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3 **Current technology and industrialization status of cell-cultivated meat**

4

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17 **ABSTRACT**

18 Interest and investment in cultivated meat are increasing because of the realization that it can
19 effectively supply sufficient food resources and reduce the use of livestock. Nevertheless, accurate
20 information on the specific technologies used for cultivated meat production and the characteristics of
21 cultivated meat is lacking. Authorization for the use of cultivated meat is already underway in the United
22 States, Singapore, and Israel, and other major countries are also expected to approve cultivated meat as
23 food once the details of the intricate process of producing cultivated meat, which encompasses stages
24 such as cell proliferation, differentiation, maturation, and assembly, is thoroughly established. The
25 development and standardization of mass production processes and safety evaluations must precede the
26 industrialization and use of cultivated meat as food. However, the technology for the industrialization of
27 cultivated meat is still in its nascent stage, and the mass production process has not yet been established.
28 The mass production process of cultivated meat may not be easy to disclose because it is related to the
29 interests of several companies or research teams. However, the overall research flow shows that
30 equipment development for mass production and cell acquisition, proliferation, and differentiation, as
31 well as for three-dimensional production supports and bioreactors have not yet been completed. Therefore,
32 additional research on the mass production process and safety of cultivated meat is essential. The
33 consumer's trust in the cultivated meat products and production technologies recently disclosed by some
34 companies should also be analyzed and considered for guiding future developments in this industry.
35 Furthermore, close monitoring by academia and the government will be necessary to identify fraud in the
36 cultivated meat industry.

37 **Keywords:** Cell-cultivated meat, Muscle satellite cell, Cell culture, Culture media, Meat industry, Meat analog

38 **Introduction**

39 Cell-cultivated meat refers to edible meat obtained through cell engineering technology, wherein cells are
40 isolated from animal tissues and proliferated using culture fluid in a laboratory instead of directly raising livestock
41 [1]. It involves collecting embryonic stem cells, immortalized cell lines, muscle stem cells, and adipocytes or
42 induced pluripotent stem cells from the target animals [2] and then manufacturing cultivated meat using skeletal
43 muscle, fat, bone, and blood [3]. Cultivated meat was first mentioned in Winston Churchill’s 1931 essay “Fifty
44 Years Hence,” in which he stated that “50 years later we will have to escape the folly of raising a chicken to eat
45 it by breeding chicken breasts or wings in a suitable media.” About 50 years later, Willem Van Eelen of the
46 Netherlands systematized the concept of cell growth, forming an atmosphere to support cell growth research in
47 the Netherlands and, consequently, Professor Mark Post’s research team at Maastricht University produced the
48 world’s first cell-cultivated hamburger patty and held a tasting event [4]. When Professor Mark Post’s research
49 team proved the production potential of cultivated meat, many researchers and companies entered the cultivated
50 meat industry. Among them, Eat Just, a cultivated meat company in Singapore, became the first in the world to
51 obtain permission from the Singapore government for manufacturing cell-cultivated meat as food and began
52 selling cultivated chicken in 2020 [5]. Besides Eat Just, Upside Foods and Good Meat have also started providing
53 cultivated chicken to customers. Upside Foods launched sales of cultivated chicken in the United States (U.S.) on
54 July 20, 2023 [6]. Good Meat revealed cultivated chicken meat to audiences in the U.S. on July 25 2023 [7].
55 Currently, various start-up companies and research teams worldwide, especially in the U.S., Korea, Europe, China,
56 Israel, the Netherlands, and Singapore, are making efforts to industrialize cultivated meat. With the increasing
57 interest in cultivated meat, the size of the global cultivated meat industry is growing rapidly, and it is expected to
58 replace the existing livestock and meat markets. Cultivated meat is important largely due to external factors such
59 as the rapidly increasing population worldwide, the resulting demand for animal protein sources, the need for
60 environmental protection due to accelerating environmental problems, and the increased interest in animal welfare
61 and ethics. In addition, all steps of the cultivated meat production process must be carried out in a sterile
62 environment, and the existing food quality management system can be applied to obtain cultured meat free from
63 external contamination, common diseases, and food poisoning [3,5]. However, despite these interests and efforts,
64 cultivated meat has currently reached only the early stages of industrialization and the growth to a stage where
65 cultivated meat can be purchased by ordinary consumers is impeded by technical limitations. Therefore, this
66 review compiles information on the history, development and current level of technology, industrialization status,
67 and future prospects of the cultivated meat industry.

68

69 **History of cultivated meat technologies**

70 Cultivated meat emerged through the gradual development of cell and tissue culturing technologies that
71 allowed the successful *in vitro* cultivation of specific animal cells (Table 1). In 1912, Alexis Carrel cultured a
72 contracting embryonic chick cardiac muscle [8], and in 1971, Russell Ross cultured smooth muscle [9]. These
73 successful tissue engineering reports gave rise to the concept of cultivating meat. In 1995, NASA received
74 approval from the U.S. FDA to research the production of cultivated meat as food [10]. Meanwhile, in 1999,
75 Willem Van Eelen secured the first international patent for the theory of cultivating meat through tissue culture.
76 Since then, multiple attempts to cultivate meat have emerged [11]. Some noteworthy examples include NASA's
77 cultivated turkey meat in 2001, cultivated goldfish meat by NSR/Tauro Applied Bioscience Research Consortium
78 in 2002, and edible cultivated frog steaks by Catts and Zurr in 2003 [10-13]. Moreover, the launching of an edible
79 cultivated beef patty made by Dr. Mark Post has secured public interest towards cultivated meat production [3].
80 Recently, Singapore made a monumental decision by approving the sale of Eat Just's cultivated chicken meat,
81 becoming the first country to declare the safety of cultivated meat [4]. More recently, the U.S. also followed suit,
82 granting pre-marketing approvals to Upside Food and Good Meat's cultivated chicken meat [15].

83

84 **Summary of the development of cultivated meat technologies**

85 Since the 1940s, cells that can continue to grow, such as HeLa cells, have been secured. Furthermore,
86 since the 1950s, efficient media such as minimum essential media by Harry Eagle have been developed for organ
87 culture, accelerating the development of cell culture technology. Cell culture processes usually consist of cell line
88 establishment, media development, bioreactor-based mass production, separation and purification, and finally,
89 commercialization. Cell lines for culturing cells must not only be highly productive but must also ensure stability,
90 and the environmental factors of media such as composition, pH, oxygen concentration, and ammonia
91 concentration affect animal cell growth. Bioreactors are selected based on the product to be made via cell culture,
92 the estimated annual production of the product, and the characteristics of the cell line [17]. Cell culture methods
93 include organ culture (maintains the original form of organs and partially cultivates organs at a liquid–gas
94 interface), primary graft culture (cultivates lumps of tissue cells at a solid–liquid interface), and cell culture
95 (separates tissue cells by enzymatic or physical methods). Organ culture does not facilitate proliferation, is
96 difficult to quantify, and has a high cost of cultivation. Cell culture forms a cell system because cells proliferate

97 when the proliferated cells are enzyme-treated, diluted, and then subjected to series culture. Cell culture
98 technology is used in various fields such as new drug evaluation and vaccine manufacturing, and the size of
99 industries related to cell culture media, culture devices, and bioreactors is also growing, increasing the importance
100 of cell culture.

101 The high production costs are a major obstacle to the commercialization of cultivated meat technology.
102 Therefore, research on cost reduction and mass production is steadily underway, and thorough facility
103 management is required to produce cultivated meat without adding antibiotics in a laboratory environment. To
104 this end, it is first necessary to remove as many contaminants as possible during the collection of muscle cells.
105 For example, when collecting adult stem cells from cow muscle tissue, a procedure that eliminates any exposure
106 to contaminants should be considered, and cells should be cultivated and examined in an antibiotic-free state for
107 a certain time to determine the presence of pollutants. These problems can be overcome, to some extent, by using
108 induced pluripotent stem cells or embryonic stem cells that continue to proliferate *in vitro*, because cells that
109 proliferate continuously *in vitro* facilitate the selection of clean cells (cells having no contaminants). However,
110 the use of these two types of stem cells necessitates the use of various chemicals or recombinant proteins, and
111 legal consideration is needed for their use in food. Future Meat, a food research company that produces cultivated
112 meat, used an immortalized fibroblast cell line to solve this problem. Because this cell line divides indefinitely,
113 non-contaminated cell clones can be selected and cultivated without antibiotics [18]. Upside Foods uses antibiotics
114 and antimycotics in the cell storage process but not during the proliferation and differentiation processes. In
115 addition, the cells hardly retain any antibiotics or antimycotics because they are exposed to a diluted solution
116 during the scale-up process [16]. Although, theoretically, this method offers a solution, its use remains
117 controversial owing to consumer safety concerns regarding the use of antibiotics or antifungal agents and the
118 potential levels of residue retained in the cells. The cell culture media must supply essential nutrients, growth
119 factors, and hormones necessary for cells, and be maintained at an appropriate pH and osmotic pressure, all of
120 which vary depending on the stage of the cell, resulting in differences in the composition of each culture [19]. The
121 culture media is an important factor in cell culture technology and is classified according to its purpose. The serum
122 media contains many different substances necessary for the survival and growth of animal cells. In contrast,
123 serum-free media has a predetermined composition, facilitating the selective growth of target cells and resulting
124 in high reproducibility of results [20]. Fetal bovine serum (FBS) is a serum separated from bovine blood extracted
125 from the mother cow's womb; it contains nutrients, growth factors, and hormones essential for cell culture and is
126 widely used owing to its relatively low immune rejection rate [5]. Despite these advantages, it is necessary to

