


Review

# Trends and Technological Challenges of 3D Bioprinting in Cultured Meat: Technological Prosppection

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**Abstract:** Cultured meat presents a possible alternative to conventional meat products and may be used to address growing food demands attributable to global population growth. Thus, a comprehensive technological prosppection of the scientific literature related to cultured meat produced by 3D bioprinting is of great interest to researchers. The purpose of this article is to review and analyze published studies related to the biofabrication of cultured meat using 3D bioprinting techniques. The growing number of related publications in recent years highlights that cultured meat has gained traction in the scientific community. Furthermore, private companies and startups have contributed to advancements in the biofabrication of cultured meat for consumption, illustrating that cultured meat as a conventional meat substitute is already becoming reality. However, like any scientific advance, 3D bioprinting of cultured meat faces challenges involving regulation, acceptance, the selection of ideal biomaterials and cell lines, the replacement of fetal bovine serum (FBS), and attaining a texture and nutritional value similar to those of conventional meat.



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**Keywords:** cultured meat; 3D bioprinting; technological prosppection; bibliometric analysis; VOSviewer

## 1. Introduction

The global population continues to expand quickly and is expected to reach nearly 10 billion people by 2050 [1]. Investment in innovations that allow for the expansion of the food sector, while minimizing the social compensation and ecological damages resulting from large-scale food production, will be necessary in order to meet future demands [2]. The current agro-food system has a considerable negative impact on the environment; livestock farming contributes to the occupation of large areas of land, in addition to high greenhouse gas emissions [3,4].

Given the challenges facing conventional meat production, traditional livestock farming is unlikely to meet the increased demand for meat required to sustain an increasing global population [5]. Cultured meat has advantages over other alternative proteins, such as plant-based proteins, algae, and insects. Because it is an animal-based protein, cultured meat can potentially replace animal products directly and thus, for example, overcome the concerns that some consumers may have about the taste and nutrition of plant-based proteins [6]. Therefore, cultured meat presents an exciting alternative dietary protein source due to its practicality, environmental and ecological benefits, and potential economic implications. An additional benefit of cultured meat is that the facilities necessary for its production do not require the extensive ecological conditions and availability of land

suitable for grazing of traditional livestock farming. However, the production process necessary for large-scale commercial production will require technological advances, financial investment, regulatory guidance, and favorable market dynamics [2]. Moreover, the current method of meat production is challenged by ethical considerations concerning animal welfare and public health. Because cultured meat is almost exclusively produced in the laboratory, there is a much lower risk of acquiring zoonotic diseases than in conventional meat production practices, and animal welfare is preserved as no animal slaughtering is required for production [7].

Cultured meat production derives from tissue engineering practices used in regenerative medicine. This technology involves culturing precursor cells and their subsequent deposition in an organized and controlled manner so as to allow for the replication of the natural animal tissue under *in vitro* conditions, which can even be reflected in different cuts of meat. The process begins with a small biopsy from the source animal to obtain the required cells, followed by the proliferation of said cells in the appropriate culture media. These cells will then differentiate into muscle, fat, and connective tissue—products that will then be combined with different biomaterials to engender the 3D-shaped tissue [8,9]. Spinning techniques, cell stratification, and 3D bioprinting have been utilized to improve the construction of cultured tissue and its resultant characteristics, with the primary goal of increasing its similarity to *in vivo* animal tissue. These techniques can produce highly aligned fibers with a good distribution that resembles animal muscle tissue [10].

Among the 3D tissue production techniques mentioned above, 3D bioprinting has stood out as one of the primary methods for the biofabrication of cultured meat. This technique can improve the distribution of macromolecules and cells within the tissue, generating final products with improved organoleptic properties. This method allows for the precise deposition of cells, micronutrients, technological aids, and biomaterials in predefined locations and shapes and offers several advantages compared to other biofabrication methods [9,11]. The term “biofabrication” refers to products that are generated from the combination of biomaterials, living cells and their bioproducts, and other biomolecules, and that are structured through bioprinting or assembly [12].

Three-dimensional bioprinting is a technique very similar to conventional 3D printing. However, instead of traditional inks and materials, 3D bioprinting uses “bioink” which consists of hydrogels, a combination of biomaterials, cells, and other biomolecules of interest. According to the standard of the American Society for Testing and Materials (ASTM), 3D printing is mainly based on the following processes. In 3D bioprinting, the primary methods are extrusion-based, jetting-based, and vat photopolymerization-based bioprinting [13]. In extrusion-based bioprinting, the bioink is deposited with high precision, obtaining customized 3D structures with good structural integrity due to the continuous deposition of filaments. The entire process of bioprinting is carried out under the control of a computer [14]. Jetting-based bioprinting can produce ink droplets with controllable size and low volume, depositing the ink in specific locations with high precision and without contact. Employing this technique, it is possible to use a variety of biomaterials as well as the incorporation of living cells [15]. Finally, Vat polymerization-based bioprinting is an emerging technology in the biofabrication of scaffolds applied in tissue engineering, used for its high resolution compared to other bioprinting technologies [16].

Nowadays, companies have been contributing to advancing studies related to the biofabrication of cultured meat using the 3D bioprinting technique. For example, 3D Bioprinting Solutions, in partnership with KFC (Kentucky Fried Chicken), plans to produce and market cultured nuggets. 3D Bio-Tissues Ltd (3DBT) has also been partnered with CPI (Independent Center for Technological Innovation) and the United Kingdom government’s High-Value Manufacturing Catapult to improve cell culture media for the cultured meat industry. Japan’s Nissin Food Holdings is partnering with the University of Tokyo to develop printed meat cubes [17]. In addition to cultured meat, a wide variety of food types have been successfully manufactured using 3D printing technology, including chocolate, cakes, and breads [18].

