THE GOOD FOOD INSTITUTE

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An analysis of culture medium costs and production volumes for cultivated meat

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Executive Summary

Emerging technologies across the alternative protein landscape are poised to transform protein production in the coming years by offering higher efficiency, greater consistency, and fewer harms to public health, the environment, and animals than conventional meat production. Within this landscape, the cultivated meat industry – meat produced through animal cell culture, sometimes referred to as cell-based meat, clean meat, or cultured meat – is a relatively young but rapidly growing field. Cultivated meat builds upon deep insights into cell biology and biological manufacturing procured through the development of much more mature industries like biopharma and industrial biotechnology, and these fields serve as informative models for scale-up and growth. At the end of 2016, only four startup companies had formed to commercialize cultivated meat technology. By the end of 2018, there are well over two dozen companies spanning at least nine countries and four continents.

Because of this increase in activity and funding, many forward-thinking industry leaders across diverse sectors are exploring opportunities to contribute to the cultivated meat industry through products, services, and enabling technology development. At the same time, the number of investments into cultivated meat companies has blossomed and these companies are progressing to larger rounds of funding and more rigorous diligence assessments. To inform these strategic business development decisions and investment decisions with insights into challenges regarding scale-up and cost reduction, The Good Food Institute's Science & Technology team has discussed findings from our preliminary cost estimate exercises for the cultivated meat industry with hundreds of individuals across dozens of meetings, workshops, and seminars.

As demand for these insights has increased substantially in recent months, we have decided to develop this analysis into a standalone white paper that is more widely shareable. However, readers should take these findings at face value – as an informative and useful exercise that sheds light on several approaches for achieving substantial progress down the cost curve, not as the final word on what the cultivated meat field can achieve nor as a final prescriptive guide for what the needs and challenges of the field will be. We anticipate that cultivated meat companies will continue to develop innovative new methods and to refine existing approaches to tackle the challenges associated with cost and scale, and their own calculations of production volumes and production costs should be taken into account alongside our independent estimates.

We initially conducted this analysis internally for two purposes: a) to decide whether we felt cultivated meat would ultimately be economically viable at scale and thus worth supporting within our organizational mission, and b) to identify the most compelling opportunities to accelerate progress down the cost curve, which could be through industry partnerships, starting new companies, and/or catalyzing basic research. As our understanding of the industry has deepened, this analysis has been continuously refined and expanded throughout the past 18 months. This publication offers a snapshot of our latest key findings, along with a detailed articulation of the rationale and justification of various assumptions and approximations contained herein (see Table 6).

The key takeaways from this analysis are:

1) It is likely that cultivated meat can achieve price parity with mainstream conventional meat once produced at industrial scale. There is no single path to achieving cost reduction of this magnitude, but rather vast opportunity to leverage a number of different and complementary approaches. We anticipate that many of these factors can be substantially further optimized than reflected in this analysis, as this analysis has intentionally incorporated conservative estimates and assumptions at many levels.

2) Cell culture medium — the most significant cost driver — can be produced completely free of animal-derived components and at scales and price points several orders of magnitude lower than current bench-scale costs without relying on fundamentally new technologies or innovations. While there are many aspects that require optimization, none of the assumptions or estimates within this analysis rely on technological "moonshots" that are unprecedented or that require novel inventions.

3) The yield and efficiency of cultivated meat production can be influenced through many variables within the design and operation of the industrial-scale manufacturing process, and a holistic model can inform tradeoffs between various parameters and design requirements and identify pain points for concerted R&D focus. For example, analyzing the residence time and yield of a batch versus semi-continuous process (and therefore the economics of each approach) can guide cell line developers in determining the desired proliferative capacity of their cells.

Other insights that emerge implicitly from this analysis are opportunities for diverse industries to contribute manufacturing paradigms and novel perspectives to accelerate cultivated meat's progression down the cost curve. There is tremendous opportunity to rethink cell culture medium entirely for the cultivated meat industry. Compared to applications in biopharma, cell therapeutics, or biomedical research, the cultivated meat industry presents a whole new paradigm for cost-benefit analysis and optimization. For example, raw materials like hydrolysates or polysaccharides from large-scale agricultural processing could serve as inputs for cultivated meat medium rather than relying on highly purified amino acids and sugars.

Similarly, operational and process design insights from a wide array of industries — not just from biopharma or other animal cell culture processes but also from food production, fermentation, and even industrial chemistry — should be leveraged for developing industrial production frameworks for cultivated meat. The manufacturing facilities for cultivated meat will likely resemble a food production environment more than a biopharmaceutical manufacturing suite, but it is important to acknowledge which operational insights from each context are indispensable and which are malleable.

This analysis serves as one resource for assessing economic viability, evaluating cost reduction claims made by cultivated meat companies, identifying commercial opportunities within the growing cultivated meat ecosystem, and articulating high-impact research projects for advancing the industry. We are confident in these findings given our current understanding of the industry, and this work has been critically reviewed by over a dozen external experts (ranging from bioprocess engineers in food and biopharma to business development leads at cell culture media suppliers). However, for any of these activities, additional sources should be consulted and any critical assumptions validated with multiple data points. As with all of GFI's white papers, this analysis will continue to be refined and revised as the field matures and more data are gathered, and the authors welcome feedback and critique from experts across a diversity of fields. We hope that this analysis will serve as a foundation for additional analyses that incorporate empirical data, creative solutions, and more sophisticated models.

Table of Contents

List of Tables and Figures

1 Introduction and motivation

The cultivated meat competitive landscape has grown at an astounding pace, blossoming from just four companies to several dozen in less than two years. However, the field still faces shared challenges around scale-up and cost reduction that will benefit from increased involvement by industry partners across diverse sectors and well-informed strategic investors. In order for these potential partners to justify their involvement in the cultivated meat industry, they need access to independent analyses of the potential of cultivated meat to ultimately address and overcome scale-up and cost challenges.

To inform these strategic decisions, we have developed this overview of cell culture medium cost analyses and production volume analyses to estimate realistically achievable production costs for cultivated meat in the relatively short term (i.e. that do not rely on theoretical or unproven technological advances). These insights assist with assessing economic viability, identifying compelling commercial opportunities within the growing cultivated meat ecosystem, and articulating high-impact research projects. Thus, this analysis is intended for a broad but deeply knowledgeable audience, including life science companies, chemicals and commodity suppliers, infrastructure developers, corporate and venture capital investors, market analysts, entrepreneurs, and research scientists.

Section 2 analyzes a number of cell culture medium production and formulation scenarios to assess their impact on the cost of the medium using a widely-used, commercially available animal-free formulation as the basis of the cost modeling exercise. Section 3 translates this culture medium cost model into a more meaningful metric — the cost contribution per kilogram of meat produced — by estimating the medium requirements for producing a batch of cultivated meat. The analysis is then extended by exploring the cost implications of various process design considerations and operational modes in the context of a theoretical industrial-scale manufacturing environment. Wherever assumptions or projections are used throughout the analysis, please refer to Table 6 for more information on their justification as well as a discussion on error estimates and the implications of error on the findings of this work. When parameters have been obtained from similar processes – for example, from parallels in large-scale animal cell culture for therapeutic protein manufacturing or cell therapy – the sources for these parameters are provided along with the rationale for their applicability to cultivated meat production.

This report reflects a generalized hypothetical conception of cultivated meat production and is not modeled on production processes, cell types, technologies, or any other strategic insights that are specific and unique to individual cultivated meat companies. In fact, this analysis purposefully avoids specifying parameters like species of origin or starting cell type(s) and ending cell type(s) because the results of analyzing an agnostic, generalized system serve as guidelines for making those decisions. For example, this exercise allows us to estimate how many population doublings are required for an economically viable industrial-scale process, which informs decisions about cell type selection, cell line modification strategies, and process design.

1.1 Implications of this analysis for informing R&D strategy

Cost calculations such as those presented here can provide critical insights for guiding research and development strategy because they can identify anticipated future bottlenecks that will be encountered in scale-up and cost reduction. This accelerates overall R&D timelines by allowing concerted effort to address these future bottlenecks prior to the point at which they become rate-limiting. For example, much of the dialogue concerning the cost of cell culture medium focuses entirely on the cost of the growth factors to the exclusion of serious discussion of opportunities to optimize the basal medium. Reducing the cost of growth factors is indeed critical in early R&D because they currently comprise over 95% of the cost of representative animal component-free media

formulations, as illustrated in this analysis. However, this analysis also reveals that even if the cost of growth factors were reduced to zero, further cost reductions in the basal medium formulation would still be required for cultivated meat to approach price parity with industrial animal meat. Thus, research should begin immediately to comprehensively explore novel basal media formulations, rather than rely on incremental refinement of formulas established several decades ago.

Likewise, exercises like this allow comparison of multiple approaches for achieving cost or volume milestones and elucidate opportunities to combine strategies to more efficiently realize these milestones. For example, Section 2.3 predicts that combining cell line adaptation to tolerate lower growth factor concentrations and/or growth factor engineering along with moderate-scale growth factor production can reduce costs to a degree similar to what could be achieved by large-scale growth factor production. In the short term, the former approach may be more accessible to start-up companies who are unable to contract the volumes necessary to capitalize on the returns to scale of industrial-scale growth factor production.

1.2 Limitations of this analysis

This analysis does not entail a full cost of goods model complete with operational costs such as labor and energy, nor does it attempt to estimate the capital expenditures required to build the production facilities described in the production volume estimation. A comprehensive cost of goods analysis is currently in progress with external partners, but is beyond the scope of this paper. This analysis also does not make any projections about how long it may take to achieve the scales assumed here, as forecasting the rate of growth of the entire industry requires a much more sophisticated analysis of the innovation ecosystem and larger market dynamics.

We decided to focus on the cost of the cell culture medium for three reasons:

1) Cell culture medium is a necessary input for all cultivated meat production, whereas other inputs — such as the scaffolding material — may only apply for certain product types or modes of production.

2) The cell culture medium is arguably the most well defined input to this process at the present moment. Any estimates concerning the scaffolding material or the overall bioprocess design, including medium or energy recycling, would necessarily be significantly more speculative than an analysis of the cell culture medium alone. While the medium formulation is likely to be adapted and optimized for every cell line and production process, we feel that currently available chemically defined media formulations provide a reasonable approximation of the formulations that will ultimately be used in cultivated meat production.

3) The cell culture medium will provide the greatest marginal cost contribution in industrial-scale production. All experts with whom we have consulted – including representatives from many cultivated meat companies as well as academics with extensive bioprocess engineering experience – agree that the cell culture medium will provide the greatest marginal cost contribution at industrial scale. Estimates we have heard from these sources range from 55% to over 95% of the marginal cost of the product attributable to the cost of the medium.

2 Determining anticipated medium costs at industrial scale

The first step of this analysis entails determining the anticipated cost of large-scale cell culture medium for cultivated meat production. Cell culture media, including hundreds of formulations free of animal-derived

constituents, are already commercially available at large scale. The development of cell culture media formulations optimized for cultivated meat-relevant cell lines can utilize existing techniques that have been pioneered within the biomedical and research fields to develop media tailored for specific cell types and cultivation processes. However, achieving a cost that makes cultivated meat economically feasible is a different task than technical optimization of media for specific cultivated meat-relevant cell lines.

