

Meat without the animals: cleaning our conscience with clean meat

by Alex Norman and Pranay Shah,
Department of Biochemistry

Abstract

In 2013 well-renowned Chef Richard McGeown cooked and presented live on air a hamburger made entirely from cultured stem cells. This was the culmination of several years of work in the lab of Dr Mark Post, a Dutch cardiologist turned food scientist. His work had constructed the burger with stem cells derived from cows that had been differentiated and grown in a laboratory. Since then the field of 'clean meat', as it is now known, has grown substantially. Many seriously consider it now as a viable option to sustain the world's population on both nutritional and environmental fronts. This progress has been made possible by scientists in academia as well as in start-ups funded by the likes of Richard Branson and Bill Gates. Although the technology is still at an early stage, increased resources and funding for clean meat research have led to several advances. Still, as the field moves forward, two main obstacles will need to be overcome: the perception of lab-grown meat by the public and governments, particularly regarding consumer uptake, regulation and legislation and secondly, the technical challenges that still remain. Among others, these challenges include the up-scaling of production to commercial levels and the engineering of more complex cellular structures to more closely replicate the taste, consistency and texture of meat.

Introduction

In his 1932 essay *Fifty Years Hence*, Winston Churchill made the following prediction: "We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium." Despite being acknowledged 86 years ago, this absurdity still remains. There have been attempts to realise Churchill's vision using meat alternatives based on vegetable-derived products but, in the opinion of

30 many, they fall short of accurately mimicking meat in all its sensations, from sight and feel to
31 taste. Now, decades of biochemical research in tissue engineering, cell culture and protein
32 science amongst other disciplines, are being brought together in the ground-breaking field of
33 clean meat.

34 Clean meat is animal meat made by the process of culturing animal stem cells in such
35 a way that they produce muscle tissue. It reproduces the 3D structure of animal fibres, closely
36 replicating those found in conventional meat. The key difference between conventional and
37 clean meat is that the latter is grown in a laboratory instead of inside a living organism which
38 is then slaughtered. In the process of clean meat production, living animals are only used at
39 the start to donate the initial stem cells (see Figure 1 for a detailed description).

40 In this piece, we describe the ‘absurdity’ that clean meat is tackling as well as how
41 scientists and for-profit companies are achieving it. We outline the obstacles that the field of
42 clean meat currently faces and give our views on its future directions, including the important
43 issue of public acceptance on shifting food production from farms to laboratories.

44

45 **The historical foundations and reasons for adopting clean meat**

46

47 On the 5th of August 2013, Dr Mark Post of the University of Maastricht in the
48 Netherlands unveiled the first burger grown in a lab, thus being the first made of clean meat.
49 This was no small feat: the technologies necessary to grow such a piece of animal tissue were
50 made possible by the preceding 15 years of stem cell and tissue research. This had not been
51 conducted for the purpose of growing muscle for human consumption but primarily in the
52 field of regenerative medicine. However, Dr Post, formerly an assistant professor of Medicine
53 at Harvard Medical School, realised that this research could be used to develop a new avenue
54 of food technology and he pivoted into the fields of vascular physiology and tissue
55 engineering, moving to the Netherlands to establish research groups in these areas.

56 That world-first clean meat burger from his research group cost over \$300,000 to
57 produce and was funded in part by Google-cofounder Sergey Brin. Since those early days

58 when the goal was a proof-of-concept example of meat grown in the lab that was fit for human
59 consumption, the cost of clean meat has gone down to \$5,280/kg (as of June 2017) [1].

60 The successful production of clean meat could, in theory, solve some of the biggest
61 problems that face humanity. One of those problems is that if the global demand for meat
62 increases due to developing world economies becoming wealthier, the current methods of
63 food production will not be able to meet this growing demand [2,3]. Some estimates even put
64 forward that livestock meat production is already at its upper limit [3]. Combined with
65 growing populations, the limited production capacity could set up the conditions necessary
66 for a drastic global shortage of meat. Arguably, meat is already on track to become a luxury
67 commodity affordable to a wealthy minority [4]. Clean meat may be able to tackle this looming
68 problem as it can theoretically be produced more cost-effectively than farmed meat.

69 A second problem that wide-scale clean meat production could solve is that there are
70 well-established environmental burdens associated with the agricultural livestock industry.
71 Around 40% of total CO₂ emissions are attributed to this industry, and factory farming also
72 consumes considerable land, energy and water [2]. Energy usage estimates have recently been
73 made for clean meat production: making 1000 kg of cultured meat would require 26 to 33 GJ
74 energy. This figure is between 7 to 45% lower than the conventional livestock energy
75 consumption [5]. The study also estimates that clean production will use 99% less land and
76 reduce water consumption by 82 to 96% [5].