As cultured meat production via Bio 3D becomes more commonplace, the research investment in this area also increases. For this reason, a comprehensive technological prospection of the scientific literature related to cultured meat produced via 3D bioprinting is of great interest to the scientific community.

Technological prospecting can use bibliometric analysis as support for exploring and analyzing large volumes of data that can be used to identify emerging trends, research components, and patterns of collaboration on a specific research topic. Thus, it is possible to obtain a comprehensive view of a field of knowledge and to identify and characterize knowledge gaps, how they can be filled, and what kind of information may be missing. The bibliometric methodology applies quantitative techniques focused on analyzing variables related to publication and citation metrics [19,20].

Therefore, this study aimed to carry out a technological prospection of recent trends on cultured meat produced by 3D bioprinting, to investigate related research topics, and to illustrate the advances and challenges which have emerged in this area of research in recent years.

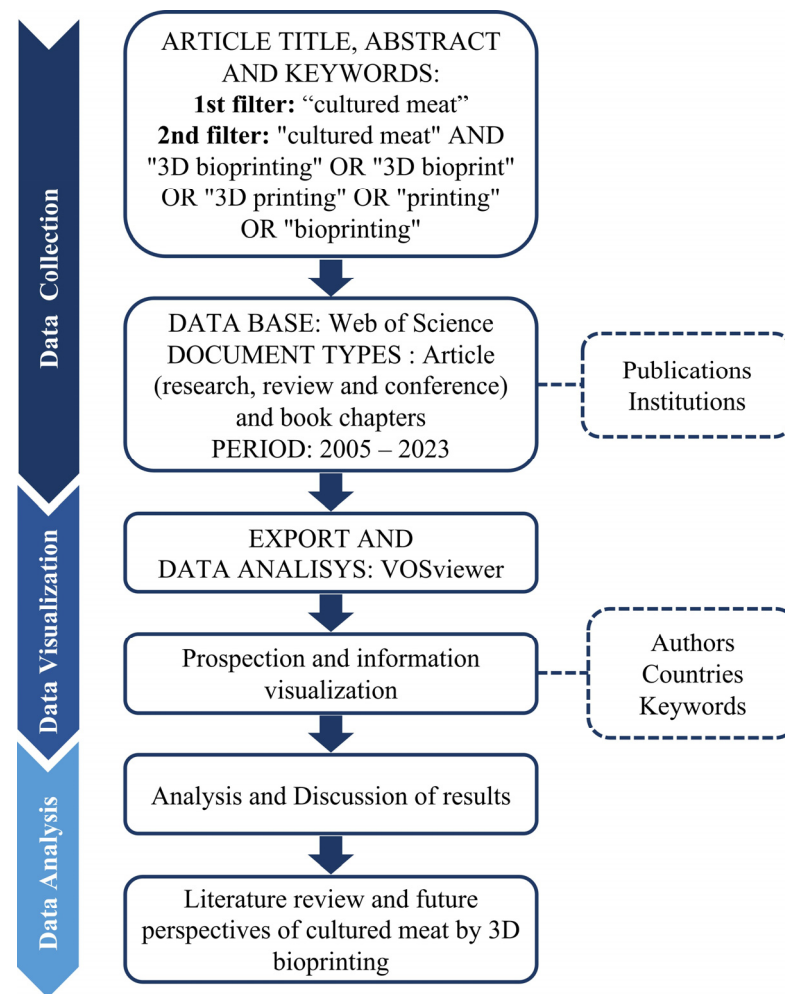
## 2. Materials and Methods

The data search for this technological prospection was performed on 1 June 2023 using the WoS database. WoS covers a wide range of studies and offers a more general and comparative view of publications in a specific field of knowledge [21]. Bibliometric analysis of the scientific publications showed that there are several terms that refer to cultured meat, such as “in vitro meat”, “cultivated meat”, “clean meat”, “cell-based meat”, “lab meat”, “artificial meat”, and “synthetic meat” [22,23]. However, the most commonly used term is “cultured meat”, hence its consideration in this study [23].

The analysis of the publications was made using two filters: the first one considered the keyword “cultured meat” for a broader analysis, and 643 documents were retrieved using this filter; filter two was applied for a more specific analysis, using the following keywords and Boolean operators: “cultured meat” AND “3D bioprinting” OR “3D bioprint” OR “3D printing” OR “printing” OR “bioprinting,” resulting in 39 documents. Among these, those articles with the highest number of citations were analyzed, in addition to the most productive countries and funding agencies. Additionally, from the co-occurrence analysis, a keyword network map was generated using the VOSviewer software.

The software VOSviewer (version 1.6.17, Leiden University, Leiden, The Netherlands) was used to perform technological prospection in this study. This software allows for creating, visualizing, and exploring maps based on bibliometric network data. Output results are displayed in interlocking circles to facilitate viewing existing connections between bibliometric data [24]. The distance between two or more circles indicates the strength of the link between the terms represented by each, and the size of the circles is correlated with the frequency of appearance of the terms [25,26]. Different term groups are represented by different colors and are named “clusters”. The number of clusters in each network map can vary depending on the number of links. Considering the connections between keywords in each cluster, the relevant themes were identified and discussed in detail.

Figure 1 shows the flowchart of each step followed in the technological prospection: (i) definition of search criteria with which to identify records in the WoS database and refine the retrieved records (data collection phase); (ii) exporting of documents to the VOSviewer software for technological prospection of publications, authors, countries, institutions, journals, and areas (data visualization phase); (iii) data analysis to identify the main themes discussed in research concerning the 3D bioprinting of cultured meat.