Thus far, cell culture medium production has been developed with research and therapeutic applications in mind. These fields do not operate under the same cost constraints as a field like food or agriculture, so while there have been efforts to reduce the cost of media for these applications, the cost pressure has not been adequate for extension to larger-scale, lower-margin applications like cultivated meat. Furthermore, the production requirements for a food application are not likely to be as stringent as for a therapeutic or research application, potentially enabling cost savings resulting from the grade of the raw materials and the good manufacturing practices (GMP) requirements of the medium production facility. Finally, animal cell culture operations that are currently considered large scale – such as those producing biologics like antibodies using CHO (Chinese Hamster Ovary) cells – are orders of magnitude smaller than the anticipated need for an application like cultivated meat, so there remains significant potential from achieving true economies of scale.

Cell culture medium production thus far has been developed for research and therapeutic applications, which do not exhibit the same cost constraints as food or agriculture. Cost savings may result from a move toward raw material grades and good manufacturing practices (GMP) that are sufficient for food. In addition, the scale of production for cultivated meat will be orders of magnitude larger than current animal cell applications, so there remains significant potential from achieving true economies of scale.

2.1 Starting model: Cost breakdown of a commercial research-grade animal-free medium formulation

We use the composition of Essential 8™ – a widely used and commercially available animal component-free medium – as our starting formulation for this analysis. Essential 8 was developed in 2011 by refining a previous formulation, the 19-ingredient TeSR medium, to eliminate unnecessary factors including albumin, which is expensive and highly variable [1]. Essential 8 has been demonstrated to work well for derivation and prolonged maintenance of stem cells without triggering differentiation, and its precise composition is publicly available. Liquid Essential 8 medium is sold at bench scale quantities for around $400 per liter and is used frequently as a serum-free medium for many cell types from several different species.

Table 1 lists the components comprising Essential 8, their concentrations, and bench-scale list pricing of each component from existing suppliers. A volume of 20,000 L is used throughout this analysis for consistency with the meat yield calculations in Section 3 that assume a batch size of 20,000 L. The values in Table 1 reflect the cost of the raw materials if a cultivated meat company were to create a batch of Essential 8 medium in-house without negotiated or bulk pricing. While the cost per liter calculated as a starting point for this model ($377 per liter) is relatively close to the ~$400 per liter retail price of Essential 8 medium, it should be noted that this is not reflective of profit margins within the life science industry. The list prices of each component in Table 1 include markup to end consumers, while a life science supplier sources these ingredients at wholesale and then applies a profit margin to the medium as a whole. Industry experts indicate that profit margins on cell culture media are typically in the range of 60-80%.

Table 1. Cost of components within Essential 8 medium and their relative cost contribution to a hypothetical 20,000 liter batch.

Components	Final Concentration (mg/L)*	Amount per 20,000 L (g)	Cost per g†	Source Supplier	Cost per 20,000 L
DMEM/F12 (basal medium)	[n/a (1X)]	[20,000 L]	[$156 for 50L]	Thermo Fisher	$62,400.00
AA2P (ascorbic acid 2-phosphate)	64	1280	$7.84	Cayman Chemicals	$10,035.20
$NaHCO_3$	543	10860	<$0.01	Alibaba, averaged across multiple suppliers	$2.39
Sodium selenite	0.014	0.28	$0.10	Alibaba, averaged across multiple suppliers	$0.03
Insulin	19.4	388	$340.00	Sigma	$131,920.00
Transferrin‡	10.7	214	$400.00	Sigma	$85,600.00
FGF-2	0.1	2	$2,005,000.00	R&D Systems	$4,010,000.00
TGF-β§	0.002	0.04	$80,900,000.00	R&D Systems	$3,236,000.00
Total cost per 20,000 L					**$7,535,958**
Cost per liter					**$376.80**

It is clear from this preliminary analysis that the vast majority of the cost of this formulation – over 99% – is attributed to the growth factors when using bench-scale component pricing as a benchmark. While these growth factors could likely be sourced for substantially less cost even at current production scales, this simply serves as the base case for this exercise. Note also that within the growth factors, just two – those present at the lowest concentrations, FGF-2 and TGF-β – account for almost all of the cost of the growth factors, comprising over 96% of the total cost of the medium in this base case.

2.2 Cost breakdown of food-grade basal medium components at scale

The same approach used to develop a cost model for Essential 8 medium using cost data for each component can be extended to the basal medium, which is listed as a single component in the Essential 8 formulation but is actually comprised of 52 components. Because the basal medium contains the bulk nutrients for cellular metabolism and other factors for maintaining physiologically relevant parameters like osmolarity and pH balance, many of these ingredients can be sourced from suppliers whose target clientele are in industries like food, agriculture, and industrial chemistry. For many components, pricing can be found at the metric ton scale; for others, pricing is available at kilogram scale. For most components, the costs in Table 2 below reflect bulk retail pricing from commercial vendors at online marketplaces like Alibaba, though it is important to note that large medium manufacturers can likely purchase these products through negotiated contracts at lower cost than what is publicly available online. The highest purity among all vendors was used, and values listed reflect the higher end of the average price range among multiple vendors to ensure that the analysis is not overly dependent upon – and thus vulnerable to pricing variabilities from – a limited number of suppliers. Pricing data from outliers (vendors selling at significantly higher or lower per-kg or per-ton prices) was not used.

* All values derived from Chen et al. [1]
† Pricing data were gathered in April 2017. Note that numbers may vary depending on quantity and supplier; these selections simply represent a starting point for this analysis based on one set of list-price values.
‡ Recombinant, expressed in rice (not purified from serum).
§ Recombinant, expressed in CHO cells.

Table 2. Cost of components within DMEM/F12 basal medium and their relative cost contribution to a hypothetical 20,000 liter batch.

Components	Final Concentration (mg/L)*	Amount per 20,000 L (g)	Cost per kg†	Cost per Metric Ton	Cost per 20,000 L
Inorganic Salts					
Calcium chloride ($CaCl_2$)	116.7	2334	$0.30	$300.00	$0.70
Cupric sulfate ($CuSO_4$-$5H_2O$)	0.0013	0.026	$2.50	$2,500.00	$0.00
Ferric nitrate ($Fe(NO_3)_3$-$9H_2O$)	0.05	1	$0.60	$600.00	$0.00
Ferrous sulfate ($FeSO_4$-$7H_2O$)	0.417	8.34	$0.10	$100.00	$0.00
Potassium chloride (KCl)	311.8	6236	$0.39	$390.00	$2.43
Magnesium chloride ($MgCl_2$)	28.64	572.8	$0.33	$330.00	$0.19
Magnesium sulfate ($MgSO_4$)	48.84	976.8	$0.65	$650.00	$0.63
Sodium chloride (NaCl)	6995.5	139910	$0.40	$400.00	$55.96
Sodium bicarbonate ($NaHCO_3$)	1200	24000	$0.50	$500.00	$12.00
Sodium phosphate, monohydrate (NaH_2PO_4-H_2O)	62.5	1250	$2.00	$2,000.00	$2.50
Sodium phosphate, dibasic (Na_2HPO_4)	71.02	1420.4	$2.10	$2,100.00	$2.98
Zinc sulfate ($ZnSO_4$-$7H_2O$)	0.432	8.64	$0.84	$840.00	$0.01
Other Compounds					
D-Glucose	3151	63020	$0.80	$800.00	$50.42
Hypoxanthine	2.05	41	$100.00	n/a	$4.10
Linoleic Acid	0.042	0.84	$50.00	n/a	$0.04
Lipoic Acid	0.105	2.1	$68.00	n/a	$0.14
Phenol red	8.1	162	$25.00	n/a	$4.05
Putrescine-2HCl	0.081	1.62	$2,985.00	n/a	$4.84
Sodium Pyruvate	55	1100	$100.00	n/a	$110.00
HEPES	3575	71500	$55.00	n/a	$3,932.50
Thymidine	0.365	7.3	$300.00	n/a	$2.19
Amino Acids					
L-Alanine	4.45	89	$30.00	n/a	$2.67
L-Arginine hydrochloride	147.5	2950	$30.00	n/a	$88.50
L-Asparagine-H_2O	7.5	150	$30.00	n/a	$4.50
L-Aspartic acid	6.65	133	$3.00	$3,000.00	$0.40
L-Cysteine-HCl-H_2O	17.56	351.2	$25.00	n/a	$8.78
L-Cystine	24	480	$25.00	n/a	$12.00
L-Glutamic acid	7.35	147	$30.00	n/a	$4.41
Glycine	18.75	375	$2.00	n/a	$0.75
L-Histidine-HCl-H_2O	31.48	629.6	$50.00	n/a	$31.48
L-Isoleucine	54.47	1089.4	$50.00	n/a	$54.47

* The formulation of DMEM/F12 was obtained from Biological Industries: http://www.bioind.com/israel/support/media-formulations/media-formulation-dmemf12/

† Pricing data were gathered in April 2017. If metric ton pricing was available, cost per kg was calculated from that bulk value.

Components	Final Concentration (mg/L)*	Amount per 20,000 L (g)	Cost per kg†	Cost per Metric Ton	Cost per 20,000 L
L-Leucine	59.05	1181	$15.00	n/a	$17.72
L-Lysine hydrochloride	91.25	1825	$30.00	n/a	$54.75
L-Methionine	17.24	344.8	$15.00	n/a	$5.17
L-Phenylalanine	35.48	709.6	$28.00	n/a	$19.87
L-Proline	17.25	345	$20.00	n/a	$6.90
L-Serine	26.25	525	$40.00	n/a	$21.00
L-Threonine	53.45	1069	$2.50	$2,500.00	$2.67
L-Tryptophan	9.02	180.4	$15.00	n/a	$2.71
L-Tyrosine	38.7	774	$35.00	n/a	$27.09
L-Valine	52.85	1057	$30.00	n/a	$31.71
Vitamins					
Biotin	0.0035	0.07	$50.00	n/a	$0.00
D-Calcium pantothenate	2.24	44.8	$15.00	n/a	$0.67
Choline chloride	8.98	179.6	$35.00	n/a	$6.29
Folic acid	2.65	53	$60.00	n/a	$3.18
i-Inositol	12.6	252	$15.00	n/a	$3.78
Niacinamide	2.02	40.4	$7.00	n/a	$0.28
Pyridoxal hydrochloride	2	40	$25.00	n/a	$1.00
Pyridoxine hydrochloride	0.031	0.62	$30.00	n/a	$0.02
Riboflavin	0.219	4.38	$25.00	n/a	$0.11
Thiamine hydrochloride	2.17	43.4	$40.00	n/a	$1.74
Vitamin B12	0.68	13.6	$15.00	n/a	$0.20

2.3 Potential scenarios to reduce cell culture medium cost

Based on the analysis in Sections 2.1 and 2.2, the key drivers of medium cost in this exercise are 1) the growth factors, especially FGF-2 and TGF-β, followed by 2) the basal medium, which is largely governed by the price of a single component, the pH buffer HEPES. Here we explore seven hypothetical scenarios to reduce the cost of medium. Cost reductions can be achieved by altering the medium formulation and/or realizing raw material cost reductions as a result of scaling based on existing proxies or pricing data for bulk orders of components.

