would remain and even C2C12 cells lose myogenic capacity after extended culture^{124,125}.

Controlled differentiation. Myogenesis was among the first differentiation programmes to be accomplished using genetic modification tools. Overexpression of a single gene, myogenic differentiation 1 (*MyoD*), is sufficient to shift embryonic fibroblasts into myoblasts capable of fusion and myogenesis¹²⁶. Forced *MyoD* expression in primary human skin fibroblasts using an adenoviral vector created muscle fibres in vivo that were virtually indistinguishable from primary MuSCs¹²⁷. Notably, while *MyoD* kicked off myogenesis, it also led to cell cycle withdrawal, making it unsuitable for cultivated meat. A combination of *MyoD*, *Pax7* and myocyte enhancer factor 2B (*Mef2b*) was predicted to generate myoblasts capable of proliferation and differentiation¹²⁸. An immortalized line of bovine embryonic fibroblasts also served as a template for myogenesis. Induced *MyoD* expression, when combined with the growth factor IGF1, yielded cells with high levels of myogenin and myotube formation¹²⁹. Thus, exogenous induction of myogenic factors can shift cells to a myogenic lineage, but may also enhance differentiation in later passages.

Parallel to the role of *MyoD*, PPAR- γ overexpression can initiate adipogenesis. Murine fibroblasts showed that exogenous expression of PPAR- γ could induce adipogenesis in cells without adipogenic potential¹³⁰. Using bovine embryonic fibroblasts with ectopic expression of PPAR- γ led to adipogenesis with Oil Red O staining marking the

adipocytes, which was potentiated by the use of the PPAR- γ agonist troglitazone¹²⁹. Troglitazone shifted both C2C12 cells and primary murine MuSCs down an adipogenic lineage¹³¹. These efforts highlight the ability of genetic tools in combination with media factors to direct stem cell fate for cultivated meat.

Genetic modification of PSCs

Genetic modification for the overexpression of myogenic and adipogenic genes could circumvent the complex stepwise differentiation protocols currently used for PSCs. The induction of MYOD in human iPSCs (hiPSCs) via the piggyBac system resulted in myotube generation¹³². Lentiviral transduction successfully generated myotubes in piPSCs and hiPSCs by ectopic expression of MYOD and supplementation of selected growth factors or FBS⁸⁸. Lentiviral-mediated overexpression of PAX7 induced differentiation towards satellite cell morphology and generated myotubes in hESCs and hiPSCs¹³³. Lentiviral-mediated expression of PPAR- γ in mesenchymal progenitors yielded cells with genetic and morphological profiles that resembled mature white adipocytes¹⁰¹. A benefit of direct differentiation through genetic modification is that inducible vectors such as doxycycline can specifically control differentiation and provide a more food-safe option that should be identified or developed in the future^{89,101,132}. Although genetic modification has proven to be a viable tool to induce stem cell differentiation, it comes with the concerns of potentially undesired mutations.

Other applications of genetic modification

DNA manipulation offers vast potential for cultivated meat. While the C2C12 cell line loses differentiation capacity with extended passages, rejuvenation factors can maintain their myogenic capacity. Nanog, a transcription factor and pluripotency marker, can prevent senescence and promote differentiation through extended passages in both human and murine myogenic cell lines¹³⁴, including C2C12 cells¹³⁵. Nanog expression in these studies was inducible, which is a critical factor for producing cultivated meat at scale in large bioreactors, as persistent expression of a factor driving stemness would prevent differentiation. Typically, antibiotics such as tetracycline or tamoxifen are used in inducible expression, but other methods, such as light-induced expression, offer greater temporal control without vast antibiotic use¹³⁶. Cellular engineering can mitigate other challenges, such as the need for growth factors in culture media, which could be synthesized by the engineered cells. The removal of FGF2 from the media has been accomplished by introducing inducible expression of FGF2 into immortalized bovine satellite cells¹³⁷. The nutritional profile of the cultivated meat could be tailored by regulating macro- or micro-nutrient synthesis: bovine MuSCs have been genetically engineered to produce antioxidant carotenoids for nutrition and aid protection against diseases associated with red meat¹³⁸. The red colour of meat, which comes primarily from myoglobin, could be enhanced while also improving myogenesis, as demonstrated with bovine MuSCs¹³⁹. Adapting cells for large-scale culture, where sterility challenges could impact production, may include puromycin resistance genes.