**Figure 1.** Data Flowchart. Methodology adopted for the technological prospection.

### 3. Results

#### 3.1. Publication Analysis

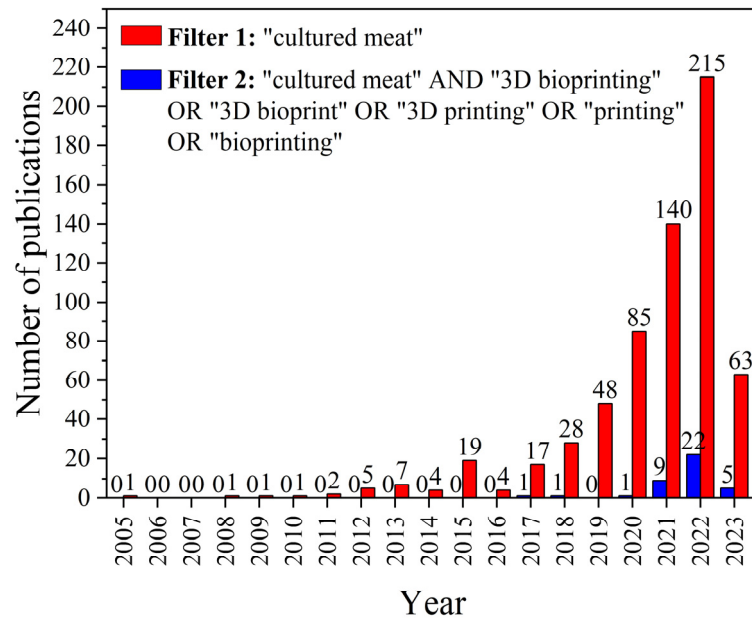
Figure 2 shows the comparison of the distribution of publications between Filter 1 and Filter 2. The first publication about cultured meat was in 2005, entitled “in vitro cultured meat production” [27]. In 2006 and 2007, there was no registry of publication on the subject. From 2008 to 2014, there were few publications on cultured meat. In 2015, there was a peak in the number of publications compared to previous years, registering 19 documents published. This considerable increase was due to the fact that laboratory-cultured meat became commercially available for the first time this year. However, in the following year (2016), the number of publications dropped to four. Since 2017, the number of publications has progressively increased, which shows the constant evolution and interest in this topic.

Analyzing publications using only Filter 2 (Figure 2), the first document was published in 2017. After this period, the number of publications was stagnant until 2020. In 2021, only nine publications were registered. In 2022 alone, 22 publications were recorded. The expectation is that the total number of publications will surpass this number by the end of 2023 for both searches. These data suggest that the use of the 3D bioprinting technology to produce culture meat is new; hence, there are still few published works on the subject.

#### 3.2. Document Analysis

Table 1 presents the 10 most cited articles between 2005 and 2023 in WoS using Filter 2. Although the number of citations does not necessarily indicate the quality of an article, it is a reliable indicator of its impact or visibility. The top five articles in this list presented

detailed content on the main topics involving the 3D bioprinting of cultured meat and alternative proteins.



**Figure 2.** Comparison of the distribution of publications between Filter 1 using the keyword “cultured meat” and Filter 2 using the keywords and Boolean operators “cultured meat” AND “3D bioprinting” OR “3D bioprinting” OR “3D printing” OR “printing” OR “bioprinting”.

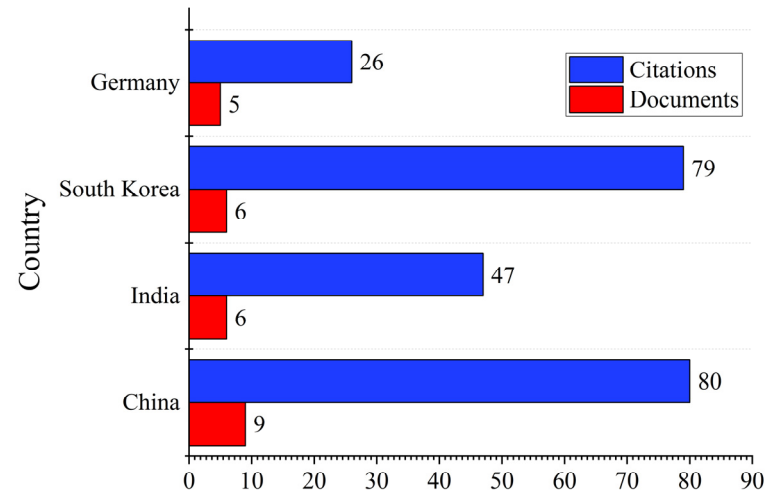
**Table 1.** The 10 most cited articles between 2005 and 2023 in WoS using Filter 2.

Title	Journal	Citations	Author
Engineered whole cut meat-like tissue by the assembly of cell fibers using tendon-gel integrated bioprinting	Nature Communications	68	Kang et al. (2021) [28]
3D Printing of cultured meat products	Critical Reviews in Food Science and Nutrition	55	Handral et al. (2022) [29]
Food of the Future? Consumer Responses to the Idea of 3D-Printed Meat and Insect-Based Foods	Food and Foodways	37	Lupton and Turner (2018) [30]
Scaffolding Biomaterials for 3D Cultivated Meat: Prospects and Challenges	Advanced Science	34	Bomkamp et al. (2022) [31]
Artificial meat and the future of the meat industry	Animal Production Science	25	Bonny et al. (2017) [32]
3D-printable plant protein-enriched scaffolds for cultivated meat development	Biomaterials	23	Ianovici et al. (2022) [33]
3D printing: Development of animal products and special foods	Trends in Food Science & Technology	13	Bhat et al. (2021) [34]
The Epic of in vitro Meat Production-A Fiction into Reality	Foods	10	Balasubramanian et al. (2021) [35]
Scaffolds for Cultured Meat on the Basis of Polysaccharide Hydrogels Enriched with Plant-Based Proteins	Gels	8	Wollschlaeger et al. (2022) [36]
Scaffolding technologies for the engineering of cultured meat: Towards a safe, sustainable, and scalable production	Trends in Food Science & Technology	7	Levi et al. (2022) [37]



### 3.3. Country Analysis

Figure 3 presents the main countries and the number of published documents and citations. Only four of the twenty-seven countries publishing on this topic met the minimum of five published documents. China had the highest number of publications on the subject, with nine publications and 80 citations. India and South Korea followed, each with six published documents. Finally, there was Germany, with five published documents.