In the first four scenarios (Scenarios A through D), we focus on the most dominant cost drivers – the growth factors, which we expect to remain the cost drivers until significant scale is reached. In these scenarios, the basal medium is purchased as a pre-mixed powder from an existing commercial supplier. Scenarios E through G examine how costs may be affected if the basal medium is produced in-house from components purchased in bulk (Scenario E) and by slight additional adjustments to the formulation (Scenarios F and G). The salient assumptions of all scenarios and their effect on medium cost per liter are summarized in Figure 1.

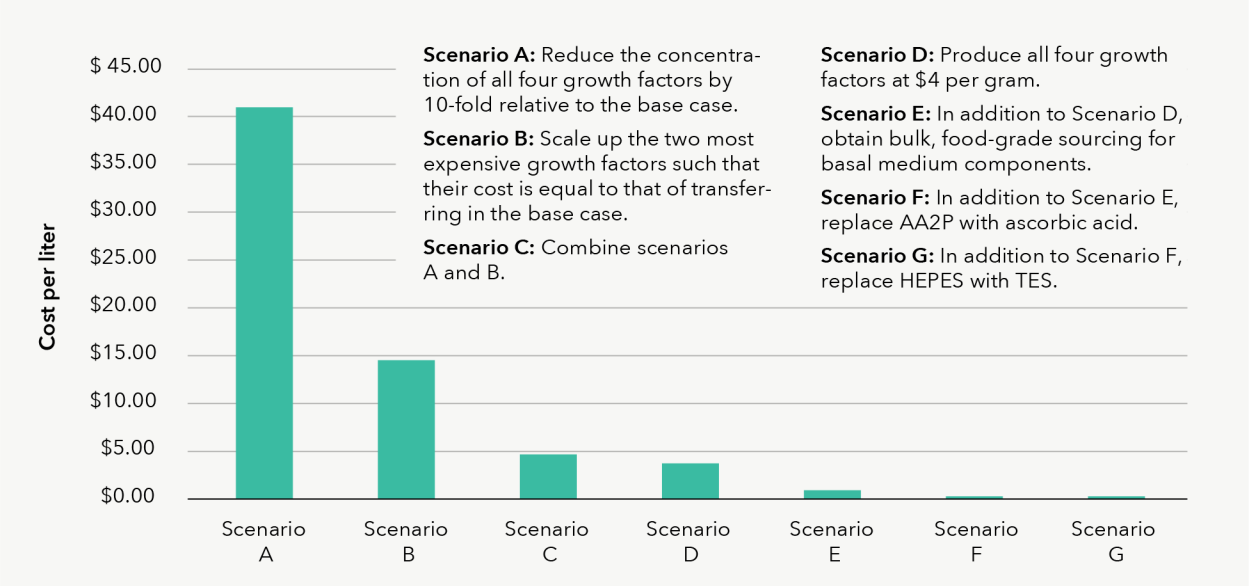

Figure 1. Visual summary of the estimated cost per liter of medium for each cost reduction scenario.

Scenario A: *Requirements for all four recombinant growth factor proteins (insulin, transferrin, FGF-2, and TGF-β) are reduced to a tenth of their current levels. This could be accomplished through many approaches. At the simplest, many cells can be adapted over the course of a few generations to tolerate significantly lower growth factor concentrations with minimal effect on cellular performance; low-serum adaptation is routine, and the same principle applies to reducing the prevalence of individual growth factors [2]. A more sophisticated approach is to engineer the proteins for higher stability (for example, through specific point mutations, truncations, or chemical modifications); for higher potency (greater binding affinity to the appropriate cell receptor(s)); and/or for lower incorporation into cells (less capable of triggering endocytosis). It has already been demonstrated that several growth factors, including IGF-1 and FGF-2, can indeed be engineered to meet these aims.* Growth factors tend to be inherently unstable molecules because they are biologically intended to act as short-term signals within the body, so in some cases it is rather straightforward to significantly improve their stability with relatively little modification. Alternatively, the cells themselves can be engineered or edited to require lower levels of growth factors (or to not require exogenous growth factors at all), or small molecule mimics can be used in place of some or all of the growth factors.† Combinations of these approaches could conceivably reduce the requirement for recombinant growth factors by much greater than a factor of ten or eliminate their need altogether.*

Scenario B: *FGF-2 and TGF-β are produced at larger scales and higher efficiency, putting costs on par with insulin and transferrin on a per-gram basis. For this Scenario, we assign a cost of $400 per gram, the higher of the two values quoted in Table 1 for insulin and transferrin, to FGF-2 and TGF-β while leaving the costs for insulin and transferrin unchanged. It is reasonable to assume that FGF-2 and TGF-β can be produced at a cost similar to transferrin as their protein structures do not indicate significant expression*

* See commercialized forms of these growth factors that have been engineered for enhanced stability, available from Sigma-Aldrich (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/SAFC/Brochure/1/long-r3-igf-i-brochure.pdf) and Enantis (https://www.enantis.com/assets/custom/Download/FGF2STAB.pdf).

† There is precedent for identifying small molecule mimics of various growth factors, but in some cases these molecules are known to also exhibit toxicity. Thus, small molecule mimics may present an additional hurdle from a regulatory perspective if they are not already food-approved or food-safe components. Native growth factors, on the other hand, should be identical to the proteins that are endogenously found within animal tissue that is regularly consumed and therefore a case can be made more easily for established safety.

difficulties relative to recombinant proteins like insulin or transferrin. FGF-2 and TGF-β are both smaller and less complex (less than 35 kDa, depending on the species and isoform, versus transferrin's molecular weight of 80 kDa). Mature TGF-β requires disulfide bonding between discrete peptide chains, which may add complexity, but it is important to note that insulin — which is even less expensive in this model than transferrin — also requires disulfide bonding between peptide chains. It is noteworthy that the initial pricing of TGF-β reflects protein expressed in CHO (which is the most common host for recombinant TGF-β) as this host selection contributes to the significantly higher per-gram cost of TGF-β. However, CHO cells would likely not be required as the host for scale-up. Millipore Sigma, for example, sells CHO-derived TGF-β1 at nearly 20 times the cost of E. coli-derived TGF-β3. The paper describing Essential 8 does not specify which isoform of TGF- β they used, but based on their listed reagent suppliers, it appears that it was probably TGF-β1. However, it seems likely that TGF-β3 would also work well, as "[t]he three mammalian isoforms of TGF-β, TGF-β1, β2, β3 elicit similar biological responses," according to Millipore Sigma's product specifications for the E. coli-derived protein. This may explain why Essential 8 developers chose not to specify the isoform of TGF β in their formulation.

***Scenario C**: Both Scenario A and Scenario B are applied simultaneously. These approaches target entirely different routes to reducing cost, so there are no anticipated antagonistic interactions; improvement on one front is not expected to hamper improvement on the other. In fact, they may interact synergistically: higher growth factor stability, for example, may automatically increase expression yields of the recombinant growth factors, making a reduction in per-gram cost even easier to achieve. However, no synergistic effects are assumed in this scenario.*

***Scenario D**: Each of the four growth factors are produced at true industrial scale, on par with enzymes like lipase, cellulase, and amylase, which are manufactured for industries like food processing, consumer products (detergents, etc.), and paper milling. We anticipate that growth factors should not be inherently more difficult to produce through recombinant expression than these enzymes, and therefore the achievable cost of production should be similar. (Again, to take one example to demonstrate similarities that may be indicative of relative ease of expression, cellulases are typically several times larger than growth factors — typically 45-65 kDa or greater, and many exhibit disulfide bonding as well [3], both of which may render these enzymes harder to make than smaller proteins like growth factors.) For this, we assign a production cost of $4 per gram, which is three orders of magnitude less than the value in Scenarios B and C. Many industrial-scale enzymes are produced at much lower cost (several industry experts say $0.10 per gram is closer to a true minimum cost for recombinant protein production from microbial fermentation[*], so the choice of $4 per gram is deliberately conservative and accounts for a significant mark-up margin as it is unlikely that cultivated meat companies or cell culture medium companies will produce these growth factors in-house). The $4 per gram figure reflects costs for moderate-scale enzymes that are typically not used for pharmaceutical, clinical, or biomedical applications. For example, cellulase and pectinase can be obtained for approximately $4 per gram at rather modest scales, as low as 10 or 25 grams.[†]*

[*] This same value — $100 per kilogram — is referenced as a target benchmark for recombinant protein production in the biomaterials industry by companies like Spiber, which has given it the moniker the "Hundred Dollar Barrier": "[I]t is often said within the fermentation industry that producing genetically modified proteins via microbial fermentation for less than $100 per kilogram is extremely difficult." (www.spiber.jp/en/endeavor)

† See, for example, cellulase and pectinase from Aspergillus niger from two different suppliers, MP Biomedicals (https://www.fishersci.com/shop/products/mp-biomedicals-cellulase-aspergillus-niger-3/p-4605331#) and Millipore Sigma (https://www.sigmaaldrich.com/catalog/product/sigma/17389).

Once we assume larger-scale growth factor production accommodating the raw material costs reflected in Scenario D, the basal medium becomes the dominant cost driver, accounting for 97% of the total cost. Scenarios E through G reflect approaches to reduce the cost of the basal medium, while holding pricing for the growth factors constant at the values postulated in Scenario D.

> ***Scenario E**: For all calculations prior to Scenario E, the basal medium cost was held constant, assuming it was being purchased as a pre-mixed commercially available powder. In Scenario E, the basal medium is reconstituted from its 52 components at costs reflecting bulk pricing for each component. Many of these costs still reflect pharmaceutical-grade certified components, but when available, cost data was gathered for food-grade components under the assumption that this grade is appropriate for a food end product. It is also assumed that this grade is suitable for cell cultivation, and that impurities will not significantly impact cell performance. In most cases, the purity of the component is similar between pharmaceutical grade and food grade (often >99% purity in both cases), but the nature of the residual contaminants may differ between these certification grades, which may be relevant for cell culture performance.*

Through several approaches, the cost of the growth factors can be straightforwardly reduced such that the basal medium becomes the predominant cost driver. In order to achieve medium costs that are suitable for cultivated meat production, innovation in raw material sourcing and formulation of the basal medium will be required.

Scenarios A through E hold the original formulation of the basal medium constant; the basal medium cost benefits in Scenario E derive solely from scale of production and tolerance of non-pharmaceutical grade materials. In contrast, scenarios F and G alter the medium formulation itself by substituting high-cost components with lower-cost alternatives. While these substitutions are not anticipated to severely impact cell performance, these scenarios are more hypothetical because the effect of these substitutions must be validated empirically (though there is precedent, for example, of medium using TES rather than HEPES as the buffer, as in Scenario G). These scenarios are intended to illustrate two rather trivial medium optimization options to significantly decrease cost. There is, of course, notable potential to drastically reduce cost using more sophisticated approaches such as statistical Design of Experiments coupled with high-throughput microfluidic screening to adjust multiple medium components simultaneously. While the formulations arrived at via these high-throughput approaches would still need to be empirically validated at large scale, these approaches offer the opportunity to significantly reduce the time and resources needed to optimize custom media for various cultivated meat applications. Ultimately, the medium formulation used for large-scale cultivated meat production will be precisely optimized for each cell line and for each production process, as factors of the cellular environment (oxygenation level, cell density, etc.) will influence cellular metabolism and therefore will impact optimal levels of various nutrients in the culture medium.