127 develop alternatives to FBS to solve animal ethical problems, reduce manufacturing costs, and facilitate mass
128 production. Several companies are studying serum-free media. Particularly, Heuros of Australia is focusing on
129 synthesizing recombinant proteins to develop culture media that do not contain antibiotics, hormones, and serum
130 [21]. However, in the case of Korea, both FBS-based and serum-free media rely mostly on imports; therefore, the
131 development of its own product is a priority for Korea [22]. For the industrialization of cultivated meat, persistent
132 mass production is essential; therefore, cultivating meat in a three-dimensional (3D) space instead of a two-
133 dimensional (2D) space can help further increase production because cells grow better when attached to a surface
134 [23]. However, the technology of the 3D culture system using muscle cells is still under study and the technical
135 difficulty for its actual application in factories is high. Therefore, to date, research has mainly been conducted on
136 technologies that promote the attachment of cells to support the formation of 3D culture systems.

137

138 **Cell types used for cultivated meat production**

139 **Embryonic stem cells**

140 Embryonic stem cells refer to stem cells present in the endocytic mass of the preimplantation embryo.
141 Embryonic stem cell research began in mice in the 1980s [24], and human embryonic stem cells were first created
142 in 1998 at the University of Wisconsin–Madison. Embryonic stem cells originate from internal cell masses and
143 have the potential to differentiate into cells of all tissues [25] and the ability to self-replicate and be pluripotent
144 [26,27]. Many studies have confirmed that embryonic stem cells can be separated and cultivated to differentiate
145 into several cells such as hematopoietic stem cells, myocardial cells, insulin-secreting cells, fat cells, and nerve
146 cells [28-33]. The characteristics of these embryonic stem cells can be effectively used to treat diseases, but they
147 pose a risk of turning into tumors after transplantation. The type of cell to which the embryonic stem cells
148 differentiate is determined by the density of the stem cells, the composition of the media, the type and content of
149 growth factors, and the FBS content [34]. Although no specific research results have yet been published,
150 embryonic stem cells are known to have the highest efficiency among various cells in producing cultivated meat
151 [35]. However, the use of embryonic stem cells has issues related to animal ethics, and the intake of cultivated
152 meat derived from embryonic stem cells is expected to have various legal challenges.

153

154 **Induced pluripotent stem cells**

155 In 2006, a research team from Yamanaka in Japan discovered induced pluripotent stem cells with
156 characteristics similar to existing embryonic stem cells through artificial reprogramming induction [36]. In 2007,

157 it was found that similar approaches were possible using adult fibroblasts from humans but not from experimental
158 mice [37]. Fibroblasts are essential for generating induced pluripotent stem cells, which require a long time (1–2
159 and 3–4 weeks for mouse and human cells, respectively), and many studies are underway to overcome the poor
160 efficiency (0.01 to 0.1%) of this process. Induced pluripotent stem cells are receiving huge attention because they
161 overcome the ethical problems and immune rejection issues caused by embryonic stem cells [38]; however, they
162 have been reported to cause somatic cell mutations and immune problems [39,40]. In 2009, a research team
163 succeeded in obtaining induced pluripotent stem cells from pigs [41]; however, the production of meat by culturing
164 these induced pluripotent stem cells in the laboratory has not yet been reported.

165

166 **Muscle satellite cells**

167 Muscle satellite cells, known as muscle stem cells, were discovered in frog muscle cells in 1961 and are
168 a source of cell nuclei for new muscle production [42,43]. Muscle satellite cells exist between the myofibroblast
169 and the basal membrane and are included in adult stem cells [43]. A previous study reported that muscle satellite
170 cells can differentiate into muscle cells, adipocytes, osteocytes, or myocytes owing to their multipotential
171 mesenchymal stem cell activity [44]. In addition, muscle satellite cells are the most commonly used cells for
172 cultivated meat production because satellite cells easily differentiate into myotubes and mature myofibrils owing
173 to their regenerative capacity [45-49]. Muscle stem cells are known to have differentiation abilities depending on
174 the species, age, and body part from which they are extracted [50]. Among the different cells used for
175 manufacturing cultivated meat, the cell culture efficiency of muscle stem cells is relatively low; however, it is still
176 most widely used because it can be directly acquired from livestock muscles and is considered to be in the most
177 advantageous position for receiving permission for use as food.

178

179 **Basic procedure for the production of cultivated meat**

180 **Selection of muscle**

181 Muscle stem cells, which are the raw materials for producing cultivated meat, can be separated from the
182 muscle tissue of slaughtered livestock or obtained through the biopsy of living donor animals, and the condition
183 of the cells obtained in this way may vary depending on the condition of the animal from which the tissue is
184 collected (Figure 1). The essential conditions that should be considered while collecting tissues include the age of
185 the livestock, the location of the muscles, and the species and gender of the animals, all of which affect the number

186 of cells and the efficiency of the culture. In addition, the younger the animal, the higher the cell culture efficiency
187 [45].

188

189 **Isolation of muscle**

190 Animal skeletal muscle consists of various types of cells such as muscle fibers, connective tissue, and
191 somatic cells, and muscle stem cells are present in muscle fibers. Therefore, efficiently separating muscle stem
192 cells from muscle tissue is important. In general, muscle is cut into small pieces and then muscle stem cells are
193 purified by removing fibrous fragments, tissues, and connective tissues using proteases (e.g., trypsin or pronase).

194

195 **Primary cell culture**

196 The tissues separated from the muscles of livestock are washed several times at ambient temperature with
197 buffers such as phosphate-buffered saline (PBS) to obtain primary cells from animal tissues [51]. Digestive
198 enzymes such as 1.5 U collagenase D, 2.4 U dispase II, and 2.5 mM calcium chloride are mixed and used to digest
199 muscle tissues. Subsequently, the digested tissues are filtered and the filtered cells are recovered through
200 differential centrifugation. The pelleted cells are recovered and suspended in culture media with basic fibroblast
201 growth factor (bFGF) in a cell culture dish (Figure 1). The primary cells that have undergone enzymatic digestion
202 are cultivated in DMEM containing various components such as 100 U/mL penicillin, 100 µg/mL streptomycin,
203 2.5 µg/mL gentamicin, 2.5 mg/mL amphotericin, 5 mg/mL transferrin, 10 mg/mL insulin, 0.15 mM non-essential
204 amino acids, 1 mg/mL hydrocortisone, 30 ng/mL epidermal growth factor, 4 mM L-glutamine, and 2.7 mg/mL
205 glucose [52]. FBS (10 %), which contains ingredients beneficial to cell proliferation such as cell adhesion and
206 growth factors, may also be added to the basal media; however, the effects of each component of the FBS have
207 not yet been clearly identified. Next, the primary cells are incubated at 37°C in an incubator with 5 % CO₂, and
208 this step can be applied to isolate whole muscle cell populations with many satellite cells from animals [51].

209 Cells obtained through an appropriate method can be cultivated and proliferated through an *in vitro* cell
210 culture system. The cells in culture are supplied with nutrients through periodic replacement of culture media, and
211 when the cells multiply above a certain level of culture container, they are subcultured. The culture media
212 replacement and subculturing frequency vary depending on the cells being cultured, and in general, the seed
213 culture is determined by the cell line growth curve and the subculture is performed at intervals of one, four, or
214 more weeks [53]. The cultivated cells are generally subcultured, and there may be some differences depending on

215 the cell type. In particular, when recovering cells, the dissociation agent that removes cells from the culture
216 container may differ depending on the cell type [51].

217

218 **Basic components of the culture media used for producing cultivated meat**

219 A safe media that is economical and can be consumed for food is essential for the production of cultivated
220 meat. The basic media consists of glucose, glutamine and other amino acids, vitamins, inorganic salts, signaling
221 molecules such as growth factors, and buffers (Table 2). The culture media of animal cells comprises additional
222 components necessary to elicit the desired cell condition, such as proliferation, attachment, and differentiation, as
223 well as to facilitate cell survival. The media for producing cultivated meat not only contains various nutrients but
224 also growth factors necessary for the growth and differentiation of muscle cells and should be suitable for the
225 development of muscle tissue into muscles [20].