**Figure 3.** Distributions of published documents and the number of citations by countries using Filter 2 (“cultured meat” AND “3D bioprinting” OR “3D bioprint” OR “3D printing” OR “printing” OR “bioprinting”).

Three countries that did not meet the criteria of two minimum published documents, although they are currently active in the production and consumption of cultured meat, were Japan, Israel, and Singapore.

### 3.4. Keyword Analysis

To critically analyze the main themes of research related to “cultured meat” linked with “3D bioprinting technology”, it is important to extract keywords with the highest occurrence among the retrieved documents. Analyzing these keywords is essential to determining trends and emerging themes and to identifying hotspots that may represent interesting future fields of research, development, and innovation.

The analysis of keywords related to “cultured meat” and terms related to “3D bioprinting” (Filter 2) resulted in the co-occurrence of 318 keywords. Among them, only 33 (10.4%) reached the threshold of at least three co-occurrences in the same document. The network of key terms is shown in Figure 4.

The keywords obtained during the search process were classified in four clusters. The terms with major co-occurrence in the networks were “cultured meat” ( $n = 25$ ) and “3D printing” ( $n = 9$ ), as expected since they were the keywords used in the search. The term “cultured meat” has been broadly used to characterize the studies of cell-based meat production. These words have great prominence and importance to the theme, having connections to all the words on the network. Analyzing the clusters, it is observed that in cluster 1, the main term is cultured meat, and it is contextualized with other terms such as 3D printing, stem-cells ( $n = 9$ ), food ( $n = 6$ ), consumer perception ( $n = 4$ ), alternative proteins ( $n = 3$ ), soy protein ( $n = 3$ ), and future ( $n = 3$ ). In cluster 2, the terms are related to bioink terms themselves ( $n = 4$ ) and with other components thereof, such as alginate ( $n = 4$ ), collagen ( $n = 3$ ), hydrogels ( $n = 3$ ), skeletal-muscle ( $n = 5$ ), satellite cells ( $n = 4$ ), and tissue engineering ( $n = 4$ ). In cluster 3, emphasis is on terms related to the regulation of cultured meat, regulatory challenges ( $n = 4$ ), 3D bioprinting ( $n = 5$ ), scaffolds ( $n = 5$ ), protein ( $n = 6$ ), muscle ( $n = 4$ ), and tissue ( $n = 5$ ). Finally, in cluster 4, the terms are differentiation ( $n = 8$ ), challenges ( $n = 7$ ), cells ( $n = 5$ ), and biofabrication ( $n = 3$ ).



## 4. Discussion

### 4.1. Overview of Main Studies

The first cultured burger was produced and tasted at a demonstration in London, England, in 2013, making that year a significant date in the history of cultured meat [38]. Dr. Mark J. Post, from Maastricht University in the Netherlands, was responsible for this landmark demonstration. He produced the cultured burger using the techniques and principles of muscle tissue engineering by cultivating *in vitro* stem cells taken from bovine skeletal muscle until edible filaments resembling natural meat were formed [39]. In 2015, the Mark Posts lab reported a drastic drop in cultured hamburger production costs, i.e., from USD 325,000 to about USD 12 per hamburger or about USD 80 per kilogram of meat produced [40,41]. In 2020, there were more than 70 startups focused on developing innovations in the cultured meat sector, and as of 2020, around 25 countries are home to at least one company focused on the production of cultured meat [17].

Among the 39 documents retrieved in Filter 2 (Table 1), the most cited article (68 citations) was authored by Kang et al. [28]. They used “Tendon-gel-integrated bioprinting” (TIP) technology to biofabricate fibers that simulate muscles, adipose tissue, and capillaries. These constituents were later manually assembled to mimic the histological structure of a conventional steak with a diameter of 5 mm and a length of 10 mm.

The second most cited study, with 55 citations, was a review article published by Handral et al. in 2022 [29]. This paper highlights the benefits of 3D bioprinting technology for the scalable and reproducible biofabrication of cultured meat. The authors emphasize that 3D bioprinting can offer unique solutions to vital questions about the biofabrication of cultured meat, mainly those concerned with regulating the nutritional contents of cultured meat, including its protein and fat components, as well as questions related to the texture of the final product.

In the third most cited study ( $n = 37$ ), Lupton and Turner [30], the authors conducted an exploratory qualitative study investigating consumer attitudes toward cultured meat produced via 3D bioprinting and insect-based foods. According to the study, participants recognized the potential and benefits of these alternative food sources. They mentioned that the safety, flavor, and similarity to the original protein sources are vital factors in the acceptance of these products. In general, however, the participants expressed levels of concern and insecurity regarding producing these products, with only a few individuals reporting real interest in tasting the food and supporting the associated technological development.