> ***Scenario F**: Once the basal medium has been reassembled from its individual components purchased in bulk, the predominant cost driver becomes AA2P (ascorbic acid-2-phosphate). This is a long-acting ascorbic acid (vitamin C) derivative that is frequently used in cell culture.* Scenario F substitutes AA2P with food-grade ascorbic acid, which is produced at much larger scales and several orders of magnitude lower cost. Even if ascorbic acid degrades more rapidly in the medium, a medium monitoring system could frequently dose it to maintain target levels. While this may ultimately require more ascorbic acid than a functionally equivalent amount of AA2P, the cost differential between the two is so great that a switch to*

* See product information: https://www.biomol.com/products/chemicals/biochemicals/l-ascorbic-acid-2-phosphate-magnesium-salt-cay16457-5?fs=2926958974

*ascorbic acid would still significantly reduce overall medium costs. For example, even if ten-fold more ascorbic acid is required than AA2P to achieve the same cell performance, switching to ascorbic acid would still reduce the cost of that component by 99.6%, thus reducing the cost of the medium by 59% overall relative to Scenario E.**

***Scenario G**: The main cost driver within the basal medium is HEPES, a pH buffer. HEPES maintains pH within a physiologically suitable range for animal cells, but several other buffers also function within this range [4]. TES buffer exhibits very similar pH buffering range, solubility, and pK_a to HEPES but can be sourced for approximately half the cost of HEPES.† In fact, media that use HEPES may be over-engineered for commercial applications: there is some indication that HEPES is only particularly relevant for cells that are handled (removed from CO_2 incubators) frequently or when metabolites are allowed to accumulate in the medium [5]. Neither of these conditions are applicable in a closed cell culture system with continuous medium perfusion and filtration, as is likely for cultivated meat production. Scenario G reflects the same conditions as in Scenario F, with the additional cost impact of substituting TES for HEPES.*

Table 3. Projected costs and fold reduction relative to the initial cost model for a 20,000 L batch of medium made under Scenarios A through G. Highlighted cells indicate changes relative to the previous scenario.

Components	Base Case	Scenario A	Scenario B	Scenario C	Scenario D	Scenario E	Scenario F	Scenario G
Basal medium	$62,400	$62,400	$62,400	$62,400	$62,400	$4,600	$4,600	$2,456
Vitamin C or precursor	$10,035	$10,035	$10,035	$10,035	$10,035	$10,035	$4.48	$4.48
$NaHCO_3$	$2.39	$2.39	$2.39	$2.39	$2.39	$2.39	$2.39	$2.39
Sodium selenite	$0.03	$0.03	$0.03	$0.03	$0.03	$0.03	$0.03	$0.03
Insulin	$131,920	$13,192	$131,920	$13,192	$1,552	$1,552	$1,552	$1,552
Transferrin	$85,600	$8,560	$85,600	$8,560	$856.00	$856.00	$856.00	$856.00
FGF-2	$4,010,000	$401,000	$800.00	$80.00	$8.00	$8.00	$8.00	$8.00
TGF-β	$3,236,000	$323,600	$16.00	$1.60	$0.16	$0.16	$0.16	$0.16
Total cost per 20,000 L	**$7,535,958**	**$818,790**	**$290,774**	**$94,271**	**$74,854**	**$17,054**	**$7,024**	**$4,879**
Cost per liter	**$376.80**	**$40.94**	**$14.54**	**$4.71**	**$3.74**	**$0.85**	**$0.35**	**$0.24**

Applying cost projections for large-scale production of the growth factors alone is able to reduce the overall cost of the medium by approximately two orders of magnitude from the starting model ("base case"). A recent publication by the Burridge lab at Northwestern University demonstrated that 97% cost reduction could be achieved even at bench scale simply by producing stable growth factor variants in-house.‡ Industry experts indicate that costs within this order of magnitude are indeed achievable for large-scale clients of animal component-free media, such as biologics manufacturers. By additionally sourcing the basal medium components in bulk and at food grade, this analysis indicates that the medium cost can be dropped below $1 per liter.

* This component may be dispensable altogether. Subsequent modifications to the Essential 8 formulation showed that ascorbic acid was not necessary in any form for short-term maintenance of stem cells. When regular ascorbic acid was substituted in place of AA2P, it was used at a far lower concentration (1 mg/L ascorbic acid, rather than 64 mg/L AA2P used in Essential 8) [14].

† See supplier reference information on suitable pH buffers for biological systems: https://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html

‡ Kuo et al., Negligible-Cost and Weekend-Free Chemically Defined Human iPSC Culture, Stem Cell Reports, 2020. https://doi.org/10.1016/j.stemcr.2019.12.007

Minor adjustments or substitutions to individual components can further reduce this cost per liter by several-fold; it is likely that this same approach can be used to reduce costs associated with many more components than just the most salient non-growth-factor cost drivers (AA2P and HEPES). In fact, several basal media formulations include far fewer components – for example, some only include the essential amino acids that cannot be synthesized by the cells. While eliminating factors like the non-essential amino acids would significantly further reduce the media cost, this must be weighed against the loss in performance as it places greater metabolic demands on the cells to synthesize these components. In addition, there are components that can be reduced or eliminated because the design requirements for a cell culture medium in the context of a large-scale production environment often do not mimic the requirements for bench-scale R&D cell culture. For example, phenol red is a colorimetric pH indicator that can be discarded altogether in the context of a bioreactor with integrated pH sensors. Cost modeling combined with empirical data about cellular performance attributes such as cell viability, proliferation rates, etc., will be a critical aspect of further optimizing the media formulation to reduce raw material costs without unduly compromising overall yield.

3 Estimating production volumes, equipment residence time, and scaling

The goal of the production volume portion of the analysis is to estimate the volume of meat that can be obtained from a given reactor size to determine scaling needs for industrial-scale cultivated meat production facilities. This analysis also allows for an estimation of residence time of the cells within the equipment, which informs facility design and production throughput – as well as biological considerations such as the required number of generations through which the production cell line must be genetically stable to accommodate various bioprocess designs. This, in turn, informs how much medium may be required per pound of meat produced, which allows us to translate the cost per liter of medium into an anticipated cost contribution of medium per pound of meat. As with the medium analysis above, this production and yield analysis helps to guide R&D strategy – for example, by informing how much effort should be expended to achieve a given number of stable cell divisions once the break-even point is determined for various instantiations of semi-continuous processes.

First, we develop a batch process schematic to guide the calculations that follow (Section 3.1). We then determine the timeframe of this process, including residence time within each reactor in the seed train and the full-scale production reactors (Section 3.2). Next, we use two different approaches to estimate the maximum and minimum volume of medium that would be required throughout the process (Section 3.3). We estimate the mass of meat that we expect to harvest from this hypothetical batch process (Section 3.4) and then extend this analysis to determine the effect of running the process semi-continuously (Section 3.5). Finally, we apply the cell culture medium costs derived in Section 2 to determine the cost contribution of the medium per pound of meat for several different medium cost and production process scenarios and compare to the current wholesale costs of conventional animal meat (Section 3.6). In Table 6, we revisit several of the assumptions and conversion factors applied throughout this analysis to assess the range of production costs that could reasonably be achieved for large-scale cultivated meat production.

3.1 Developing a hypothetical batch production process

The production schematic in Figure 1 depicts the hypothetical process that serves as the basis for the calculations that follow. This represents a strictly batch process, where each harvest results from inoculation directly at the seed train stage from a starter culture (likely in the form of a cryopreserved vial). Continuous and semi-continuous processes exhibit considerable efficiency gains for bioproduction processes that capitalize on exponential cell expansion and these are addressed in Section 3.5. However, the first portion of this analysis focuses on the least optimal production scheme to ensure that this feasibility analysis does not rely upon overly optimistic assumptions.

The volumes of each scale-up stage throughout the seed train were determined by selecting 20,000 liters as the volume of the largest proliferation reactor and working backwards. A 20,000 liter reactor was selected as the end-stage volume because that volume represents the upper end of the scale at which animal cell culture is already conducted. Although much larger-scale fermenters exist for microbial (bacterial, algal, fungal, etc.) cell culture, these cells are typically smaller, more robust, and often exhibit cell walls that render them less susceptible to the pressures present in reactors at scales of 100,000 liters or larger. Animal cells may not be suitable for these scales.

Figure 2. A hypothetical batch production process schematic, from inoculum through seed train to a maximum reactor volume of 20,000 liters for the proliferation stage. When the cells are seeded onto scaffold in the second stage of the process, the volume of the tissue perfusion reactor is 8,000 liters to account for the final tissue volume and allows for an equal volume of porosity (void space) within the tissue where the nutrient media flows. This model assumes no additional cell proliferation after transition to the scaffold.

To determine the volumes and residence time in the seed train reactors, 200,000 cells/ml ($2x10^5$) was selected as the minimum tolerable inoculation density. While densities as low as 100,000 cells/ml may be viable for some cell types* (and this number can potentially be pushed lower, either through selection or more sophisticated genetic techniques that allow cells to tolerate low density without triggering apoptosis), we have conservatively decided to double this.

We assume that the maximum cell density at each proliferation stage (while the cells are in suspension growth) is $4x10^7$ cells/ml, based upon numbers reported for single-cell suspensions of stem cells.† Traditional large-scale animal cell culture applications (such as CHO cell biologics manufacturing) typically maintain cells at lower densities (around $1.5x10^7$ cells/ml) in fed-batch systems but can reach higher densities in perfusion systems. It is likely that the maximum cell density can be increased even higher than $4x10^7$ cells/ml, which will have a significant positive effect on the yield of the process. Densities upwards of $1x10^8$ viable cells/ml have already been reported in the literature for animal cell culture [6] and numbers approaching $3x10^8$ cells/ml were reported for perfusion processes at a recent cell engineering conference (ECI Cell Culture Engineering Conference, May 2018).

To determine the size of the tissue perfusion bioreactor for the maturation stage, the total cell volume (calculated in Section 3.4) of 4 m^3 or 4,000 L is doubled to account for void space for the flow of nutrient medium through the cells and scaffold. In order to mimic the texture and density of meat, a void fraction of less than 50% is desirable, as vasculature (the biological equivalent of void space within a tissue) accounts for less than 50% of the volume of conventional animal meat. In practice, it may be that cells mature on a scaffold with much greater void space than would be desirable for the texture of meat (to facilitate nutrient flow and access) and then compressed into more dense tissue upon harvesting, thus circumventing the greatest challenge in the tissue engineering field – providing nutrient access within dense, thick tissues. Thus, this 50% void space assumption is simply for the

* See application note for HeLa cell splitting protocols: http://www.percell.se/116.pdf
† See product specifications for one such commercial supplier of high-density stem cells: http://www.accellta.com/category/singles%E2%84%A2

purposes of estimating the volume of the tissue perfusion vessel (and therefore the volume of medium needed to circulate through it) and does not reflect the void fraction of the final product.