226

227 **Basic components of culture media**

228 **Glucose**

229 Glucose is the most common energy source used in cell culture. Glucose enters the cell based on manual
230 transport below the concentration gradient or ATP-dependent active transport through transport proteins on the
231 cell surface. Once inside the cell, it acts as a reducing agent for oxidative stress in the form of NADPH, which is
232 produced through the pentose phosphate pathway. Glucose is also the main energy source that produces ATP
233 through the process and is used at a concentration between 5.5 and 55 mM, and the amount required varies
234 depending on the cell type [54].

235

236 **Amino acids**

237 Amino acids are essential for producing proteins and low molecular weight compounds such as
238 nucleotides and short-chain peptides [55]. Amino acids can be divided into two groups: essential and non-essential
239 amino acids (EAA and NEAA, respectively). While NEAA can be newly synthesized by animals, EAA must be
240 obtained through diet. Cells capable of synthesizing certain amino acids *in vivo* do not exist *in vitro*; thus, there is
241 a difference between what is considered “essential” in cell culture and what is considered “essential” in the whole
242 organism [56]. For example, when culturing cells *in vitro*, the 13 amino acids in Eagle’s media (arginine, cysteine,
243 glutamine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine)
244 are considered EAAs although most of these amino acids are considered NEAA *in vivo* [57].

245

246 **L-glutamine**

247 L-glutamine is one of the most important amino acids included in cell culture because it is a major
248 contributor to protein biomass and is easily transported into cells. Glutamine is an essential amino acid that can
249 be easily metabolized as a supplementary alternative energy source at the time of growth and proliferation because
250 it becomes a precursor to biomolecules containing carbon and nitrogen, such as intermediate molecules used in
251 the synthesis of various amino acids and nucleotides [58].

252

253 **Inorganic salts**

254 Inorganic salts are not only important in maintaining cell osmotic pressure but are also important
255 components of enzyme cofactors, receptors, and extracellular matrix proteins. Inorganic salts are composed of
256 cations and anions that are completely dissociated in solution [59]. The minimum essential culture contains six
257 inorganic salts (calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, sodium phosphate,
258 and sodium bicarbonate) [57].

259

260 **Vitamins**

261 Vitamins are an important component for cell maintenance and growth. Most vitamins are generally
262 supplied through cultures as a single compound that can be directly absorbed by cells [60]. Vitamins are classified
263 as fat-soluble or water-soluble and can act as enzyme cofactors, antioxidants, or hormones [61].

264

265 **Buffer**

266 Buffer solution is essential for cell culture systems because it plays a role in maintaining the pH of the
267 cell culture at a certain level (typically 7.4 ± 0.4 for mammalian cells) despite changes in acid or base composition
268 [62]. The buffer system for cell culture is generally composed of a buffer such as the bicarbonate system or HEPES
269 [63,64].

270

271 **Serum and serum alternatives**

272 Serum has been widely used to cultivate animal cells *in vitro* and plays a role in promoting the growth of
273 various mammalian cell lines. Serums contain attachment factors, macronutrients, micronutrients, growth factors,
274 hormones, and protective elements, which promote rapid cell growth, but also have the risk of contamination by

275 viruses or prions [65]. FBS, which includes important substances such as growth factors, is still widely used in
276 cell culture and is not free from expensive costs and ethical problems; however, suitable serum replacements have
277 not yet been developed. Serum must be replaced owing to economic (high price) and ethical (using fetuses taken
278 out of the mother's womb) concerns, regardless of whether regulatory agencies allow it or not. In addition, it is
279 important to use low-cost and safe materials for the development of the cultured meat industry. It is reported that
280 the most frequently attempted method by cultivated meat companies is to create growth factors with recombinant
281 protein manufacturing technology to replace FBS [66].

282

283 **Serum-free media**

284 In the past few decades, serum-free media have been reported not only for mammalian and insect cell
285 lines but also for primary cultures [67]. Serum-free media is generally composed of basic cultures and supplements
286 to which chemical components or growth factors may be added as supplements. In general, supplementary
287 components can be divided into necessary and special factors. To grow in serum-free media, all cell lines require
288 essential factors such as transferrin and insulin. Special factors include adhesion factors, binding proteins, and
289 hormones. Compared to serum-containing media, serum-free media shows poor performance in terms of growth
290 promotion and differentiation efficiency. While identifying and replacing all functional components in serum is
291 challenging, computer-aided design and synthetic biology can be used for efficiently identifying chemically
292 known components [68-70]. Mass production of cultivated meat can be promoted under safe conditions by adding
293 key ingredients and adapting cells to serum-free media through a systematic approach that can gradually replace
294 serum by replacing essential nutrients or growth factors [71]. To successfully produce a serum-free media for
295 cultivated meat production, a basic medium based on DMEM and Ham's F-12 nutritional media should be selected
296 and additives such as insulin, transferrin, and selenium should be added to it; generally, at least 10 additives,
297 including hormones and growth factors, should be considered. Various Plackett–Burmann and response surface
298 methodology designs of experimental methods should be reviewed to optimize the media composition. Small-
299 scale cultivation using the first selected media composition must be performed and then an adaptation period must
300 be provided. Through this step, the culture conditions should be adjusted for each growth stage (passage) to reduce
301 the amount of serum used and to finally enable the use of serum-free media.

302

303 **Hormones and growth factors**

304 Hormones are endocrine signaling molecules that are available in various structural forms. Hormones are
305 found in serum, and the common hormones listed as components of FBS include insulin, cortisol, growth
306 hormone, parathyroid hormone, triiodothyronine (T3), thyroxine (T4), thyroid hormone, follicle-stimulating
307 hormone, testosterone, progesterone, prolactin, and lutein. Most hormones act by binding to receptors located on
308 the cell surface or cytoplasm, starting a signal cascade that can regulate the expression, metabolism, and growth
309 of genes. However, some hormones only affect certain cell types. Most cells require cell-specific additives because
310 cell growth and differentiation are limited when only basic media is used. Growth factors are a broad class of
311 signaling molecules that bind to receptors on the cell surface and initiate signal cascades to stimulate growth,
312 proliferation, and differentiation. Recently, protein-based growth factors that can be produced through
313 recombination methods were used [72].

314

315 **Recombinant proteins**

316 Genetic engineering is a technology that facilitates the manipulation of genes of microorganisms to
317 produce recombinant protein growth factors necessary for the production of cultivated meat [66]. The cultivated
318 meat industry is exploring ways to customize recombinant protein growth factors based on livestock species
319 (sequence homology) and increase production efficiency (discovering edible microorganisms with high
320 recombinant protein production efficiency) [73]. Nutritional components are extracted and used from organisms
321 such as plants, animals, and protozoans without using recombinant proteins (or in parallel). They are used as food
322 materials with low culture costs such as spirulina, chlorella, and yeast. Vegetable raw materials (e.g., soybeans)
323 that we often see at the table are also being studied to replace FBS; however, the quality deviation is large when
324 cultivated in open fields instead of controlled machines [66]. A strategy to develop an alternative to FBS by
325 processing livestock by-products from the slaughter process or using a co-culture system is also being explored.
326 This strategy is based on the organ-on-a-chip concept, which involves cultivating various organs of the body in a
327 laboratory environment. Different organs in livestock produce different substances (including growth factors) that
328 interact with each other. For example, if you grow liver cells, fat-derived stem cells, and muscle cells together and
329 find a combination with a positive mutual influence, you can grow muscle cells without adding growth factors.
330 However, it is not easy to find the right combination between various cells, and it is not easy to scale up this
331 combination [66].

332

333 **Differentiation of cells**

334 Stem cells must be differentiated into muscles and other types of cells at some point in the culture process
335 to obtain complete cultivated meat, and complex interactions such as extracellular signaling molecules,
336 intracellular signaling pathways, and precursor activity occur during this process.