The fourth most cited article which resulted from this search ( $n = 34$ ), written by Bomkamp et al. [31], discussed the properties of vertebrate skeletal muscle that must be successfully replicated in order to obtain an acceptable product, as well as the current state of scaffold innovation within the cultured meat industry. They highlighted the materials and promising fabrication techniques that may be applied in the development of cultured meat. The authors also provided recommendations on how to support the growth of high-quality cultured meat production while minimizing biofabrication costs for future research on scaffolds. Interdisciplinary collaboration is crucial to overcoming the challenges arising from the innovation that is the biofabrication of cultured meat. Specialists in tissue engineering, food science, chemistry, material science, mechanical engineering, and many other fields will need to operate at the intersections of their collective knowledge in order for this technology to reach its full potential.

Finally, the fifth most cited review ( $n = 25$ ), Bonny et al. [32], analyzed the advantages and disadvantages of different methods of artificial meat production, comparing “cultured meat” with “modified livestock systems” and “meat substitutes”. They concluded that new technologies for 3D printing of cultured meat have emerged to meet consumer demands and address concerns regarding food safety, environmental impact, and animal welfare. However, there remain many challenges for this technology to overcome before it can achieve market accessibility on a large scale.

The other articles, with fewer citations, provided relevant technical discussions, such as the best type of cell to be cultured and the best cell sources. They detailed differ-



ent biofabrication techniques and explored which factors influence the choice of culture medium and scaffold molding. They also investigated which bioreactors would be most suitable for the process.

#### 4.2. Status of Cultured Meat in the World

Considering that China is the world's largest consumer of conventional meat [42], a shortage of traditional meat could soon present a major sustainable development challenge for the country. As domestic meat production is increasing at a rate lower than the demand, China must keep exploring and adopting more effective alternatives [22]. China's Ministry of Agriculture and Rural Affairs has included cultured meat and other "future foods" such as plant-based eggs as part of its food security project to overcome this challenge. This strategic initiative could accelerate the country's regulatory timeline for cultured meat, drive more research and investment in the alternative protein industry, and encourage wider consumer acceptance [43–45].

India's leadership in cultured meat may reflect a national policy aimed at further developing the manufacturing sector, namely, the "Make in India" policy. This policy includes new initiatives that stimulate foreign direct investment and protect intellectual property (Creative India), facilitating business and thus promoting the creation of technology-based industrial start-ups (Start-up India) [46].

Mumbai was the first city in the world to host a research center for laboratory-grown "clean meat", namely, the Center for Excellence in Cellular Agriculture, based on the culture of animal cells extracted via a painless procedure. The priority of their studies is to promote technological advancement in this industry with the development and optimization of the most relevant cell lines [47].

Australia has become the latest country to join the emerging laboratory cultured meat industry, with two producers. One producer is Vow Food, a biotechnology start-up based in Sydney that has attracted about USD 20 million in venture capital. Vow Food intends to reproduce meats that are already produced and marketed on a large scale in Australia, with a focus on premium quality [48]. According to Glen Neal, general manager of risk management and intelligence at FSANZ (Food Standards Australia and New Zealand), culture meat could be on shelves in 2023 [49].

In Japan, companies are already working to bring cultured meat to local consumers. To ensure the successful integration of the new product into the population, Japan's Ministry of Health has assembled a team of researchers to investigate its safety. The team will help to advise the authorities on any health risks associated with cultured meat and the regulations that will be needed for this sector [50,51].

Recently, the Israeli biotechnology company "Future Meat" (Jerusalem, Israel) opened its first factory which produces meat grown from chicken-, pork-, and lamb-derived cells [47]. This achievement marks a major step along the path of technological development for the cultivated meat market, serving as a catalyst to developing the product on an industrial scale. Another Israeli company, "MeaTech 3D Ltd." (Ness Ziona, Israel), announced the printing of a cultured steak of 104 g made from fat and muscle cells using its own 3D bioprinting technology. It is believed to be one of the largest cultured steaks produced in recent years [52].

In December 2020, the Singapore Food Agency (SFA) (Singapore) was the first regulatory authority in the world to approve the commercialization of a cultured meat product, after it was considered safe for consumption [53]. According to a study by Singapore Management University (SMU) (Singapore), conducted with locals and USA consumers, the product was better accepted by Singaporeans than Americans. This greater acceptance by Singaporeans may be a reflection of a local cultural trait called "Kiasuism" which describes a desire to be pioneer when compared to other nations, expressed by a greater acceptance of new foods [54].

With the higher co-occurrence of keywords (Figure 4), cultured meat can be considered the food of the future. In 2012, the United Nations' Food and Agriculture Organization

(FAO) (Rome, Italy) estimated that global demand for meat would reach 455 million tons by 2050 (increasing 76% compared to 2005) [55]. This increase in demand, combined with sustainability policies, has been revolutionizing production processes, especially in food chain sectors, as can be seen in the emergence of cultured meat production [56]. Traditional meat production has some disadvantages when compared to this new technology, such as animal slaughtering, inadequate breeding environments, the transmission of some diseases, and the generation of greenhouse gases (GHG) such as methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) [57,58].

#### 4.3. Biofabrication of Cultured Meat

##### 4.3.1. Animal Source

Despite its benefits, producing biofabrication cultured meat takes time and effort. The first challenge lies in choosing an appropriate animal for the cell biopsy. The selection of the donor animal should not be random, as numerous factors affect satellite cells (adult skeletal muscle stem cells), such as age, sex, and rearing conditions [59].

Advances in engineering production are required in order to improve cell culture efficiency and scalability and tissue engineering to select biomaterials and form 3D meat products that mimic their animal-derived equivalents. Furthermore, advances in food engineering and nutrition are still needed in order to make a final product palatable, sensorially acceptable, and nutritionally interesting [60].