3.2 Estimating residence time for each stage of production

Expanding the culture from $2x10^5$ cells/ml to $4x10^7$ cells/ml requires a 200-fold increase, which represents between seven and eight cell doublings. To be conservative, we assign a residence time in each scale-up stage sufficient for eight cell doublings.* To estimate doubling time we extrapolate from Accellta, a stem cell company with high-density cultures, which claims a 50- to 100-fold increase in seven days.† This translates to about 28 hours per doubling (assuming six doublings, or 64-fold increase, in seven days). 24 hours per doubling is an approximation routinely used in animal cell culture, and 24hr doubling times have been demonstrated for some populations of mesenchymal stem cells [7], so 28 hours represents a reasonably conservative assumption.‡ Layering these assumptions, each stage will require 9.3 days; for the sake of incorporating another cautious cushion, we round this up to 10 days per stage.

The 200-fold increase between inoculation of each stage and progression to the following stage means that each reactor in the seed train will be $1/200^{th}$ the volume of the subsequent reactor. Thus, the reactor that feeds into the 20,000 liter reactor will be 100 liters. The reactor prior to that will be 0.5 liters (500 ml), which can be inoculated from a single 2.5 ml frozen vial of cells (assuming cryopreservation at $4x10^7$ cells/ml), which represents an appropriate volume and density for routine cell cryopreservation, shipping, and storage within a cell bank.§

For this production scheme, no additional cell proliferation is assumed after the cells are seeded onto the scaffold for differentiation and maturation. Thus, the time assigned to the maturation stage of the process is derived from a range of studies differentiating skeletal muscle from intermediate cell types like mesenchymal cells. A survey of the literature indicates that 10 days is a reasonable timeframe, but this timeframe will vary depending on many factors including the desired final cell type (fibroblasts, for example, tend to differentiate and mature more quickly than muscle cells) and the cell type used for the proliferation stage (for example, myoblasts will mature into myotubes more quickly than mesenchymal cells – in as little as three days**). We believe there is significant potential to identify protocols or modify/select cells to accelerate this process because most work to date has aimed to optimize differentiation efficiency or reproducibility; for biomedical or basic research applications, relatively little work has been done to explore approaches to accelerate skeletal muscle differentiation.

This results in a total residence time – from a 2.5 ml frozen vial of cells to nearly one quadrillion mature cells†† (20,000 L times $4x10^7$ cells/ml, subsequently seeded onto scaffold and matured) – of 40 days. Because of the multitude of conservative assumptions applied in arriving at this process timeframe, this likely represents an absolute maximum process length. Indeed, several companies' stated estimates and one published estimate of the timescale of the production process are in the range of two to four weeks [8].

* While 1:200 may seem like an aggressive split, this a reasonable inoculation density for this application because these tanks allow inflows of medium as a fed batch. Thus, the inoculum can first be seeded into a small volume with fresh medium added over time to reach the final tank volume. For example, the 100 L inoculum can initially be added to 900 L of fresh medium to functionally provide a 1:10 split, and fresh medium to a final volume of 20,000 L can be flowed in as the cells proliferate and increase in density.

† See product specifications: http://www.accellta.com/category/singles%E2%84%A2

‡ However, it should be noted that doubling times vary considerably between species and cell type – for example, in some studies human primary skeletal muscle stem cells exhibit doubling times over twice as long as those of C2C12, a common mouse skeletal muscle line [15].

§ Some scale-up systems utilize even larger volumes and higher densities of cryopreserved cells, such that a frozen stock can directly inoculate a 20 L reactor and shave nine days off of the seed train process [16].

** See product user guide: https://fujifilmcdi.com/assets/CDI_iCellSkeletalMyoblastsPrototype_UG.pdf

†† See Section 3.5 for details on how this cell number metric is converted into cellular biomass.

3.3 Determining culture medium requirements for cultivated meat production

We will first treat the medium requirement for this process as a boundary-setting exercise. The minimum volume of medium that could be used throughout the process illustrated in Figure 1 is equivalent to the volume of the largest reactor, 20,000 liters. This assumes that all medium added to each reactor throughout the seed train remains in the system (through transfer to the subsequent, larger reactors) and fresh medium is simply added at each step to account for the increased tank volumes.

The upper bound will be defined by assuming that the entire medium is changed every two days, which reflects the frequency with which medium is changed in typical bench-scale cell culture applications. Assuming five volumes within each scale-up stage (ten days divided by a medium change every two days) and five volumes at the tissue maturation stage (ten days in the maturation bioreactor, divided by a medium change every two days), this translates to a total medium volume per batch of:

$$(5 \times 0.5\ \text{L}) + (5 \times 100\ \text{L}) + (5 \times 20{,}000\ \text{L}) + (5 \times 8{,}000\ \text{L}) = 140{,}502.5\ \text{L} \approx 140{,}000\ \text{L}$$

Alternatively, the culture medium requirement can be estimated through another established method, which is to run a long-term cell culture process by alternating 80% recycling with a fresh volume every three days (i.e., replace 20% of the medium on day 3, replace 100% on day 6, replace 20% on day 9, and so forth). This strategy arrives at a total medium requirement of approximately ten times the volume of the reactor [9]. Applying this method to the process diagram in Figure 2, the average volume of the reactor over the course of this process is 7,025 liters; ten times this volume is 70,250 liters. This estimate is close to the average between the *high use case* and *low use case* scenarios presented above: the average between 20,000 and 140,000 liters is 80,000 liters, and this average value is used in the more complex production scheme analyses in Section 3.6.

The true volume of medium required is expected to lie within these upper and lower bounds, and it will not necessarily reflect a literal volume of medium but rather the *volume equivalent* of the medium components that will be required. In traditional cell culture for research or biomedical applications, the cell culture medium is refreshed in its entirety. As cells metabolize, nutrients are depleted and waste products are generated, some of which become toxic at elevated concentrations. The medium will also begin to acidify over time. The practice of completely replacing the medium with fresh medium adds significant expense and contributes to unnecessary waste, as many of the nutrients and growth factors are still present in the medium after two days of culture.

Restoring nutrient, osmotic, and pH balance need not require replacement of the entire medium volume. At production scale, some version of medium recycling will be required to reduce waste and cost. With appropriate sensors and filtration methods, metabolic waste products can be removed, non-metabolized components like salts, buffers, and growth factors can be retained and recycled, and the depleted nutrients like sugars and amino acids can be replenished as they are consumed by the cells. Some research in the area of media recycling and adaptive control to introduce concentrated nutrient feeds in response to monitoring individual components shows promise. For example, a dialysis system used to remove toxic metabolites from human stem cell suspensions was able to retain upwards of 85% of growth factors including FGF and TGF-β [10]. Determining the level of recycling that is achievable for each component of the medium requires a significantly more complex analysis informed by metabolic modeling and/or empirical data from spent media analysis and is outside the scope of this paper, but will be a critical aspect of further refining medium formulation strategy.

3.4 Estimating cultivated meat yield for batch process

To determine the amount of meat we anticipate our hypothetical batch process could produce, we determine the number of cells that will be generated, estimate the volume those cells will occupy once mature, and then convert this total cell volume measure into a mass measure in terms of kilograms of meat. Each of the assumptions and conversion factors used in this calculation are delineated below. It is important to note that there are many factors that are not accounted for in this simplified analysis. For example, cellular losses due to inefficient differentiation, cell death, or losses in cell harvesting or transfer were not quantified in this analysis. On balance, we feel the yield we estimate is a reasonable approximation of what may be realistically achieved at scale due to the conservativeness of the assumptions applied to each parameter.

Cell density: We assume the same cell density in the proliferation reactor (from which we ascertain the total number of cells generated) that was first introduced in Section 3.1, based upon numbers reported for single-cell suspensions of stem cells.

Size of the largest reactor: In accordance with the production schematic introduced in Section 3.1, the largest proliferation volume in this process is 20,000 liters ($2x10^4$ liters), which is equivalent to $2x10^7$ milliliters. We select this stage of the process to capture how many cells are being generated within each batch. These cells are then seeded onto scaffolds in the maturation bioreactor, where they will be much more dense than they are in the proliferation bioreactor (on par with the cell density of conventional meat), but the total cell number is assumed to not change after harvesting from the proliferation bioreactor.

Average volume per cell: An average vertebrate cell volume is approximately 5,000 μm^3, so this analysis will use this number while acknowledging that this is perhaps a substantial underestimate. The two most relevant cell types for meat – skeletal muscle cells and adipocytes – are both enormous cell types relative to nearly all other cells.* In fact, skeletal muscle cells are the largest cells in the vertebrate body [11]. Skeletal muscle cells in small vertebrates like mice are on the order of 5 nl (5,000,000 μm^3), while skeletal muscle cells in larger vertebrates can be half a meter long with volumes approaching 1 ul ($1x10^9$ μm^3).† However, fully mature skeletal myotubes are the result of the fusion of up to hundreds of immature precursors as evidenced by the presence of hundreds of nuclei. The volume of the mature, multinucleated muscle cells roughly approximates the volume of the hundreds of smaller cells that fused to form them, so this approximation is likely to hold true for muscle cells in the context of this analysis. Adipocytes, on the other hand, are not formed through fusion of smaller cells. The average adipocyte has a volume of 600,000 μm^3, though this can vary by more than an order of magnitude in either direction; even small adipocytes are several times larger than the average cell size assumed in this analysis.

Note that this volume calculation also does not account for the volume occupied by scaffolding or vasculature, which may ultimately comprise a significant fraction of the final product. ("Vasculature" or void space volume is accounted for in setting the size of the tissue perfusion bioreactor, but scaffolding is not accounted for anywhere in this analysis.) Once these aspects are taken into consideration, it is likely that the meat yield of a batch of this size will be much larger. The implications of more optimistic assumptions are discussed in Table 6, but for now the analysis that follows assumes an average cell volume of 5,000 μm^3 as the only parameter dictating the volume of meat harvested.

* See reference sizes for various vertebrate cell types here: http://book.bionumbers.org/how-big-is-a-human-cell/

† The difference in muscle cell volume between small animals and large animals may point towards differences in meat yield between species (for example, chicken versus bovine muscle cells have very different maximum sizes *in vivo*). However, it is unclear whether these differences based on body size will be relevant within the cultivated meat production environment, where the length of the fibers will be dictated by parameters like the scaffold thickness rather than the length of a muscle within an animal body.

Density of meat: A cubic meter of ground meat weighs about 881 kg.* Note that this assumes some fraction of fat, as the weight of a cubic meter of material with a density equivalent to water would be 1,000 kg. This conversion factor can be adjusted for various products based on the anticipated degree of incorporated fat in the final product.

Using these conversion factors, we can calculate the anticipated yield (kg of meat per batch) as follows:

$$(4x10^7 \text{ cells/ml}) \times (2x10^7 \text{ ml/batch}) \times (5x10^3 \mu m^3/\text{cell}) \times (10^{-18} m^3/\mu m^3) \times (881 \text{ kg}/m^3) = 3{,}524 \text{ kg/batch}$$

The conversion factors above relate to the following attributes, respectively:

(cell density) x (size of largest reactor) x (avg. volume per cell) x (unit conversion) x (density of meat) = yield

For all subsequent analyses, we round this number down to 3,500 kg of meat for each batch of the process outlined in our schematic. Again, because several conservative estimates were layered into arriving at this number, it is quite likely that this is an underestimate (and potentially significantly so). The anticipated impact of errors in each of these parameters is discussed in Table 6.