Industrial use of engineered stem cell lines. While adult stem cells can be viable for potentially up to 50 doublings, immortalized cell lines can achieve 100 or more doublings while maintaining genetic stability¹¹⁶, eliminating the need for routine reseeded from live animals. This can enhance the consistency and stability of the process, and the processes can be protected by patents, which would be advantageous in commercial settings.

The most prolific company for patents on the generation of engineered immortalized cell lines has been UPSIDE Foods, although they are also developing spontaneous immortalization strategies. Among UPSIDE Foods' earlier patents in 2016 is the immortalization of chicken muscle cells by overexpressing TERT along with CRISPR-CRISPR-associated endonuclease 9 (Cas9)-based knockout of cell cycle

regulators p15 and p16 (ref. 140). To substantially reduce the cost of media and reliance on animal serum, UPSIDE Foods has filed a patent application to genetically engineer porcine cells to replace growth factors with small molecules¹⁴¹. The company has also filed patent applications to genetically modify cell lines to overexpress glutamine synthetase to convert the waste product ammonia into a useful amino acid¹⁴² and genetically engineer cell lines containing specific proteins from exotic, endangered or extinct species to enhance meat characteristics¹⁴³. While the details and verification of the techniques used are not currently publicly available, GOOD Meat uses a strategy of extracting cells from adult animals and then producing banks of immortalized cells that proliferate indefinitely. In November 2022, Steakholder Foods announced the successful differentiation of porcine adipose cells from piPSCs, although the details of the process and involvement of genetic modification are not currently known.

Safety and regulation of cultivated meat

The first approval of a cultured animal cell food product was issued by the Singapore Food Agency (SFA) in 2020 for growing chicken cells in a controlled environment. Just Eat's chicken product, made with 70% cultured chicken cells with the remainder being plant protein, "was safe for consumption at the intended levels". The SFA reviews the safety of cultured meat products at three levels. First, "the safety of the individual inputs in the production process and the products", including cell lines, culture media and reagents with toxicology reports on each. Second, "the production process and controls" to ensure that the process is contamination free. Finally, "the product must meet the standards in our food regulations", so that additives or heavy metals, among others, in products are within regulatory limits while also not exceeding the levels of allergenic proteins expected in traditional meat sources¹⁴⁴.

In November 2022, UPSIDE Foods completed the first US voluntary pre-market consultation for a human food made from cultivated animal cells¹⁴⁵. The Food and Drug Administration (FDA) reviewed the company's production process and final product. A complete nutritional evaluation profiled major nutrients, analysed for potential environmental contaminants (for example, heavy metals and microbial contamination) and compared the results with traditional poultry data. In addition, every ingredient used in the manufacturing of cultured meat needs to be quantified in the final product or considered for risk for it to remain in the final product. UPSIDE Foods' application, detailing the non-confidential safety and production of cultivated chicken, has been made publicly available¹⁴⁶. According to this document, the "Nutrient composition of UPSIDE Foods' cultured chicken has been analyzed and is within expected and is observed within safe ranges"¹⁴⁶. The pre-market consultation concluded when all questions relevant to the consultation were resolved, meaning that the FDA had 'no further questions' about the firm's safety conclusion.

Some of the cell lines (for example, chicken fibroblasts) that are used to produce UPSIDE Food's cultivated chicken product are immortalized via a genetic modification approach to induce constitutive expression of the chicken TERT gene¹⁴⁷. UPSIDE Foods "concluded that the intentional genomic alteration of poultry cells through introduced cisgenic events results in a safe and suitable alternative to conventional poultry meat"¹⁴⁶. The cisgenic approach of reintroducing genes already present under altered expression produces "an endogenous cellular pathway found in normal tissues". The company asserted that, relative to plants, animal cells traditionally consumed as food do not typically harbour nor produce toxins. Overall, UPSIDE Foods stated that the potential harmful effects of off-targeting and potential pleiotropy are minimal to non-existent in animal cells. A second pre-market consultation for a human food made from cultured animal cells, GOOD Meat's chicken cell cultured product, was announced in March 2023¹⁴⁵.