##### 4.3.2. Cells

The main challenge in tissue culture for meat production is the obtention of an ideal cell source since a large number of homogeneous initiator cells is essential to promoting cell proliferation and differentiation effectively [61]. The process of cell proliferation and expansion is directly related to the industrial scalability of cultured meat production [62]. Currently, some of the most studied cell lines used in cultured meat production are satellite cells [63–65], pluripotent stem cells [66], mesenchymal stem cells (MSCs) [67], and dedifferentiated fat cells (DFAT) [68].

Since all the cell lines used in cultured meat are adherent, it is necessary to have an appropriate matrix serving as a support (scaffold) in order that the cells can successfully grow and differentiate [69]. Hydrogels are widely used as a cellular matrix in bioinks, and they can be adapted to mimic or replace native tissue due to their high water content, mechanical properties, and 3D network structure [70,71]. Bioinks must have specific properties for tissue engineering, such as biodegradability, biocompatibility, printability, high mechanical integrity, chemical stability, non-immunogenicity, non-toxicity, and insolubility in the cell culture medium [72]. These bioinks are made by combining cells and structural-based biomaterials to biofabricate tissue-like constructs [73,74].

##### 4.3.3. Biomaterials

It is in this context that biomaterials are applied. Several supporting biomaterials have been used successfully in adipose tissue engineering and may be viable options for cultured meat production. The material used for cultured meat must be biocompatible and biodegradable, safe for consumption, and mimic the texture of traditional meat [75].

Amongst the different potential biomaterials, biopolymers stand out. This material class has been extensively studied in the biofabrication of cultured meat as they provide a temporary mechanical support structure for cell adhesion and proliferation, simulating the extracellular matrix (ECM) [76]. Biopolymers can be divided into two categories: the naturals and the synthetics. However, it is essential to note that biopolymers derived from animals should be avoided because one of the principal drivers for cultured meat production is animal welfare [77,78]. For this reason, biopolymers derived from algae and plants have been widely studied for their application in cultured meat production. The main biopolymers derived from algae used in this process are alginate [79–82], agarose [77,83]

and Konjac gum [84], and derived from plants are soy protein [85–87], glutenin [88], starch [89,90], and bacterial cellulose [91].

Alginate was the biomaterial with the highest co-occurrence in the network generated from our keywords search (Figure 4), linked with the term “bioink”. This polymer is a natural polysaccharide extracted from brown algae and has been used as a matrix in the 3D cell culture, providing support for cell growth and integration [92]. Alginate is also widely used in the food industry as an additive [93]. Due to its capacity to produce hydrogels via ionic crosslinking, alginate is one of the most promising and most applied natural biopolymers in tissue engineering [94,95] and, consequently, in the biofabrication of cultured meat. However, pure alginate lacks functional groups that stimulate cell adhesion and proliferation. It also has low mechanical properties [82,96]. Alternative studies have shown that combining alginate with other biomaterials—for example, collagen and gelatin—revealed promising results, mainly improving cell adhesion and proliferation. However, the use of animal-derived materials is contrary to the concept of cultured meat, where the main purpose is to avoid the slaughter of animals. Therefore, studies using biomaterials from other sources, such as plants, algae, and microorganisms, have been conducted [84].

Ianovic et al. [33] used the 3D printing technique to print scaffolds using modified alginate-based bioinks, enriched with isolated pea and soy protein. The bioinks enriched with pea and soy proteins showed better stability and rigidity than those produced with modified alginate, increasing muscle cell proliferation by up to 90%. These results may be of great interest in the field of cultured meat biofabrication.

#### 4.3.4. 3D Bioprinting

Three-dimensional bioprinting is one of the most promising technologies with which to biofabricate scaffolds for tissue engineering, both for regenerative medicine and for the food sector, and especially with respect to the biofabrication of cultured meat [97]. The main 3D bioprinting techniques used for the biofabrication of scaffolds loaded with cells are extrusion, jetting, and Vat photopolymerization. The extrusion-based bioprinting technique can be pneumatically driven (air pressure) or mechanically driven (piston or screw).

This technique is the most commonly used for various applications in tissue engineering due to its versatility and wide availability [98]. The main advantages of the extrusion-based method are its scalability and ability to print a wide range of high-viscosity materials and high cell concentrations [99]. However, high-viscosity fluids are the most suitable for this technique, providing adequate mechanical support for 3D scaffolds [100]. The jetting-based bioprinting technique is capable of printing biological materials with optimized speed, accuracy, and resolution [101]. Despite its advantages, this technique can only be used for low-viscosity materials, which leads to a tendency towards nozzle clogging [102]. In vat photopolymerization-based bioprinting, noted for its stereolithography technique, it is possible to obtain 3D scaffolds with high speed and high resolution [59]. This technique is based on the polymerization of light-sensitive polymers. UV radiation used in conventional stereolithography can be harmful to cells and can trigger mutations [103]. In common, these 3D bioprinting techniques have limitations according to the properties of the bioinks, such as their viscosity, directly impacting the bioprinting quality of the material [100,104,105]. Despite the numerous advantages of 3D bioprinting, the success of this technology depends directly on a few factors, including the biomaterial properties, the crosslinking process, print fidelity, stability, and cell viability, as well as the physical, mechanical, and rheological properties of bioinks [106,107]. One of the most challenging properties in the 3D bioprinting process is cell viability.

In addition to the technical challenges, 3D bioprinting presents ethical issues that need to be addressed, such as intellectual property issues, safety regulations, and concerns about misuse of the technology [108].