3.5 Extending cultivated meat yield estimates to semi-continuous processes

The batch process used for the analyses in Sections 3.1 through 3.4 is ultimately not the most desirable platform for large-scale production because, as with any process relying on exponential expansion, greater efficiency is experienced at the latter stages of the scale-up process. To produce 3500 kg of meat from a 2.5 ml frozen vial of cells, the cells must undergo 24 doublings (three stages with eight doublings each). This is already within the proliferative capacity of cells like some mesenchymal stem cells [12]. If this proliferative capacity can be extended†, semi-continuous processes that exhibit significantly higher efficiency are possible.

We will explore two semi-continuous process scenarios. Note that in both of these scenarios, "harvest" refers to harvesting from the proliferation tank for seeding onto scaffold in the maturation bioreactor, wherein the cells still mature for 10 days. In one scenario, 50% of the cells are harvested for subsequent maturation; in the other, 90% of the cells are harvested for subsequent maturation. The mechanics of the harvesting process between the proliferation stage and the maturation stage need not be defined here, for the purposes of this analysis. In the case of a semi-continuous process with constant perfusion, the "harvested" cells could simply refer to those that flow freely from the tank in the media bleed with no additional requirements to concentrate them —they would simply adhere when flowed through the scaffold, perhaps with a brief incubation step to allow sufficient time for attachment.

In the first semi-continuous scenario, once the 20,000 liter proliferation bioreactor reaches harvest density, only 50% of the cells will be harvested for seeding onto scaffold and subsequent maturation. The remaining 50% of the cells will be supplemented with fresh medium to restore the total volume to 20,000 L and allowed to proliferate for another 28 hours (one doubling) to once again reach harvest density. This continues repeatedly (as indicated by the number of harvests per production run in Table 4 below) until the final harvest, at which point the entire volume is harvested for seeding and subsequent maturation. In the *low media use* case, the additional medium requirement for each additional harvest is 10,000 L. In the *high media use* case, each harvest also requires an additional 20,000 L of medium (five volume changes throughout the ten days that the batch matures once seeded

* See reference table for bulk density of various food products: http://www.mpd-inc.com/bulk-density/

† There are a number of ways to extend the proliferative capacity of cells including both genetic and non-genetic methods – including reversible immortalization methods using gene editing techniques [17] – but a discussion of these approaches is beyond the scope of this paper.

onto scaffold; this maturation will occur in a tissue perfusion bioreactor half the size (4,000 L) of the maturation reactor postulated in the batch schematic, since only 50% of the cells are seeded into this bioreactor). Thus, the *high media use* case requires a total of 30,000 L of medium for each additional harvest.

In the second scenario, 90% of the cells are harvested for seeding and subsequent maturation once the 20,000 liter proliferation tank reaches harvest density. The 10% of cells remaining after a 90% harvest will require a ten-fold increase (approximately 3.2 doublings) to achieve harvest density again, which will require about 3.7 days between harvests. In this scenario, the *high media use* case will require more medium for each additional harvest than the *low media use* case even in the proliferation phase because of the recovery time for the culture to repopulate. The *low media use* case requires 18,000 L of additional medium (90% of the tank volume) to refill the tank after each harvest. The *high media use* scenario requires 18,000 L of medium added immediately to support the first two days of proliferation, after which the entire medium is exchanged for a fresh 20,000 L for the remaining 1.7 days until the next harvest. In addition, each harvest in the *high media use* case requires 36,000L of medium (five volume changes throughout the ten days that the batch matures once seeded onto scaffold, in a tissue perfusion bioreactor 90% of the size (7,200 L) of the maturation reactor postulated in the batch schematic) for a total of 74,000 L of additional medium for each harvest step introduced.

Table 4 can be used to determine which of these semi-continuous approaches may be most appropriate (or other processes between or on either end of these harvesting fractions) based on various additional considerations. For example, if the cell line is stable through 33 generations, the 50% harvesting scheme yields slightly more meat. However, ten harvesting events must occur in the meantime, in contrast to just four harvests for a 90% harvesting scheme at the same number of cell generations. If the act of harvesting presents a non-negligible risk of culture contamination, a 90% harvesting scheme may be preferable in the long run because of the loss of crashed cultures due to contamination introduced at harvest.

Table 4. Proliferation capacity requirements of the cell line, total meat yield for a multi-harvest production run, and overall length of the production run for several operational modes of semi-continuous production.

	50% harvesting scenario			90% harvesting scenario		
Number of harvests per production run	**Total meat yield per production run (kg)**	**Required proliferation capacity (doublings)**	**Approximate total length of the production run (days)**	**Total meat yield per production run (kg)**	**Required proliferation capacity (doublings)**	**Approximate total length of the production run (days)**
1 (batch)	3,500	24	40	3,500	24	40
2	5,250	25	41	6,650	27.2	44
3	7,000	26	42	9,800	30.4	47
4	8,750	27	44	12,950	33.6	51
5	10,500	28	45	16,100	36.8	55
6	12,250	29	46	19,250	40	59
7	14,000	30	47	22,400	43.2	62
8	15,750	31	48	25,550	46.4	66
9	17,500	32	50	28,700	49.6	70
10	19,250	33	51	31,850	52.8	74

Note that for both of these versions of a semi-continuous process, a production facility would have to incorporate multiple maturation bioreactors for each proliferation bioreactor, and this additional capital expenditure and process complexity should also be factored into process design considerations.

3.6 Determining the culture medium cost contribution to cultivated meat production

Now that we have derived the timescale of the process (Section 3.2), upper- and lower-bound estimates of the medium requirements (Section 3.3), and estimated yields of meat for batch (Section 3.4) and semi-continuous (Section 3.5) processes, this information can be integrated with the cell culture medium cost analysis in Section 2 to determine the culture medium's contribution to the total cost of the product. As noted previously, the culture medium is expected to be the largest marginal cost driver at scale.

For batch processes, this calculation is straightforward: required liters of medium are multiplied by cost per liter and then divided by kilograms of meat obtained from the process. Table 5 shows the culture medium cost contribution per kilogram of meat for each medium cost scenario for the *high use* (140,000 liters per batch) and *low use* (20,000 liters per batch) assumptions as well as for an average of the two (80,000 liters per batch) to reflect a realistic single baseline for the more complex analyses that follow.

Table 5. Medium raw material cost contribution per kilogram of meat for batch production using high, low, and average volumes of medium at various raw material costs.

	Base case ($376.80 /L)	Scenario A ($40.94 / L)	Scenario B ($14.54 / L)	Scenario C ($4.71 / L)	Scenario D ($3.74 / L)	Scenario E ($0.85 / L)	Scenario F ($0.35 / L)	Scenario G ($0.24 / L)
High media use	$15,072.00	$1,637.60	$581.60	$188.40	$149.60	$34.00	$14.00	$9.60
Avg. media use	$8,612.57	$935.77	$332.34	$107.66	$85.49	$19.43	$8.00	$5.49
Low media use	$2,153.14	$233.94	$83.09	$26.91	$21.37	$4.86	$2.00	$1.37

As shown in Table 5, the lowest-cost culture medium scenarios would render the process economically viable for the majority of commodity meat products only if the lowest end of the estimates for media usage in a batch production environment are achievable.* Although a batch process is unlikely to economically outcompete the majority of conventionally produced meat without further optimization, this is a useful exercise for determining what culture medium cost is appropriate for batch production of higher-end products such as some types of fish or other exotic meats. For example, a batch production process at this scale would likely be feasible for something like sushi-grade tuna (which retails for upwards of $70 per kg) even in the highest culture medium use case at a culture medium cost of $0.35 per liter.

To examine the effect of more sophisticated but ultimately more efficient semi-continuous production, Figure 3 explores the influence of three variables (cost per liter of medium, harvesting scheme employed (50% or 90% harvesting), and number of harvests per production run) on the medium cost contribution per kilogram of meat. There are three medium costs considered: $0.85 per liter derived from Scenario E, which represents an achievable raw material cost from scaling alone with no change to the formulation itself; $0.24 per liter derived from Scenario G, which is achieved with relatively straightforward formulation modifications; and $0.18 per liter, which assumes the cost can be further decreased by 25% from Scenario G. There are several possible approaches to arrive at this cost or lower – including assuming a *functional* per-liter cost achieved with adaptive control and medium recycling or assuming lower production costs for the growth factors – some of which are described in Table 6.

* For comparison, the retail price of products like ground beef, boneless chicken breast, and ham are around $3 to $4 per pound, which translates to $6.60 - $8.80 per kilogram. Note that organic meats can retail for a few times this price. However, the most relevant comparison is the wholesale price of meat, which is typically around 50% of the retail price (https://www.ers.usda.gov/data-products/meat-price-spreads), since that more closely reflects the cost of production.

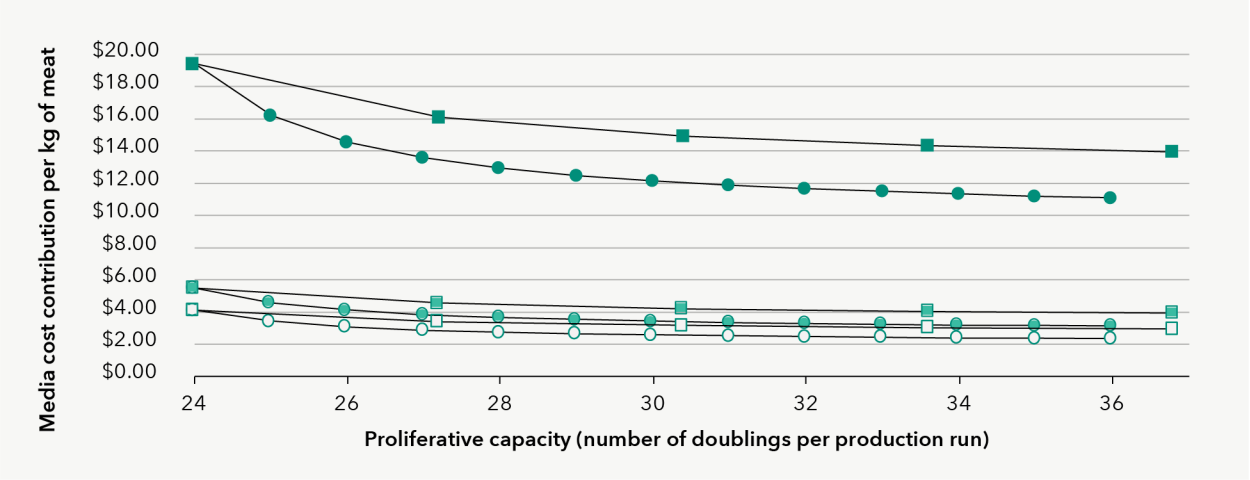

Figure 3. The culture medium cost contribution per kg of meat within various production process scenarios. Circles indicate a 50% harvesting scheme and squares indicate a 90% harvesting scheme as described in Section 3.5, where each marker represents a harvesting event. Dark green series assume a medium cost of $0.85/L, light green series assume a medium cost of $0.24/L, and white series assume a medium cost of $0.18/L.