337

338 **Differentiation of muscle cells**

339 Muscle regeneration can be divided into several stages, which are characterized by different expressions
340 of myogenic regulatory factors (MRFs) [74]. In the stationary phase, the mitosis of satellite cells is activated by
341 various stimuli such as stretching, exercise, damage, and electrical stimulation [75]. Satellite cells express c-Met
342 receptors and M-cadherin (calcium-dependent cell adhesion protein) before they are activated, which is rare
343 because they are regulated downward in the early stages of muscle regeneration [76]. Satellite cells in the early
344 stages of activation express Pax7 and MyoD (myoblast determination protein), and Pax7 is a gene that is
345 specifically expressed in the myoblasts at the proliferation stage [74,77]. Pax7 is regulated downward as
346 differentiation progresses, and satellite cells can perform self-renewal by activating Pax7 and deactivating MyoD
347 to enter the cell cycle again [78]. As the differentiation stage progresses, myoblast expresses genes such as Myf5,
348 Myf6 (MRF4), MyoD, and myogenin. MyoD is the first gene to be expressed and MyoD is expressed later [79].
349 Myogenin is expressed in cells expressing both Myf5 and MyoD [74]. MyoD regulates the formation,
350 proliferation, and lifespan of myocytes, and mice lacking both Myf5 and MyoD do not form myoblasts [74,80].
351 In addition, myogenin and Myf6 are responsible for differentiation and are necessary genes for late muscle
352 regeneration [81]. Following the expression of MyoD, MyoG and Myf6 are expressed, and after muscle damage,
353 satellite cells are stimulated by various signals generated in the damaged environment, and they eventually
354 differentiate, move to the damaged area, and then re-enter the cell cycle [82]. After the proliferative phase, muscle
355 cells exit the cell cycle and differentiate into mature myocytes through the regulatory activities of MRFs (such as
356 Pax7, MyoD, Myf5, MyoG, and Myf6), which can be used as markers to determine the differentiation stage of
357 muscle cells.

358 To produce cultivated meat, cells must be converted into tissues. A separate process is required for muscle
359 differentiation, but sufficient research on this topic has not been conducted to date. Support-based culture is better
360 than floating culture for maintaining the characteristics of the cells because supports provide an environment
361 similar to that of the body. Therefore, supports are essential for the muscle differentiation process. Supports can
362 be divided into two main types: i) scaffolds: sponge-shaped structures, and dozens of cell-sized ii) microcarriers.

363

364 **Scaffolds**

365 Meat is made of myocytes of the skeletal muscle. Myocytes are attached cells, and for cell proliferation
366 and differentiation, a scaffold called a substratum or an external template is required [83]. Scaffolds for the
367 production of cultivated meat not only affect the structure but also the texture and taste of cultivated meat. In
368 addition, the scaffold and by-products used must be edible, and non-animal-derived raw materials may be used
369 [19]. Scaffolds can be manufactured in a form that has flexible properties or can be mechanically pulled and
370 unwound to promote cell differentiation. Recently, a method of manufacturing scaffold for cultivated meat was
371 developed by extruding hemp, maize, and pea protein fibers [84]. When using the scaffold method, embryonic
372 cells or adult cells are attached to the scaffold or microcarrier and cultivated again by injecting media from a static
373 culture device or a rotary culture machine. The cells cultivated in this way can be used as cultivated meat when
374 myotubes are formed. The scaffold method is more appropriate than the self-organizing method to manufacture
375 hamburger patties, sausages, and minced meat, which are meat used without bones [19]. However, self-organizing
376 methods are advantageous in making highly structured meat such as steaks. Scaffolds have superior muscle
377 differentiation performance compared to microcarriers because they simulate the cell's extracellular matrix in the
378 body. However, there is a disadvantage in that it cannot be used in a floating incubator; thus, it can be said that
379 microcarriers were developed to solve this problem. The scaffold for use in cultivated meat production should
380 have the following characteristics [66]:

- 381 (1) Cell adhesion: Ideally, nearly 100% of cells should be able to attach to the scaffold.
- 382 (2) Badge permeability: The badges should flow well to the inside. To facilitate the recovery of waste and the
383 supply of nutrients, the micropores inside the scaffold should not be blocked until the last stage of culture.
384 To this end, spheroids that are clustered between cells should not be created.
- 385 (3) Muscle differentiation: Scaffolds should not affect the inhibition of muscle differentiation in cells. Because
386 the charge, elasticity, and hydrophilicity of the scaffold surface act in combination, it is a difficult property
387 to control in the process of manufacturing the scaffold.
- 388 (4) Bioreactor compatibility: The scaffold should be compatible with the bioreactor. In the first step of the
389 cultured meat manufacturing process, the scaffold should be placed in the bioreactor with the cells and it
390 should endure in the culture media for 3–4 weeks for the cultured meat to be made.
- 391 (5) Edibility: Scaffolds should be made from edible materials so that the product obtained by growing cells
392 attached to scaffolds can be eaten without having to separate them from the scaffold.

393

394 **Microcarriers**

395 Muscle stem cells that make up the cultivated meat are adherent cells and are generally proliferated *in*
396 *vitro* in planar culture plates. Because this culture method reduces cell production rate due to adhesion
397 dependence, developing a culture method that can increase cell concentration per unit volume is important. Among
398 the methods developed, a 3D cell culture method using a microcarrier is drawing huge attention.
399 Microcarriers are effective in cell proliferation scale-up because they increase the surface area available for cells
400 to proliferate by attaching cells to microspheres of approximately 100–300 μm in diameter. In 1967, van Wezel
401 used microcarriers for the first time. He cultivated cells using an ion exchange gel. Recently, with the development
402 of microporous microcarriers, microcarriers have been applied not only to attached cells but also to floating cells
403 [85]. In the beginning, only solid microcarrier forms were used. However, today, microcarriers prepared using
404 various materials, such as dextran, gelatin, collagen, and cellulose, are available [53]. These microcarriers are
405 mixed and stirred together with media and cells in a 3D bioreactor, forming a microcarrier complex with cells
406 [70]. However, the culture method using a microcarrier has a problem in that the activity and proliferation of cells
407 are inhibited due to shear stress generated in the process of continuously mixing the cell–microcarrier complex.
408 In addition, when applying microcarriers to cultivated meat, it is important to standardize the technology for
409 forming muscle tissue by differentiating mesenchymal stem cells [86]. Finally, like in the case of scaffolds, it is
410 necessary to manufacture microcarriers using materials that can be consumed immediately without separating
411 microcarriers and muscles.

412

413 **Bioreactors**

414 The use of bioreactors for manufacturing cultivated meat is important in terms of tissue engineering. The
415 optimal conditions required for producing cultivated meat in bioreactors are low shear stress and constant
416 perfusion. Various types of cell reactors are required depending on the type of cultivated meat to be produced,
417 and the shape of the scaffold used varies depending on the bioreactor. The bioreactor can be of different shapes
418 or types such as rotary shape, tank stirring type, static culture type, and plastic bag. Thus, the use of customized
419 scaffolding should be considered accordingly. The scaffold can be selected based on the shape of the cultivated
420 meat and the prevailing political culture. The production of cultivated meat using a scaffold depends on the media
421 component and the appropriate supply of oxygen [19]. NASA has traditionally used rotating bioreactors. In the
422 case of a rotating reactor, the tertiary structure of cultivated meat is well-formed only when the balance of forces
423 such as centrifugal force, drag, and gravity is well maintained [87,88]. The concentration of animal cells to be

424 cultivated is approximately 108 cells/mL or 30 kg/m³ by weight, and the volume of the reactor can range from 10
425 mL to hundreds of litres [89]. In economic terms, the optimization and automation of the reactor must precede. It
426 is expected that combining 3D organ printing, bio-photonics, and nanotechnology with bioreactor research would
427 facilitate the production of various types of cultivated meat [90].

428

429 **Analysis of the efficiency of cell cultures**

430 The efficiency of cell cultures is generally evaluated based on the degree of cell proliferation. Cell
431 proliferation is a process in which cells grow and divide, increasing the size of a cell group. The degree of cell
432 proliferation depends on the species and cell type, and cell growth and division depend on the appropriate cell
433 cycle-based regulatory activities [91]. The degree of cell proliferation can be measured using various principles
434 and methods.

435

436 **Cell count method**

437 It is the simplest experimental method to evaluate cell proliferation. In this method, all proliferated cells
438 are collected in a culture container and the number of cells is counted using a hemocytometer or an automated
439 cell counter to check whether the cell proliferation rate has increased compared to that observed when the cells
440 were first cultivated. At this time, in many cases, the number of cells present in one zone is not large, so the
441 number of all cells present in areas 1, 3, 7, and 9 is counted to increase measurement accuracy. In counting cells,
442 the total number of cells is predicted by counting only cells across areas 1, 3, 7, and 9 or cells across the thin line
443 according to the measurement method to prevent overlapping cells present in each zone. To measure only
444 surviving cells among all cells, the surviving and dying cells can be distinguished using the Trypan blue reagent
445 before counting [92]. Subsequently, the total cell concentration and the total cell number are calculated as follows:
446 Cell density (cell/mL) = Average of the number of cells counted in the four areas × Trypan blue dilution (2X) ×
447 Chamber volume (10⁴)
448 Total number of cells: Cell density (cell/mL) × Total volume (mL)

449

450 **Absorbance measurement method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide** 451 **(MTT) assay)**

452 In this method, the rate of change in the absorbance of a cell culture solution is measured by treating it
453 with a specific reagent without recovering the cells present in the culture container. Representative reagents

454 include MTT (Roche, Product Name: Cell Production Kit I), BrDU (Abcam, Product Name: BrdU Cell Production
455 ELISA Kit), and WST-8 (Dojindo, Product Name: Cell Counting Kit-8). The type of reagent used may vary
456 depending on the product; nevertheless, all reagents evaluate cell proliferation using similar principles. Cell
457 proliferation can be evaluated by treating the culture with substances whose structure changes based on cellular
458 metabolism or mechanisms and then measuring the cell-induced changes in the color and absorbance of the culture
459 solution. In general, analysis is conducted in small cell culture containers such as 96-well and 24-well plates, and
460 this method can be performed simply by replacing most solutions without recovering cells. MTT is a positively
461 charged tetrazolium salt, which can readily penetrate cells. Within the cell, it is converted into formazan by the
462 mitochondrial dehydrogenase. Formazan is water-insoluble; thus, it must be solubilized with acid and/or detergent
463 before absorbance measurements. Common solubilizing chemicals include acidified isopropanol, DMSO,
464 dimethylformamide, SDS, and a mixture of detergent and organic solvents. Several factors such as MTT
465 concentration, incubation period, and number of metabolically active cells can affect the signals emitted. Thus,
466 optimization of these factors must be considered. Finally, the resulting purple color is measured at 570 nm, with
467 the usual reference wavelength at 630 nm [93].