In the extrusion-based bioprinting process, the bioink is extruded through a needle under pressure, which can cause shear stresses between the bioink and the wall of the

printing nozzle. If these stresses exceed a specific limit, they can rupture cell membranes, causing reduced cell viability [109]. Chand et al. [110] used computational simulation to evaluate the maximum shear stress during the extrusion-based bioprinting process. They concluded that in addition to the shear stress that normally occurs in the printing process, the geometry of the nozzle also influences cell viability. In the jetting-based bioprinting process, there are few detailed studies on the impact of droplet velocity and volume on cell viability. Ng et al. [111] used a thermal inkjet bioprinting system to evaluate the influence of drop impact speed and drop volume on cell viability and proliferation. They observed that increasing the concentration of cells in the bioink resulted in slower droplet impact speed, providing greater cell viability and improved print quality by reducing droplet splatter. It was concluded that due to the limited use of biomaterials compatible with the method, in addition to its photopolymerization process, the control of impact velocity and droplet evaporation directly influences cell proliferation and viability [16].

The cross-linking process is another fundamental factor for 3D bioprinting and is directly related to the mechanical properties of 3D structures, as well as to the gelation kinetics, post-print structure fidelity, and viability of encapsulated cells [112]. The most common cross-linking mechanisms applied in the 3D bioprinting field of bioinks are ionic cross-linking, UV photopolymerization, and physical cross-linking [113]. Ionic cross-linking occurs between polymer chains and oppositely charged divalent or multivalent ions. A typical example of ionic crosslinking is the electrostatic interaction between negatively and positively charged alginate chains with  $\text{Ca}^{2+}$  ions [114].

Photocrosslinking has been widely used in 3D bioprinting as it provides spatial and temporal control over the gelation of bioinks [115]. Ultraviolet light sources are most commonly used in the photocrosslinking of bioprinted materials [116]. Usually, photoinitiators are used, and their choice is fundamental in the bioprinting of bioinks. The absorption peak of the photoinitiator is related to the wavelength used. The wavelength has a direct impact on cell viability bioprinted bioinks. Therefore, depending on the wavelength, harm can be done to a cell's nuclear DNA, causing genomic and carcinogenic mutations [117]. To overcome the effects of UV light on cell viability, researchers have investigated the use of visible light in photocrosslinking. Normally, visible light does not impact the biological conditions of bioprinted scaffolds [118].

Finally, in physical crosslinking, no crosslinking agent is incorporated into the reaction. This is an advantage of physical crosslinking, as the use of crosslinking agents can affect the integrity of the substances [119], in addition to their potential cytotoxicity to living cells. The main parameters that can promote physical crosslinking are pH variation, temperature, thermodynamics, and physical–chemical interactions: for example, hydrophobic interactions, hydrogen bonding, and charge interaction [120].

#### Biofabrication of Cultured Meat Using 3D Bioprinting

Three-dimensional bioprinting technology allows for the deposition of bioinks in a layer-by-layer manner, with precise control of the functional component deposition and spatial arrangement [121]. Three-dimensional bioprinting has several advantages in the biofabrication of cultured meat, such as the possibility of meeting specific needs in terms of calories, nutrients, shape, texture, and flavor of the product [122].

The texturing and flavoring of cultured meat comes from two essential steps in the production process: the first phase occurs before the 3D bioprinting process, and consists of cell proliferation, commonly performed in a bioreactor, aiming at the obtention of the maximum possible number of cells; the second phase takes place after 3D bioprinting, in which the bioprinted scaffolds already containing the cells are taken to the bioreactor for cell differentiation and tissue maturation [123,124].

##### 4.3.5. Bioreactor

The bioreactor promotes a favorable environment for cell expansion and supports the diffusion of nutrients through the pores of the scaffolds [125]. It allows for large-scale

cell culture with filtering and replacement medium process throughout the proliferation stage [126]. In recent years, bioreactors have been developed to monitor and mechanically stimulate cell growth. Cacopardo and Ahluwalia [127] redesigned a bioreactor to reproduce the mechanical changes of a liver microenvironment and, at the same time, to monitor the mechanical properties of cell-loaded gelatin hydrogels in vitro. The authors observed that increases in scaffold elasticity are related to an increase in cellular stress. According to the authors, this study can be implemented to model other processes, and thus evaluate the parameters that influence tissue stiffness, the complex system of cell division, ECM differentiation and synthesis, and density and crosslinking.

Todros et al. [128] designed a bioreactor for the radial stimulation of porcine-derived diaphragmatic scaffolds with the aim of promoting cell alignment and the development of radially oriented muscle fibers. Regarding the biofabrication of cultured meat, cell and fiber alignment influences the quality of the meat product, mainly with respect to its texture. Therefore, bioreactors with monitoring and mechanical stimulation represent a positive development in the field of cultured meat, inspiring other researchers.

#### *4.4. Technological Challenges*

The three-dimensional bioprinting of food is one of the emerging technologies of Industry 4.0, enabling the production of on-demand, complex, and personalized food [129]. This technology has several advantages in terms of health, economy, and the environment, with the potential to revolutionize the manufacturing of alternative food sources. Through the use of this technology, an improvement in the functional and nutritional properties of printed foods is expected with the advent of Industry 4.0 innovations, thus increasing the chance of consumer acceptance [130].

While 3D bioprinting is a promising technology in the biofabrication of cultured meat, it is still at an early stage, with many challenges still to overcome, as it requires non-animal-derived scaffold compositions that are both printable and edible [33], in addition to challenges related to regulation, scalability, acceptance, and cost. Another much discussed point is the continuous supply of cells and/or tissues obtained from animals via biopsy and the methods involved [131]. In addition, the use of fetal bovine serum (FBS) in cell culture media is contrary to the basic principle of cultured meat. It is necessary to find reliable alternatives to FBS, ensuring sustainability and ethical development [59].