This analysis informs decision-making regarding trade-offs between various production processes. For example, developing a cell line that can accommodate 60 doublings per semi-continuous production run allows one to achieve similar per-kilogram production costs with relatively expensive ($0.85/L) medium to batch production using much less expensive ($0.24/L) medium. Likewise, switching to a 50% harvesting model at $0.24/L allows a similar cost benefit as a 90% harvesting model using $0.18/L medium. This analysis also indicates fruitful areas for allocation of resources for strategizing current and future research and development. For example, extending cell lines' proliferative capacity from 24 to about 30 doublings produces much greater returns than further extending their capacity from 30 to 36 doublings.

Similarly, Figure 4 illustrates the relative merit of pursuing lower medium costs versus pursuing greater meat yield in the context of a 50% semi-continuous harvesting scheme. Table 6 discusses various aspects of the model that can be adjusted to achieve 2-fold or 3-fold higher meat production per batch.

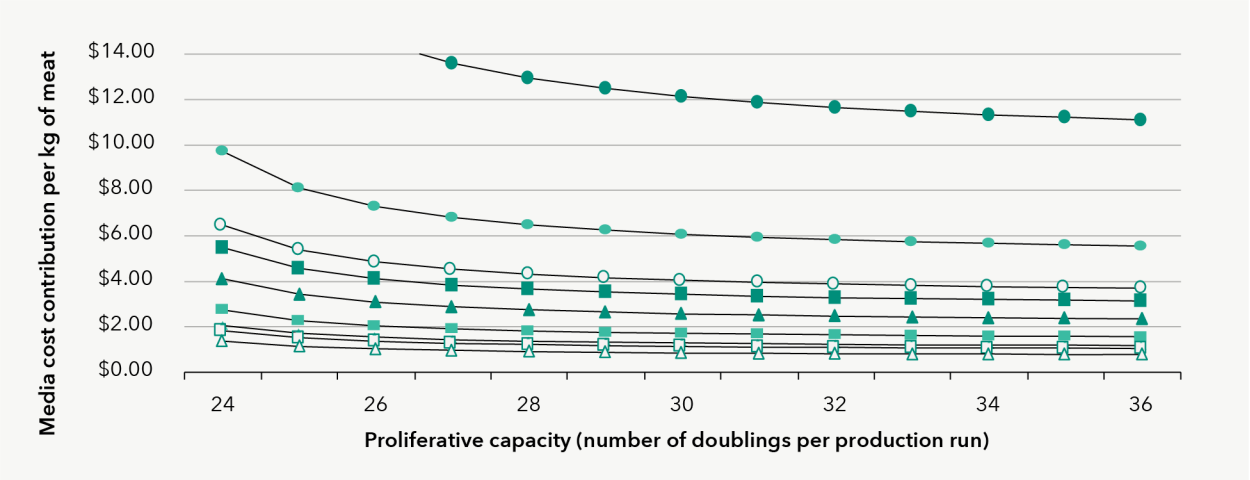

Figure 4. The culture medium cost contribution per kg of meat for various medium costs and meat yields. Circles indicate a culture medium cost of $0.85/L, squares show a culture medium cost of $0.24/L, and triangles show a culture medium cost of $0.18/L. Dark green series represent a yield of 3,500 kg per 20,000 L batch; light green series represent a yield of 7,000 kg per 20,000 L batch; and white series represent a yield of 10,500 kg per 20,000 L batch. A 50% semi-continuous harvesting scheme is applied to all scenarios.

4 Parameter justifications and error estimation

In Table 6, we summarize the assumptions and conversion factors that have been incorporated throughout this analysis and we assess – quantitatively when possible, but otherwise qualitatively – the anticipated impact on overall cost and yield if these factors have been over- or under-estimated. The top portion of the table lists factors where the assumptions were conservative, and thus are areas where the process may look more optimistic in practice than its conceptualization in this analysis. The bottom portion of the table examines factors that were excluded or oversimplified within this analysis, and for which accounting for them in a more advanced iteration may decrease the overall efficiency or yield of the process and/or increase costs.

Table 6. Estimation of magnitudes and downstream implications for assumptions, conversion factors, or unaccounted variables in this analysis.

Conservative assumptions and their implications	
Assumption	**Implication**
Cultivated meat will require all of the components in Essential 8 medium at their current concentrations and supplied in purified form.	Essential 8 is a rich medium that supplies all amino acids, some components that are only relevant for typical R&D applications (for example, the pH indicator phenol red), and high concentrations of growth factors. The concentration of many of these components can likely be reduced without a significant loss in cell performance, and the components that are currently sourced as purified individual components could be sourced as bulk mixtures (for example, protein hydrolysates to provide the majority of the desired amino acid content). As another example, the concentration of one of the most expensive basal medium components – the pH buffer – is largely dictated by the ambient CO_2 concentration in the cells' incubator; current formulations assume an R&D standard convention of 5% CO_2, but altering the sparge air composition in cultivators could significantly decrease buffer requirements. We estimate that eliminating unnecessary components or reducing the concentration of components that are present in excess in the current formulation could further reduce medium costs by at least 20%.
$4 per gram is a reasonable figure for large-scale recombinant protein production.	Conversations with industry experts indicate that recombinant proteins can be produced for as low as $0.10 per gram, albeit at lower purity than current growth factor standards. If this level of purity is tolerable or innovations in low-cost recombinant protein purification techniques are achieved, this would significantly decrease media cost. Even assuming $2 per gram applied to Scenario G would allow a medium cost of $0.18 per liter; $1 per gram would allow a medium cost of $0.15 per liter.
The cell culture medium components will not be recycled.	Only some of the components are degraded or metabolized by the cells, while others simply maintain pH balance or osmotic balance. This latter category can be recycled if a proper separation/filtration is incorporated. The pH buffers in particular are responsible for a substantial fraction of the basal medium cost, accounting for nearly 38% of the total medium cost even in Scenario G where HEPES has already been replaced with the less expensive TES buffer. If sodium bicarbonate and TES alone can be recycled with 75% efficiency, the cost of each "refill" of medium throughout the cultivation process would cost $0.17 per liter, compared to $0.24 per liter for the existing Scenario G medium.
4×10^7 cells/ml is the maximum achievable cell density in the proliferation stage.	As mentioned in Section 3.1, animal cell densities exceeding 1×10^8 cells/ml have been reported. Above this density, it may become difficult to prevent spontaneous differentiation or to ensure uniform nutrient access and aeration, but it is not inconceivable to imagine that a density of perhaps twice the value used in this analysis could be achieved by adapting or modifying cells and/or the bioreactor fluid dynamics. This would either double the number of cells produced in a process of this scale or reduce the tank volume required for the proliferation phase by 50%.
The cells will require 28 hours for doubling.	As discussed in Section 3.2, it is possible that cell doubling times of 24 hours or even slightly lower could be achieved. This would shorten the production cycle by approximately four days (10% of the total production cycle). It may be possible to reduce doubling times further, but there are fundamental limits to the speed of cellular replication due to the size and complexity of animal cells. Attempts to overcome these limits may result in error-prone cellular replication with reduced cellular performance.

Conservative assumptions and their implications	
Assumption	**Implication**
The average volume of a cell is 5,000 μm^3.	The average cardiomyocyte (a non-fused type of muscle cell) has a volume of 15,000 μm^3, so this may serve as a reasonable estimate of the contribution of each fused cell to the total volume of a skeletal muscle cell. Thus, this analysis could be adjusted to increase the cell volume conversion factor by three-fold (15,000 μm^3 versus the current 5,000 μm^3). The reason this larger number was not used in this analysis is to eliminate any assumptions regarding the ability of cultured muscle cells to fully recapitulate the phenotype and physiology of skeletal muscle cells isolated from animals. If 15,000 μm^3 is used as the average muscle cell volume and the final product is assumed to contain 5% fat cells with an average fat cell volume at the lowest end of mature adipocytes, or 60,000 μm^3 (for a final product that is 20% fat by volume), the average cell volume of that mixture is nearly 2.5 times greater than 5,000 μm^3, with a similar impact on overall meat yield per batch.
The cells will not proliferate any further after seeding onto the scaffold.	It is unlikely that cells that are in an active proliferative state will immediately cease to divide upon seeding onto the scaffold. In fact, it may be preferable to seed onto the scaffold at a lower cell density and then achieve an additional few rounds of proliferation in situ because it may facilitate deeper and more uniform penetration into the scaffold. In this case, the actual cell number present on the scaffold prior to the transition towards differentiation and maturation could be 2-fold, 4-fold, or possibly 8-fold (or more) greater than the cell number arrived at in this analysis.
The final tissue will consist of 50% void space, so the tissue perfusion bioreactor is twice the volume of the anticipated final cell volume.	Reliable benchmarks are difficult to find for the fraction of conventional meat that is occupied by the void space of native vasculature so it is not straightforward to define a minimum void space, but 50% is likely to be an overestimate because a final product with 50% void space would not mimic the density of meat. The consequences of necessarily overestimating this value in this analysis are that the volume of medium required in the maturation phase of the process is therefore also an overestimate. Thus, the amount of culture medium that will be continuously perfused through the tissue for the last ten days of the process may be approximately 2- to 4-fold less than indicated in this analysis.
The mass of the final product will consist entirely of cells.	This assumption ignores any contribution of the scaffolding to the final product. While this may be a close approximation in cases where the scaffold is biodegradable and will be passively disintegrated or actively disassembled by the cells as they mature on it, the fact that many companies are exploring scaffolding materials that would not biodegrade in this timescale or under these conditions suggests that the scaffold is likely to contribute to the mass of the final product. For companies exploring predominantly plant-based products infused with a relatively small fraction of cells, the fraction of the final product contributed by the scaffold could well exceed 50%, but a fraction closer to 10-15% of the final product mass is more realistic for products where the scaffold simply serves as the 3D structure and support for the cells.
The cells will require ten days to mature fully after seeding onto the scaffold.	This estimate assumes that the cells must be guided through multiple stages of differentiation and that they are also likely accumulating significant mass during this maturation phase (for example, adipocytes will accumulate lipids and muscle cells will increase in length, fuse, and synthesize significant levels of structural muscle proteins). If the cell population in the proliferation phase is fairly naïve (an iPSC or ESC population, for example), ten days may not be sufficient to achieve complete sensory mimicry of meat – it is difficult to say with certainty since there is little information in the literature on complete differentiation in the context of multi-cell-type co-cultures. However, if some early stages of this differentiation are occurring while cells are still in the proliferation phase or if a company is working with a relatively mature cell type like a proliferative myosatellite cell, this maturation time could be significantly decreased – to as little as three days or perhaps less. Alternatively, sensory analysis may indicate that cells that have not matured fully may sufficiently recapitulate the sensory attributes of conventional meat; therefore, complete maturation may not be necessary.
Miscellaneous rounding errors always on the conservative side	At several additional points throughout this analysis, numbers were rounded up or down in accordance with avoiding an unduly optimistic outcome. For example, the actual number of days needed to saturate each reactor in the proliferation phase is 9.3 days, but this was rounded up to 10 days. As another example, the actual meat yield arrived at in Section 3.4 is 3524 kg but this was rounded down to 3500 kg for all subsequent analyses. These decisions may entirely compensate (or may even over-compensate) for some of the simplifications below that may lengthen the production cycle or reduce meat yield.