468

469 **Cell cycle analysis**

470 The cell cycle analysis method is an experimental method to determine the cell cycle and compare the
471 degree of cell proliferation by measuring the DNA content in the cells using the fluorescence-activated cell sorting
472 (FACS) technique. Representative reagents include propidium iodide (PI)-based products, which are inserted
473 between DNA sequences and express red fluorescence. Since PI does not penetrate living cells, the process of
474 recovering cells in culture from a culture container and fixing them using 4% paraformaldehyde or 70% ethanol
475 is required for cell cycle analysis. Thereafter, the cells are treated with PI solution and the degree of fluorescence
476 expressed is analyzed using FACS equipment. Compared to the content of the G1 group, the DNA content ratio
477 in the cell doubles during DNA replication in the S phase and then decreases during the G2/M phase. These
478 characteristics can be used to analyze the cell cycle. The lowest DNA content is observed in the G1 phase, whereas
479 the highest DNA content is observed in the G2/M phase. When DNA content is in between the contents of the G1
480 and G2/M phases, the phase of the cells can be determined based on the DNA content of the S phase, and the
481 proportion of cells present in each period can be compared to see if cell proliferation is proceeding normally. If
482 cell proliferation is not normal, the accumulation of G1-phase cells and a gradual increase in their proportion
483 would be observed [94].

484

485 **Safety of cultivated meat**

486 Cultivated meat is the most representative alternative protein food produced using proliferating cells
487 collected from animals and a method of culturing animal tissues using cell engineering technology. Unlike other
488 alternative protein foods such as vegetable alternative protein foods and edible insects, cultivated meat is expected
489 to be a competitive future alternative because it can provide animal protein-based alternative protein foods similar
490 to actual meat. However, unlike vegetable alternative proteins and edible insects that have already been
491 commercialized, cultivated meat has never been consumed except for experimental purposes, is a food based on
492 new materials and new technology, and is distinctly different from other alternative protein foods. In particular,
493 if there is a problem related to actual safety, it can pose a direct threat to consumer health and cause economic
494 losses to producers. Therefore, a strong framework for evaluating the safety of cultivated meat safety is necessary;
495 however, the legal bases related to cultivated meat are currently insufficient worldwide [45,95].

496

497 **Status of safety management of overseas cultivated meat**

498 Currently, cultivated meat is not a natural sister product of conventional meat, and producing cultivated
499 meat having substantial equivalence with conventional meat is challenging because it would necessitate the
500 incorporation of additional processes during production. Because cultivated meat belongs to the new material food
501 category, approval and safety verification are essential before its marketing. In the case of the U.S. and the
502 European Union, where the development of cultivated meat is progressing rapidly, efforts are being made to
503 ensure the safety of cultivated meat based on regulations for new food materials. The management of new
504 ingredients in the U.S. is based on the FDA's Federal Food, Drug, and Cosmetics Act and the Code of Federal
505 Regulation. On June 21, 2023, the USDA announced that it had approved Upside Foods and Good Meat's cell
506 culture chicken for sale [1,96]. This made the U.S. the second country after Singapore to license animal cell culture
507 food. The FDA works closely and shares jurisdiction with the U.S. Department of Agriculture's Food Safety and
508 Inspection Service (USDA-FSIS) over human foods from certain animal species to label them safely and
509 accurately. These two agencies are working with manufacturers to ensure that cultivated meat products meet all
510 applicable FDA and USDA-FSIS requirements.

511 Considering the overall process of manufacturing cultivated meat, various laws and regulations in
512 addition to GRAS should be used to check the safety of substances used in manufacturing cultivated meat, and
513 the final product should also be managed to meet food safety standards [97]. Discussion on the USDA Federal

514 Meat Inspection Act related to existing meat is also essential because, currently, the definition of meat differs
515 from state to state in the U.S., and Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control
516 Point (HACCP) are expected to be used in combination with GRAS. On the other hand, because GRAS does not
517 include genetically modified (GM) foods, if GM cells or other components are used during the manufacturing
518 process, the application of regulations related to GM foods in addition to GRAS will be necessary.

519 In the European Union, the regulation and management of cultivated meat is expected to be carried out
520 by Novel Food of the European Food Safety Authority. Novel Food is a system that manages the safety of new
521 food materials in the European Union. It was first enacted in 1997 and its main purpose is to protect domestic
522 consumers through safety evaluation of products before marketing. In these regulations, cultivated meat is
523 included in the food consisting of isolated or produced cell cultures derived from animals, plants, microorganisms,
524 fungi, or algae. Therefore, administrative, technical, and scientific data related to these products must be submitted
525 and they must be registered as Novel Food [98,99].

526

527 **Safety management of cultivated meat in Korea**

528 In Korea, new food ingredients can be used as temporary food ingredients after their safety and soundness
529 are reviewed under the supervision of the Ministry of Food and Drug Safety [100]. This system is called a new
530 (temporary) food raw material recognition system and its targets include agricultural products, livestock products,
531 marine products, microorganisms, and substances used as raw materials for manufacturing food. The temporary
532 recognition system, similar to GRAS in the U.S. and Novel Food in the European Union, evaluates the safety of
533 raw materials that have not yet been consumed as food in Korea. There are a total of 54 types of items licensed
534 through this system, and edible insects, a type of alternative protein food, have been sold after safety verification
535 based on relevant laws [101].

536

537 **Considerations for verifying the safety of cultivated meat**

538 The types of tissues in meat are largely muscle, fat, blood vessels, and tendons, and each tissue is
539 composed of different cells. Although research on the co-culture of muscle cells with other cells is underway,
540 presently, methods of increasing similarity to meat through processing techniques are mainly applied [102,103].
541 Currently, research is mainly being conducted on how to manufacture cultivated meat using muscle stem cells.
542 As a consideration for verifying the safety of cultivated meat, the scope and management of cell donor animals
543 should be confirmed when collecting cells. One of the great advantages of cultivated meat is that it can produce

544 various types of meat if the required cells can be collected. When producing cultivated meat, a method of
545 collecting cells from cell donor animals is to use the tissue of the conductor immediately after biopsy or slaughter.
546 However, problems related to the welfare of the target animal may occur when proceeding with a biopsy, and
547 safety problems that may occur when using a conductor immediately after slaughter should be considered. In
548 addition, standards related to the management and specification of target animals will be important for securing
549 cells of uniform quality [8]. Currently, the substances used in the manufacture of cultivated meat include
550 extracellular matrix, basic culture fluid, and serum [45]. None of these substances are currently allowed to be used
551 during food manufacturing. Therefore, setting the direction of the policy based on the domestic situation is
552 necessary; it is important to decide whether these materials should be replaced with edible materials or considered
553 residual substances as in Canada and Israel. Accordingly, research on the development of alternatives or safety
554 verification of the existing substances will be necessary in the future.

555 The management of cells used in the manufacture of cultivated meat is extremely important. In particular,
556 it is essential to review the safety and stability of cells before and after freezing. Currently, cells are stored through
557 cryopreservation and, in principle, they are stored for a long time at ultra-low temperatures so that there is no
558 change in the function of the cells [104]. So far, there have been no cases of analysis using actual raw materials.
559 In the future, allergy and toxicity tests should be conducted on the raw materials before additional research results
560 or products are released, and safety verification before marketing is essential. Allergic evaluation should include
561 allergy tests on donors and hosts, analysis of sensitivity to physicochemical treatment, homogeneity of known
562 allergens and amino acid sequences, daily protein intake evaluation, and serum screening [105]. In addition,
563 considering that growth factors are present in the serum used in cell culture, it may be necessary to include
564 carcinogenicity tests in addition to single-administration toxicity tests, repeated-administration toxicity tests, and
565 genotoxicity tests. Although the institutional foundation for the regulation and management of cultivated meat in
566 Korea is still insufficient, the future direction of cultivated meat safety management can be grasped based on the
567 current status at home and abroad and the expected considerations when checking the safety of cultivated meat.
568 Currently, the Ministry of Food and Drug Safety is conducting various studies to establish safety management
569 standards for cultivated meat, and it is expected that a management system will soon be introduced to safely
570 produce and distribute cultivated meat [106].