This voluntary pre-market consultation process is distinct from the FDA's regulation of genetic engineering in whole animals, where any 'intentional genomic alteration', including a cisgenic event,

would be considered an unapproved drug necessitating a new animal drug approval for food use. This could add many years and considerable expense to the commercialization of meat from genetically engineered animals¹⁴⁸.

However, before cultivated meat can be sold commercially, the cell manufacturing establishment needs a grant of inspection from the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) for the harvest and post-harvest processing operations, and the product itself requires a USDA mark of inspection. A formal agreement was reached in 2019 whereby the FDA and USDA-FSIS jointly regulate human food made from cultured cells of livestock, poultry and catfish¹⁴⁹. Cultured meat facilities are subject to FDA inspections rather than having on-site USDA inspectors as is the case for abattoirs and meat-processing plants. The USDA will oversee the processing and labelling of cell-cultured meat products, as it does with conventional meat regulated under the Federal Meat Inspection Act or Poultry Products Inspection Act¹⁵⁰. However, in a quirk of existing US regulatory authority, the FDA will have sole jurisdiction over cells cultured from seafood (other than catfish), game meat and/or foods intended for animal consumption. As of June 2023, UPSIDE Foods completed the final step in the US pre-market regulatory review process for cultivated meat by obtaining a grant of inspection from the USDA for its cultivated chicken.

No cell-based food products are commercially available in the European Union (EU) at the current time. The Novel Foods Regulation (Regulation (EU) No 2015/2283), which defines novel foods as any food without a "significant" history of consumption in the EU before 15 May 1997, explicitly mentions that its scope includes food from the culture of cells or tissues from animals, plants, microorganisms, fungi or algae. In addition, if genetic engineering is to be used in the production process, then the products would have to comply with the regulation on genetically modified food and feed (Regulation (EC) No 1829/2003). A mandatory pre-market authorization procedure, including an application process and safety assessment by the European Food Safety Authority, would be required before products could be sold. Such assessments would include the compositional, nutritional, toxicological and allergenic properties of the novel food, its proposed use and its anticipated intake as well as information on production processes and the additives and ingredients that are used in the bioreactor. As of 1 March 2023, Singapore remains the only country with an approved cell-based food product on the market. A global summary of the current status of general and specific regulatory frameworks for cell-based food products is provided by the World Health Organization (WHO)¹⁵¹.

Conclusions

The cultivated meat industry is growing quickly with an expanding list of start-up companies and investments from companies in the traditional meat industry, non-profit organizations and, recently, governmental grants. The Good Food Institute, a non-profit organization that supports alternative food research, stated that "access to continuous cell lines from species used for cultivated meat production remains a major barrier for new research endeavors". The cell lines selected will dictate the barriers and thus solutions needed to start with a few stem cells and expand them exponentially and then differentiate them into a cultivated meat product. These will include the selection of media optimized for the cell line that drive cost¹⁵² and the culture method for scaling up as two-dimensional culture will be replaced with micro-carriers or suspension culture in bioreactors¹⁵³. To ultimately mimic traditional meat, products would primarily be made of skeletal muscle myofibres as the primary source of nutrients. Fat cells and connective tissue-producing cells can also be critical for flavour and texture, respectively. Adult stem cells, which are already primed to create the components of meat, can be collected, but have limited expansion capacity in culture. Genetic modification of those adult stem cells could substantially increase the expansion capacity but has limitations on

regulatory and consumer acceptance. PSCs can address the limitations on expansion and differentiation ability, but currently require more specialized culture conditions as well as complex, multi-step differentiation protocols that are less suitable for cultivated meat at a large scale. However, the growing interest and ongoing research in this field could not only overcome these obstacles but provide innovations that make cultivated meat more cost-effective, sustainably produced, nutritional, flavourful and widely accessible.

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Acknowledgements

We thank the UC Davis Cultivated Meat Consortium led by D. Block, along with K. Baar, for discussions supporting this work. The National Science Foundation has supported the Cultivated Meat Consortium (2021132) and L.R.S. has received support from the Good Food Institute.

Competing interests

The authors declare no competing interests.

Additional information

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