Oversimplifications and their implications	
Oversimplification	**Implication**
The medium cost only captures the raw material cost of each component.	Cell culture medium requires labor for preparation and incurs other costs in its production – for example, maintenance of a sterile facility and in some cases filtration, solubilization, or other manipulations of individual components. Industry experts indicate that the raw materials comprise approximately 80% of the total production cost of cell culture medium, while labor and preparation comprise the remaining 20%. This analysis also does not account for markup that third-party medium suppliers will add. We anticipate that as the cultivated meat industry matures, most companies will outsource their media production to a dedicated cell culture media manufacturer. While the profit margins they apply will likely be lower than the current 60-80% margins on cell culture media due to the anticipated scale and price elasticity of the cultivated meat market, this may add another 10-40% to the price the cultivated meat company pays. However, the markup associated with outsourcing to a third-party vendor is expected to be largely negated by the improved negotiated contract pricing they can obtain for each of the medium components since they will be purchasing volumes that represent the aggregate demand of multiple cultivated meat companies.
The medium cost contribution is only one fraction of the total production cost of meat.	This is certainly a salient oversimplification of this analysis. The justifications for focusing on the cell culture medium are presented in the introduction. In the absence of a more holistic cost of goods analysis that accounts for all other aspects of production – including not just other material inputs like the scaffolding but also labor cost, energy, facility capital expenditure and footprint, etc. – focusing on the cell culture medium allows one to at least gain insight into the aspect of the process that bioprocess experts and cultivated meat companies anticipate will be a leading cost driver at scale. As mentioned previously, current estimates of the medium cost contribution to total production cost at scale are in the range of approximately 50-90%, so we feel comfortable that the total cost of production will likely fall within an order of magnitude of the cost of the medium raw materials. However, as medium cost decreases as depicted in each of the scenarios in this analysis, its relative contribution to the overall production cost will obviously decrease.
Essential 8 reflects the complexity of the media formulations required for cultivated meat even though it only contains four growth factors.	Essential 8 medium has been optimized specifically for human pluripotent stem cells, and it is certainly true that the growth factors in particular will need to be adjusted for cells of different type and species of origin, and for different stages within the production cycle. While different growth factors will certainly be required, we do not anticipate that the concentrations or the total number of growth factors for any one formulation will be different in a way that substantially impacts the cost. Among the four growth factors in Essential 8, insulin and transferrin are likely to be required at similar levels by virtually all cell types and species. These comprise the vast majority of the growth factor cost in Scenarios D through G because they are present at much higher levels than FGF-2 and TGF-β: FGF-2 and TGF-β only account for 0.0016% of the total medium cost in Scenario G. Thus, even if these two factors are replaced by many additional factors at approximately the same concentrations, the overall medium cost will not be impacted. In fact, in some cases differentiation is triggered by the *removal* of various growth factors or other nutrients, which would reduce the cost of the differentiation medium.
The medium does not account for additional factors that may be necessary for large-scale cultivation.	Some medium components may need to be added to render this formulation suitable for large-scale growth in the context of cultivated meat production, and those factors may vary between the proliferation phase and the maturation phase. For example, components like bulky polymers or surfactants may be needed to adjust the viscosity of the medium, reduce foaming, and protect cells from shear stress in long-term, large-scale cell culture applications. While these may add cost, it is not anticipated that this additional cost would exceed the cost savings from reducing or eliminating components that are overabundant or unnecessary for large-scale, closed-system growth rather than bench-scale R&D work (as discussed earlier in this table).
There is no lag phase upon inoculation at the beginning of the seed train.	Upon inoculation from a frozen vial of cells, there is often a lag phase of potentially a couple of days before the cells exhibit their maximum doubling time. The extra several hours that this may add to the production cycle is likely more than compensated for by the rounding buffer applied above at each step of the seed train. In addition, the data from which the 28-hour doubling time was calculated assumes cellular proliferation rates from a fresh inoculum, and thus already accounts for a lag phase.

Oversimplifications and their implications	
Oversimplification	**Implication**
Cells cultivated at high density will tolerate the same nutrient concentrations intended for cells at lower densities.	Essential 8 medium has not been formulated for use in applications with the cell densities assumed here (either in the proliferation phase or in tissue perfusion). The nutrient concentrations in this formulation likely reflect levels that are optimized for factors like efficiency of cellular uptake (which is dictated largely by concentration for both passive and active uptake) and osmotic balance, and these parameters are not likely to change significantly as cell density increases. However, the metabolic demands per liter of a high-density culture are certainly higher than those for a low-density culture. Thus, the metabolizable components of the media (glucose, amino acids, lipids, etc.) are likely to require more frequent replenishment or higher rates of continuous dosing into a high-density culture in order to maintain desirable concentrations and support robust cell growth. These metabolic demands will scale roughly linearly with the number of cells present in the cultivator. The medium use cases outlined in Section 3.3 provide a fairly generous estimation of media usage (with a *complete* medium change every two days in the high use case). Therefore, this oversimplification is partially or perhaps wholly balanced out by the fact that only some of the medium components are being depleted while many do not need to be replenished nearly as frequently – or at all – in the context of a medium recycling and perfusion platform.
There is no cellular death or senescence throughout the process.	Every large-scale cell culture process will experience some cellular death and some cells will fail to replicate as expected. Optimized fed batch animal stem cell culture processes routinely maintain upwards of 90-95% viability until approximately one week, where viability drops rapidly as metabolites in the media reach toxic levels. Perfusion culture, by contrast, allows the initial high viability to be maintained for much longer time periods [13]. Perfusion culture is more likely to mimic the situation in large-scale cultivated meat production along with media recycling and toxic metabolite filtration, but a loss of viability on the order of 5-20% is possible.
100% of the proliferated cells adhere to the scaffold.	Most animal cell types – including all those relevant for cultivated meat – are naturally anchorage-dependent and adhere readily to surfaces that exhibit appropriate surface chemistry. Even cells that have been adapted to suspension growth readily adhere when the culture is no longer agitated. Optimization of the scaffold's biomechanical properties can facilitate adherence, and coatings or embedded proteins that promote cell adhesion can be incorporated into the scaffold if needed.[*] In parallel, the influence of many medium components on promoting cell adherence is well characterized, so the medium conditions at the time of seeding onto the scaffold can be optimized for maximizing attachment.[†] It is anticipated that loss of cells due to failure to adhere will be low in an optimized process.
100% of cells differentiate along the desired lineage.	It is implicitly assumed that all of the cells generated in the proliferation stage ultimately differentiate into a desired cell type for cultivated meat. While differentiation efficiency is rarely 100% into any final cell type, a more pertinent question is what fraction of cells end up as a cell type that is acceptable in the final product. As alluded to above, pertaining the questionable necessity of maturing fully to recapitulate the sensory properties of meat, it may not be problematic if a fraction of cells have not matured fully by the time of product harvest. The possibility that some cells may stochastically differentiate down undesirable pathways may be more problematic, but it is important to note that undesirable does not equate with intolerable. For example, a low concentration of red blood cells or fibroblasts that spontaneously form within a tissue may be undesirable in the sense that the predominantly desired cell types are muscle and fat, but they may not detract from the final product and in fact may even enhance its sensory performance. But differentiation into osteocytes (bone cells) would be problematic if it occurred at a high enough frequency to be noticeable. If this spurious differentiation occurs while the cells are in suspension, a flagging and sorting system may be able to remove them prior to seeding onto scaffold. Process optimization, including much of the R&D already underway at cultivated meat companies and other tissue engineering and cell therapy companies, is specifically aimed at increasing the efficiency of differentiation in order to thereby decrease these off-course events. Thus, their frequency by the time cultivated meat is commercialized at large scale is anticipated to be very low.

[*] See technical document on attachment factors that can be added to surfaces to promote adherence: https://www.sigmaaldrich.com/technical-documents/articles/biology/attachment-factors-for-cell-culture.html

[†] See technical document on the influence of medium components on cell adherence: https://www.sigmaaldrich.com/technical-documents/articles/biology/cell-culture-troubleshooting-poor-cell-attachment.html

Oversimplifications and their implications	
Oversimplification	**Implication**
100% of the cultivated cells are harvested.	Because no scalable platform yet exists for the tissue perfusion cultivators required for the maturation stage of the cultivated meat production process, it is difficult to estimate the degree of product loss at harvest. However, given that the entire tissue (including the underlying scaffold, in cases where the scaffold is not biodegraded or absorbed into the tissue itself) will be harvested as the final product, it is unlikely that there will be meaningful product loss at this stage. A small number of cells may not adhere to the scaffold (which is already addressed above), but in the absence of coatings that are suitable for cell adherence on the walls of the bioreactor or other undesirable surfaces, the cells that *do* adhere should only adhere to the harvestable tissue itself.

5 Conclusions and next steps for model refinement

While this analysis serves as a relatively simple first iteration of a model that will increase in complexity and accuracy as additional data are gathered, the assumptions and oversimplifications summarized in Table 6 trend towards an optimistic outlook for improving upon these metrics as the cultivated meat production process is refined. Many of the assumptions in the first portion of Table 6 indicate areas where actual production may be better (less expensive / more productive) than this analysis portrays by several-fold or even an order of magnitude or greater. For example, there is reason to believe that the cell volume estimate is too low by a factor of three or more; that the growth factor cost is too high by ten-fold or more; and that the maximum cell density during proliferation could be at least doubled. Improvements on any *one* of these variables would halve the medium cost per kilogram, and improvements on several fronts could drastically increase the yield, efficiency, and economic viability of the process. By contrast, most of the oversimplifications in the latter portion of Table 6 only threaten to decrease the yield, efficiency, or economic viability of the process by a factor of 20% or less, and many of them are predicted to be ultimately inconsequential or directly negated by one of the conservative assumptions.

Overall, this analysis indicates that based on achievable costs for the major raw material inputs (the cell culture medium components) to cultivated meat production, it is likely that cultivated meat is capable of ultimately being cost-competitive with conventional meat production at scale. While there are still significant biological and engineering challenges to be addressed to make cultivated meat production at these scales a reality, none of the fundamental assumptions within this cost and production volume analysis hinge upon technological breakthroughs. In addition to assessing economic viability at scale, this analysis also serves to inform strategic areas for concerted R&D effort to optimize the variables that have the greatest impact on cost and yield when implemented in a large-scale production environment. As academic and commercial research generate additional data, this model will be iteratively refined as a public tool for the emerging cultivated meat industry.

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The Good Food Institute is a nonprofit that serves as a think tank for the plant-based and cultivated meat, egg, and dairy fields. Our team of scientists, entrepreneurs, lawyers, and policy experts is focused on using food innovation and markets to transform our current system from industrial animal agriculture to plant-based and cultivated meat.

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Note: *This report reflects a generalized conception of cultivated meat production and is not modeled on production processes, cell types, technologies, or any other strategic insights that are specific and unique to individual cultivated meat companies. Specific technological advances or scientific approaches that comprise the intellectual property of the companies that developed them — and thus are not available in the public domain — are not covered in this report.*

Furthermore, this report should be considered a living document, subject to frequent revision and updates as new information becomes available. Please refer to the first page for the date of last revision.

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