571

572 **International Standards for cultivated meat**

573 As cultivated meat is approved as a food, the establishment of international standards is becoming more
574 important. Table 3 shows the current international standards for cultivated meat.

575

576 **Current status of the industrialization of cultivated meat**

577 Several cultivated meat companies and research institutes are striving to develop cultivated meat products,
578 and related industries are also growing rapidly as interest in and research on the cultivated meat industry is rapidly
579 increasing (Table 4). The Netherlands and the U.S., where the cultivated meat industry developed early, have the
580 infrastructure and source technology for producing cultivated meat. Furthermore, several new cultivated meat
581 products have been developed, but most of them have not reached the stage of being sold in the market due to
582 legal problems and barriers such as productivity and economic feasibility. According to the consulting firm
583 McKinsey, the cell embryo-rearing market is expected to account for 1% of the total meat market by 2030,
584 equivalent to \$25 billion (KRW 28.2 trillion), with an annual production of 1.5 million tons [120]. According to
585 another consulting firm, Kearney, the global cell culture market is expected to grow at a 41% compound annual
586 growth rate from 2025 to 2040, and the cell culture market is expected to grow to \$450 billion (approximately
587 533 trillion won) by 2040, accounting for 35% of the total meat market. Industries related to cultivated meat are
588 attracting attention owing to the environmental destruction- and animal welfare-related issues caused by the
589 traditional livestock industry. Domestic and foreign companies that have shown remarkable results in cultivated
590 meat research in Korea are as follows:

591

592 **1) Mosa Meat**

593 · It is famous for being founded by Mark Post, a professor at Maastricht University who introduced the world's
594 first cultivated meat hamburger patty to the public.

595 · This company is developing beef patties as its major product, and Singapore is being prepared as its first market.

596 · The Dutch government has issued a letter agreeing to conditions for tasting cultivated meat and seafood
597 products in a controlled environment.

598 · This company separated satellite cells from the muscles of slaughtered cattle, differentiated them *in vitro*, and
599 made a burger patty, which was used at the tasting event.

600 · For muscle differentiation, this company uses a method in which muscle cells are scattered around the collagen
601 column and cells clump together to produce a ring that is differentiated into a muscle-like shape.

602

603 **2) Upside Foods**

604 · This company developed cultivated chicken and held the world's first cultivated chicken meat tasting in 2017.

605 · It is the first cultivated meat company to pass the U.S. FDA stability assessment.

606 · On July 20, 2023, it began sales of cultivated chicken meat in the U.S.

607

608 **3) Aleph Farms**

609 · This company specializes in making cultivated beef meat.

610 · This company makes marbled steaks using 3D bioprinting technology.

611 · It does not use genetic manipulation and cell immortality.

612 · It unveiled the world's first cultivated meat steak in 2018 and plans to build a mass production facility by 2024.

613 · In 2019, it succeeded in producing very small-sized cultivated meat at an altitude of 400 km at a space station.

614 · In April 2023, Aleph Farms announced the development of its first product brand, Aleph Cuts, in preparation
615 for the launch of its cultivated Petit Steak product in Israel and Singapore later in 2023.

616 · It announced the launch of its product in 2022 but failed to meet the schedule; thus, it has targeted production
617 for 2024.

618

619 **4) Steakholder Foods**

620 · This company was previously known as MeaTech 3D.

621 · It works with Umami Meats, a Singaporean aquaculture seafood company.

622 · It introduced Omakase Beef Morsels, a first-of-its-kind, highly marbled 3D-printed 100% cultivated beef cut.

623 · It introduced 3D printed ready-to-cook cultivated fish.

624 · It uses advanced 3D printer technology designed to produce grown meat products that mimic the texture, taste,
625 and appearance of conventional meat.

626 · It produces meat products using fusion technology, which is based on the technology of extruding paste material
627 through a narrow nozzle to create a fiber texture that best simulates meat fibers.

628 · It uses DropJet technology, which is a technique that drops a gel-based material to create a 3D structure and is
629 ideal for seafood products.

630

631 **5) Good Meat**

632 · This company conducted the world's first cultivated meat sales.

- 633 · In December 2020, for the first time in the world, this company sold cultivated chicken nuggets with food sales
- 634 authorization from the Singaporean government.
- 635 · It conducted the first cultivated meat tasting at a Singaporean restaurant in 1880.
- 636 · As of February 2023, it has received the highest cumulative investment among cultivated meat companies.
- 637 · It passed the U.S. FDA stability assessment on March 21, 2023.
- 638 · A select group of diners in the nation’s capital sat down to a historic meal of charcoal-grilled cultivated chicken
- 639 in July 2023, marking the first-ever sale of Good Meat in the U.S.
- 640 · On July 25, 2023, a restaurant serving Good Meat’s cultivated chicken was opened, with tables available
- 641 starting the week of July 31.
- 642 · As of March 2023, there is only one restaurant in Singapore that sells Eat Just’s chicken, and the quantity sold
- 643 is limited to six servings per week.

644

645 **6) DaNAgreen**

- 646 · It is a bio company with the vision of a healthy future for all life on earth.
- 647 · It possesses the tissue engineering source technology for making mini organs, muscles, and tissues by culturing
- 648 and differentiating stem cells on a 3D cell culture scaffold. Using this technology, it developed a 3D cell culture
- 649 kit protocol, commercializing the source technology of the support that allows stem cells to grow in a similar
- 650 environment to that of the living body.
- 651 · It is focused on developing a one-step production technology that produces raw materials for cultivated meat
- 652 by putting cells and supports into one bioreactor and a mass production system of cultivated meat using a
- 653 support made of vegetable protein.
- 654 · This company is preparing to register for the U.S. FDA and the Ministry of Food and Drug Safety assessments.

655

656 **7) SeaWith**

- 657 · SeaWith is a startup established in 2018 with the goal of “Borrowing the power of the sea, adding our own
- 658 technology, and increasing the value of life.”
- 659 · This company is developing technology jointly with the Chungnam National University, Korea Maritime
- 660 Science and Technology Institute, Sungkyunkwan University, and Korea Agricultural and Fisheries
- 661 University, and its distinguishing feature is the utilization of sea resources.

662 · It is focused on developing a technology that can reduce the production cost of cultivated meat from more than
663 100,000 won per 100g to around 2,000 won by creating a 3D structure and its own culture media that cultures
664 cells using algae.

665 · This is the only company that develops cultivated meat using algae and has favorable conditions for mass
666 production because seaweed production is high.

667

668 **8) SPACE F**

669 · This company aims to produce sustainable and safe future alternative food, promote global environmental
670 protection through animal protection, and enrich human life through cell agricultural technology.

671 · It is focused on developing optimal cell line extraction and cell culture source technology using muscle stem
672 cells.

673 · This company manufactures muscle stem cells through tissue engineering-based 3D differentiation techniques
674 using cell-attached supports. It produces muscle stem cell lines with high purity and rapid division functions
675 to realize muscle tissue and produces them at a composition ratio similar to that of existing meat.

676 · It aims at optimizing mass culture systems by developing automated production facilities for culture products.

677 · The goal of this company is to produce cultivated meat with muscle, fat, and connective tissue ratios similar to
678 conventional meat using self-developed new growth factors, edible cultures, and supports.

679

680 **9) TissenBioFarm**

681 · The production method of this company is different from that of other cultivated meat companies.

682 · This company specializes in bio-fabrication for manufacturing artificial organs. These technologies can be
683 implemented to develop various meat parts.

684 · This company aims to produce high-quality customized cultivated meat that takes into account individual tastes
685 and the health of consumers using high-functional edible bio-ink and the original technology for cultivated
686 meat production.

687 · Currently, this company is applying for domestic/PCT patents related to cultivated meat production methods
688 and businesses.

689

690 **10) Cell MEAT**

- 691 · Cell MEAT is a food-tech startup with the goal of sustainable protein supply for Earth and mankind's
692 coexistence.
- 693 · It is focused on developing technology that uses engineering technology to realize the unique physical texture
694 of meat by part.
- 695 · In 2021, Dokdo shrimp prototypes were introduced, and starting with crustaceans, cattle, pork, pork neck, and
696 pork belly are planned to be developed.
- 697 · This company is developing inexpensive cell culture and technology to produce serum-free media.
- 698 · It aims to manufacture cultivated meat quickly with reduced production unit price using serum-free cell culture.
- 699 · It aims to lower the production unit price to 5,000 won per kilogram.