The prospect is that in the future, with viable biotechnological alternatives and regulation of the technology, the biofabrication of cultured meat via 3D bioprinting will be scalable, sustainable, and efficient, with the expectation that cultured meat will have texture and flavor characteristics reminiscent of, or even better than, natural meat, as well as being more economically viable.

#### *4.5. Acceptance and Regulation*

In addition to the technical challenges to be overcome in the biofabrication of cultured meat, such as with respect to the appearance, structure, texture, flavor, and nutritional composition of cultured meat [132], cultured meat production must consider several social issues, including consumer appeal and acceptance [133,134]. Acceptance of cultured meat has been the subject of studies in several countries [135–137]. Pakseresht, Kaliji, and Canavari [138] performed a systematic review which aimed to identify, evaluate, and summarize the empirical results of published studies, providing a broad overview of recent empirical evidence on consumer acceptance of cultured meat. For the review, the authors considered the WoS, Science Direct, and Scopus databases throughout 2008–2020. After screening, 43 articles met the selection criteria defined by the authors. They concluded that the most important factors influencing consumer acceptance/rejection of cultured meat include public awareness, perceived naturalness, and food-related risk perception. Ethical and environmental concerns have made consumers willing to pay higher prices to purchase meat substitutes, but not necessarily cultured meats. In addition, food neophobias related



to safety, health, and uncertainty also appear to be important barriers to wide acceptance of this technology.

Another challenge facing the production of cultured meat that was evident in the present study (Figure 4) is the regulation of this product. Regulatory approaches differ substantially between countries and continents [139]. The Good Food Institute (GFI) has encouraged the development of alternative food sources among research groups and companies from different countries [140]. The GFI surveyed countries that are using existing food regulations or developing new regulations to evaluate novel products such as cultured meat [141]. Details of this survey by country and region are presented in Table 2.

**Table 2.** Status of regulation of cultured meat by country and region.

Country/Region	Status
Australia-New Zealand	According to Food Standards Australia New Zealand (FSANZ), concerning the regulation of “novel food”, cultured meat manufacturers must apply for inclusion of their products in the approved new foods list if they wish to sell them in any country. This requires an assessment of safety in the production process carried out by FSANZ, which is likely to last at least 14 months. The safety assessment is intended to establish and confirm that the food does not present a health risk.
Canada	Cultured meats are characterized as “novel foods” and detailed information must be submitted in a pre-market approval application. Information includes evidence that the food is safe for consumption, such as molecular characterization, nutritional composition, toxicology and allergenicity, and types and levels of chemical contaminants.
United States of America	The services of the US Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) are well documented. On 7 March 2019, the FDA and USDA released a formal agreement outlining their respective oversight roles and responsibilities under this framework and how they will collaborate to regulate the production of cultured meat and its entry into trade.
European Union	The Novel Food Regulation (Regulation (EU) No. 2015/2283) governs pre-marketing authorizations for foods produced from animal cells or tissue culture. However, if genetic engineering is used in the production of cultured meat, the Regulation on Genetically Modified Food and Feed (Regulation (EC) No. 1829/2003) may apply.
United Kingdom	With its departure from the EU, the UK no longer participates in the EU’s common food authorization procedures. From May 2021, any cultured meat company that wants to sell its products in the UK needs to apply for authorization from the UK Food Standards Agency (FSA).
Singapore	The Singapore Food Agency (SFA) has published guidance on its requirements for the safety assessment of novel foods, including specific requirements for the information to be submitted for the approval of cultured meat products. On 1 December 2020, the SFA approved the sale of cultured chicken meat from Eat Just Inc., which was the first approval of cultivated meat in the world.
Japan	Depending on the production method, cultured meat already falls under the existing regulatory regime in Japan and may not require pre-market assessment or approval. However, the Japanese government is working to develop a specific regulatory framework, ensuring food safety and consumer acceptance.

There is still no definitive regulation for the production and marketing of cultured meat. Many regulatory issues still need to be addressed through legislation and policies, such as food fraud and mislabeling. In recent years, administrative and public bodies have promoted debates and meetings with the aim of structuring appropriate regulation [142].

## 5. Conclusions and Perspectives

This technological prospection provided a valuable evaluation of the current data regarding 3D bioprinting technology development as it is applied to cultured meat produc-

tion. It was possible to map and analyze the main published documents. The increasing number of publications revealed by this prospection illustrates the growing interest in cultured meat within the scientific community in recent years. When the production of cultured meat is linked to 3D bioprinting technology, the resultant publications are more recent, suggesting that the implementation of this technology is current and promising. Companies and startups have contributed to advancing biofabrication technology to produce cultured meat for consumption. The 3D bioprinting of cultured meat is already a reality. However, like any scientific advance, the 3D bioprinting of cultured meat faces challenges and prospects. The application of biomaterials in an effort to attain the structure, texture, and nutritional value of conventional meat is one of the challenges. In addition, the selection of ideal cell lines and the formulation of culture media for the partial or total replacement of fetal bovine serum (FBS) are still needed. All these processes result in the high cost of this technology. Another challenge is the lack of specific regulation for this type of product, which faces a constantly evolving regulatory scenario. Although acceptance of this technology is still limited among consumers, ensuring that these products meet food safety standards and receive appropriate certifications is key to their global acceptance. The expectation is that cultured meat will significantly reduce the slaughter of animals and the generation of greenhouse gases and promote the conservation of natural resources compared to conventional meat production. Another important point is the food safety of cultured meat, as it is produced in a controlled environment, and the possibility of nutritional customization through 3D bioprinting, which allows for manipulation of the nutritional composition of the cultured meat. As research and development continues to advance, 3D bioprinting of cultured meat has the potential to revolutionize the food industry, providing a more sustainable and healthy future.

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