700

701 **11) IntegriCulture**

- 702 · This company aims to commercialize cell agriculture, which involves making food ingredients from animal-
703 derived cells.
- 704 · On February 21, 2023, this company used cultured duck liver cells as food material.
- 705 · This company is currently collaborating with the corporation “Plan·Do·See” to develop innovative restaurant
706 foods.

707

708 **12) Joes Future Food**

- 709 · This company held the first tasting event for cell-cultured meat in China.
- 710 · It developed serum-free culture media based on its experience in researching stem cell differentiation
711 technology.
- 712 · Research is underway to produce pork products (pork belly, fat, skin, etc.) through cell culture.

713

714 **13) Vowfood**

- 715 · On March 13, 2023, this company held the cultivated Japanese quail dumpling tasting event in Texas.
- 716 · On March 28, 2023, they made headlines by launching mammoth meatballs at the Nemo Museum in
717 Amsterdam.
- 718 · The best cells for food are stored in their proprietary multi-species cell library.

719

720 **Future perspectives on cultivated meat**

721 The industrialization of cultivated meat is a strategy that has the potential to address the various challenges
722 of the food industry, and the following are some key points to consider:

723 (1) Sustainability: Cultivated meat has the potential to reduce environmental impacts related to the traditional
724 livestock industry. This is because it is predicted to require fewer resources such as land, water, and energy
725 and generate lower greenhouse gas emissions. Moreover, the industrialization of cultivated meat production
726 is expected to contribute to a more sustainable food system [121].

727 (2) Livestock welfare: Cultivated meat production can help overcome several animal welfare-related issues
728 because it does not require massive breeding and slaughtering of animals. It is expected to provide an
729 alternative to potentially reducing animal suffering by producing meat without ethical problems unlike
730 traditional livestock industries [122].

731 (3) Food security: As the world's population grows, cultivated meat has the potential to solve food security
732 problems. It is expected to meet the rapidly increasing demand for food by facilitating sustainable and
733 efficient meat production [123].

734 (4) Health and nutrients: Cultivated meat can be produced with a specific nutritional profile, potentially
735 providing consumers with healthier options. For example, the composition of the final product can be
736 precisely controlled to reduce the saturated fat content or increase the content of beneficial nutrients,
737 thereby promoting healthier options to meet individual dietary preferences and requirements [124].

738 However, there are several controversies over cultivated meat, which are summarized as follows:

739 (1) Consumer acceptance: Achieving customer acceptance of cultivated meat is challenging owing to concerns
740 about naturalness, taste, and long-term health effects, all of which can reduce consumer preference for
741 cultivated meat. Therefore, overcoming these obstacles and building trust with consumers is critical for the
742 growth of the cell-cultured meat industry [125].

743 (2) Technological limitations: Currently, cultivated meat production is expensive and requires a complex
744 production process. As the possibility of mass production at low cost remains a key challenge, more
745 research and development efforts are required to optimize production methods and reduce costs.

746 (3) Regulatory framework: The development and commercialization of cultivated meat require clear regulation
747 and supervision. Therefore, establishing a strong regulatory framework to ensure safety, quality, and
748 accurate labeling of cultivated meat products is essential [126].

749 (4) Disruption of the traditional livestock industry: Widespread expansion of the cultivated meat market could
750 potentially disrupt the traditional livestock market and have economic and social implications for those who

751 rely on it. The transition to the cultivated meat industry must address these concerns and provide alternative
752 opportunities for affected communities [127].

753 Overall, the future of the industrialization of cultivated meat is considered promising in terms of
754 sustainability, animal welfare, food security, and health benefits. However, resolving controversies and challenges
755 related to consumer acceptance, technological limitations, regulations, and social impacts is important for the
756 successful integration of cultivated meat into the food industry.

757

758 **Conclusion**

759 The future prospects for the industrialization of cultivated meat are promising; it is expected to have the
760 potential to revolutionize the food industry because there has been considerable progress in technology. If
761 technology development continues as it is now, production costs will decrease and the economic feasibility of
762 cultivated meat will continue to increase. However, the technical difficulty will still be high because it is difficult
763 to improve the taste and texture of cultivated meat to achieve characteristics similar to those of traditional meat.
764 Furthermore, because cultivated meat is a new food, along with overcoming the controversy over its safety, efforts
765 to resolve conflicts with the traditional livestock industry will be needed. To permit the ingestion of cultivated
766 meat, a strong regulatory framework and clear guidelines for transparent and accurate labeling must be developed.
767 Moreover, the possibility of companies exaggerating or creating deceiving technologies related to cultivated meat
768 to induce unfair investment cannot be ruled out. Therefore, continuous monitoring by academia and the
769 government is necessary to ensure the industrialization, technology development, and licensing of safe and
770 authentic cultivated meat.

771

772 **Conflict of Interest**

773 The authors declare no potential conflict of interest.

774

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781

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- 1084

Table 1. History of cultivated meat technologies

Year	Milestones	Reference
1912	First cultivation of embryonic chick cardiac muscle tissue by Alexis Carrel	[8]
1971	First reported smooth muscle culture by Russel Ross	[9]
1995	NASA got approval from the U.S. FDA to conduct research on cultivated meat as food in spacecraft.	[10]
1999	First international patent for the theory of cultivating meat using tissue culture was granted to Dr. Willem Van Eelen.	[11]
2001	NASA began research on cultivated turkey meat production in space	[10]
2002	Morris Aaron Benjaminson succeeded in cultivating muscle tissue collected from goldfish in a Petri dish.	[8]
2002	The NSR/Touro Applied BioScience Research Consortium produced the first edible fish fillet-sized cultivated meat from goldfish.	[12]
2003	Oron Cats and Ionat Zurr used frog stem cells to cultivate edible steaks	[13]
2005	First academic paper on cultivated meat was published in the Tissue Engineering journal.	[14]
2007	The University of Amsterdam, Utrecht University, and Eindhoven University developed foundational technologies for cell culture, media development, and bioreactor design for cultivated meat production.	[13]
2013	Dr. Mark Post launched a cultivated burger patty in a hamburger to introduce the concept of cultivated meat to the public.	[3]
2020	Singapore approved Eat Just's cultivated chicken meat as food.	[4]
2022	Upside Foods received the first pre-marketing approval for cultivated chicken in the U.S. from the FDA.	[15]
2023	Good Meat's cultivated chicken meat became the second product to receive pre-marketing approval in the U.S.	[7]
2023	The USDA approved the sale of Good Meat's cultivated chicken.	[16]

Table 2. Components of the culture media used for producing cultivated meat

Components	Main characteristic
Basic components	
Glucose	<ul style="list-style-type: none"> · Main energy source, reducing agent for oxidative stress. · Glucose demand differs depending on the cell type.
Amino acids	<ul style="list-style-type: none"> · Production of proteins, nucleotides, and short-chain peptides. · Cells cultured <i>in vitro</i> obtain essential amino acids (arginine, cysteine, glutamine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine) from the media.
L-glutamine	<ul style="list-style-type: none"> · Major contributor to protein biomass and transportation into cells. · L-glutamine acts as a supplementary alternative energy source.
Inorganic salts	<ul style="list-style-type: none"> · Maintenance of cell osmotic pressure. · Examples: calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, sodium phosphate, and sodium bicarbonate added to the culture medium.
Vitamins	<ul style="list-style-type: none"> · Responsible for cell maintenance and growth.
Buffer	<ul style="list-style-type: none"> · Maintains the pH of the cell culture. · Examples: bicarbonate system or HEPES.
Serum	<ul style="list-style-type: none"> · Main factor for mammalian cell growth.
Hormones and growth factors	<ul style="list-style-type: none"> · Stimulation of cell growth, proliferation, and differentiation. · Examples: insulin, cortisol, growth hormone, thyroid hormone, sex hormones, lutein, etc.
Recombinant proteins	<ul style="list-style-type: none"> · Contribute to increased production efficiency for developing cultured meat.

1089 **Table 3. International standards for cultivated meat**



Country	Standards
Singapore	<ul style="list-style-type: none"> - Cultivated meat means meat made from animal cell culture. - Require the following information to be submitted for the safety evaluation of cultivated meat: <ul style="list-style-type: none"> • Description of the entire manufacturing process, product characteristics, information related to cell lines used, and information related to the scaffolding material used. • Information on the purity and gene stability of cell culture during the manufacturing process. • Safety assessment of possible risks in the manufacturing of cultivated meat. • Research on safety, such as digestive analysis.
United States	<ul style="list-style-type: none"> - The FDA and USDA prioritized the resolution of standards for regulatory agencies—the most important issue in the cultivated meat industry. - The FDA has come to manage and supervise cultivated meat products up to the pre-production stage of cells, including cell collection, cell banking, and cell growth and differentiation. - USDA will take over the product once it is developed and use the agency’s expertise in managing and supervising the production and labeling of cultivated meat products. - A joint regulatory agreement between the FDA and USDA has somewhat resolved the conflict (regarding standards) with the existing meat industry.
European Union	<ul style="list-style-type: none"> - In Europe, the regulation and management of cultivated meat is expected to be carried out by Novel Food of the European Food Safety Authority.

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- Novel Food is a system that manages the safety of new ingredients in the European Union and was first enacted in 1997.
 - The main aim of Novel Food is to protect domestic consumers through pre-market stability assessment.
 - Like in the case of the U.S., the Korean Food Safety Administration's Novel Food is expected to include various laws and regulations to manage cultivated meat products excluding GM foods.

Canada

- Unlike in the U.S. and European Union, Novel Food in Canada includes GM food.
 - There has been no official announcement regarding the Canadian government's policy on the regulation and management of cultivated meat. However, the Canadian government is expected to apply Novel Food because cultivated meat is not included in the current definition of meat under Canadian law.
 - Novel Food in Canada aims to protect consumers through the stable management of new and GM foods sold in Canada.
 - Cultivated meat will be used through a process that has not been used previously.
 - Cultivated meat is classified as food and is expected to be jointly managed by the Food Inspection Agency, the Ministry of Environment, and the Ministry of Agriculture and Food.
 - According to Novel Food in Canada, if the raw materials used are not edible, it is necessary to evaluate their stability and check whether they remain in the final product, and the main targets can be predicted based on the support, culture, and oxygen transport materials.
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1091 **Table 4. The cultivated meat products of different companies**

Company	Country	Products	Achievements	Reference
Mosa Meat	Netherlands		<ul style="list-style-type: none"> · Pioneer in cultivated meat production. · Presented the world’s first cultivated meat hamburger patty to the public. · Separated satellite cells from the muscles of slaughtered cattle. · Differentiated satellite cells <i>in vitro</i> to produce a burger patty. 	[107, 108]
UPSIDE Foods	United States		<ul style="list-style-type: none"> · Conducted the world’s first cultivated chicken meat tasting. · First company to pass the U.S. FDA stability assessment. · First company to sell cultivated chicken meat in the U.S. 	[6]

3D Bio-Tissues United Kingdom



- The first cultivated steak from pork cells in the U.K.
- Cultivated steak production using serum-free and animal-free cell booster.
- Produced steak with meat fiber and structural integrity similar to conventional meat.

[109]

Aleph Farms Israel
(Aleph Cuts)



- Produced marbled steaks using 3D bioprinting technology.
- Produced cultivated meat without resorting to genetic manipulation or pursuing methods related to cell immortality.
- Produced very small-sized cultivated meat at a space station.

[110, 111]

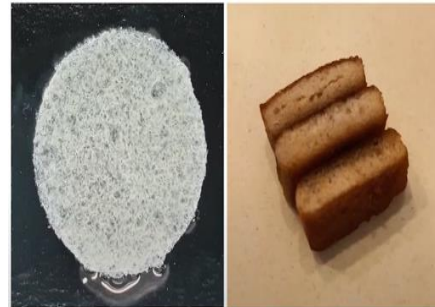
Good Meat Singapore



- Produced the world's first cultivated chicken nuggets in Singapore.
- Passed the U.S. FDA stability assessment in 2023.
- This company's cultivated chicken is sold at a restaurant in Singapore.
- Currently, the sale of cultivated chicken in the said restaurant is limited to six servings per week.

[112,113]

DaNAgreen South Korea



- Possesses the tissue engineering source technology for making mini-organs, muscles, and tissues by culturing and differentiating stem cells on a 3D cell culture scaffold.
- Commercialized the source technology for the support that allows stem cells to grow.

[114]

SeaWith

South Korea



· Utilized sea resources (algae) for cultivated meat production.

[115]

SPACE F

South Korea



· Developed optimal cell line extraction and cell culture source technology using muscle stem cells.

· Produced cultivated meat with muscle, fat, and connective tissue ratios similar to conventional meat using self-developed new growth factors, edible cultures, and supports.

[116]

TissenBioFarm

South Korea



· Possesses technology that is different from the technology used by other cultivated meat companies.

· Uses bio-fabrication technology for manufacturing artificial organs to develop various meat parts.

[117]

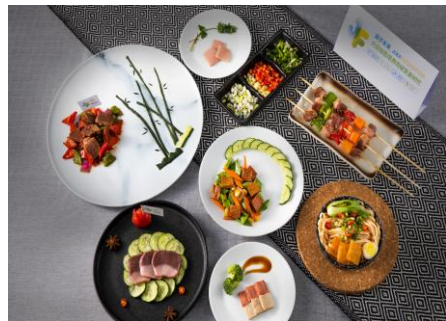
Integrilcluture Japan



- Used cultured duck liver cells as food material.
- Collaborated with the corporation “Plan·Do·See” to develop innovative restaurant foods.

[87]

Joes Future Food China



- Conducted the first tasting event for cell-cultured meat in China.
- Uses stem cell differentiation technology and serum-free culture media.
- Research is underway to produce pork products (pork belly, fat, skin, etc.) through cell culture.

[118]

Vow Food Australia



- Conducted cultivated Japanese quail dumpling tasting event in Texas.
- Launched mammoth meatballs at the Nemo Museum in Amsterdam.

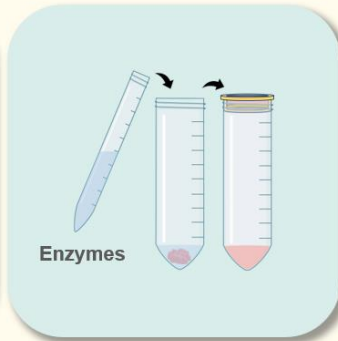
[119]



1. Muscle tissue collecting



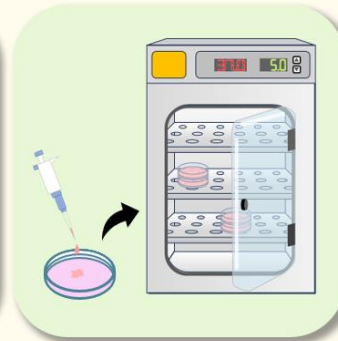
2. Mincing



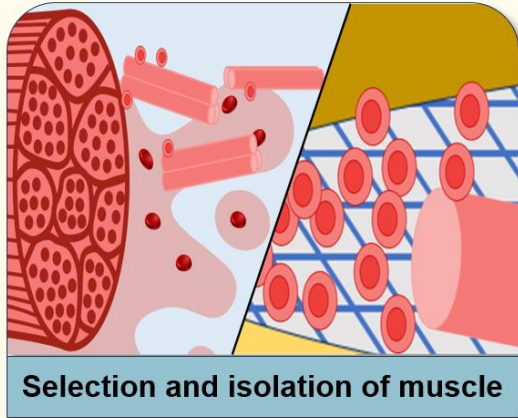
3. Digestion & filtration



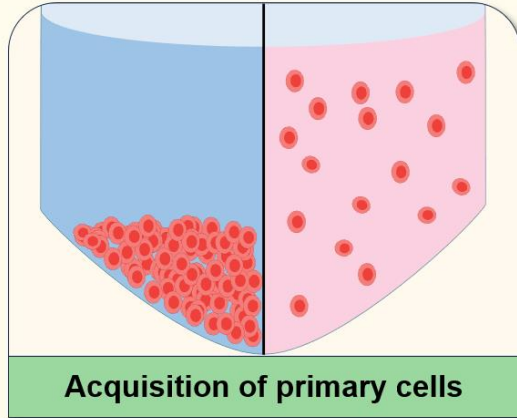
4. Centrifugation & suspension



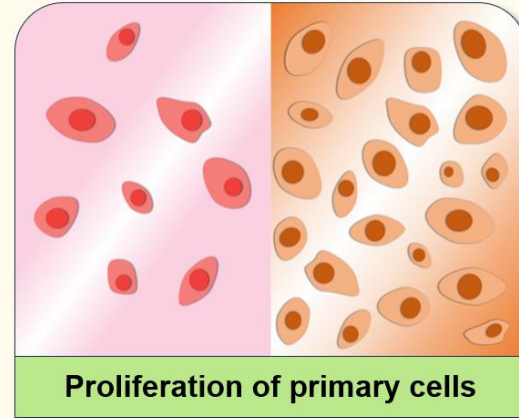
5. Cell culture & incubation



Selection and isolation of muscle



Acquisition of primary cells



Proliferation of primary cells

1093

1094 Fig. 1. Basic procedure for cultivated meat production from primary cells