77 Furthermore, there are considerable problems regarding the ethics of factory farming
78 animals, which is the large-scale industrial process that produces the vast majority of the meat
79 consumed world-wide. The issue of animal welfare divides the public opinion however, since
80 fierce proponents on either side of the animal rights debate are often unwilling to make
81 compromises or come to agreements. For instance, animal rights campaigners are perceived as
82 extremists, and potentially do more harm than good when they use aggressive campaign
83 slogans and scare tactics.

84

85 Still there are good arguments that raising and slaughtering approximately 70 billion
86 land animals every year—most of them ‘broiler chickens’ raised specifically for meat

87 production rather than for eggs—could likely be a pressing moral concern. Modern day
88 scholars like Peter Singer have long argued the moral importance of animal sentience, and
89 Yuval Noah Harari has even described industrial factory farming as ‘perhaps the worst crime
90 in history’ [6]. Recently, detractors of animal factory farming have used educational
91 documentaries to cause viewers to acknowledge the detachment that exists between a meat
92 consumer and the meat producer (this detachment is strongest in the Western world, where
93 the food is usually bought pre-packed in supermarkets.) Films such as *Cowspiracy* give an
94 insight into the world of factory farming and show viewers the harsh methods that allow the
95 mass production of meat to supply the current demand.

96 Finally, there are also consequences to human welfare that arise from modern day
97 factory farming. The greatest concerns are the use of growth-promoting hormones and the
98 gross overuse of antibiotics. Eighty percent of antibiotics used in the USA are given to
99 livestock [7] and the inevitable development and proliferation of antibiotic resistance is
100 rapidly manifesting as a global catastrophe. Furthermore, the incredibly densely populated
101 spaces within factory farms are a major repository of potential human pathogens. Given that
102 an estimated 60% of all human infectious pathogens are zoonotic in nature, this dense
103 population represents an unimaginable public health concern [8]. In this respect, clean meat
104 offers an alternative because the conditions of a laboratory-based facility will be fully
105 sterilised and there will be no need for antibiotic use. Moreover, few contagious pathogens
106 that pose a public health threat infect tissue or muscle cells themselves, so in theory the pool
107 of cells used for clean meat will have a reduced capacity as a repository for potential human
108 disease-causing agents.

109

110 **Clean meat in 2018 - what, how and who?**

111

112 The ability to produce clean meat is seen by many as a necessary development for
113 there to be sustainable food production in the future. By being able to grow meat in a lab, the
114 issues mentioned above can be addressed without altering current eating habits, as the

115 overriding aim of the clean meat field is to produce meat without being more expensive than
116 conventional meat.

117 Although tissue engineering techniques have only just become advanced enough to be
118 used to grow animal tissue in laboratories for food, various meat alternatives have already
119 been produced. The science behind these does not require the production of animal tissue
120 from cells but the use of non-meat-based analogues to replicate the texture and taste of meat.
121 Hence, whilst acellular production of meat substitutes relies on trying to mimic meat's flavour
122 and texture with alternative products, clean meat production aims to grow the actual cells
123 that make up muscle tissue.

124

125 **Acellular meat alternatives**

126

127 Today, acellular-based meat substitutes are ubiquitous in supermarkets in the UK and
128 US. The brand *Quorn* has been available for over three decades (it launched in 1985). Most
129 products are based on soy (e.g. Tofu), wheat proteins (*Seitan*) or mycoprotein (*Quorn*) as these
130 sources comprise high quantities of protein, enabling them to resemble meat's texture.
131 However, despite continuing improvements in technology, these products are not able to fully
132 mimic meat in terms of texture or taste. With this in mind, Stanford professor Patrick O'Brown
133 launched Impossible Foods in 2011 to develop better meat substitutes and combat industrial
134 animal agriculture. After 5 years of development, the company launched the *Impossible Burger*
135 which they claim to use 95% less land, 74% less water, and emit approximately 87% less
136 greenhouse gas than a burger made from cow meat [9]. Importantly, this burger resembles
137 meat more closely than any of its predecessors due to the intensive research put into creating
138 the composition. A chemical called leghaemoglobin, extracted from soybeans, causes the
139 burger to leak a red substance that resembles bleeding. The discovery of this compound, and
140 the ability to mass produce it in genetically modified yeast was the greatest leap forward in
141 developing a more accurate meat analogue. Yeast can be engineered to produce
142 leghaemoglobin more efficiently than soybeans. Further research into how to mimic the taste,
143 texture and appearance of meat led to the development of a blend of vegetable fats and

144 proteins that are used inside the *Impossible Burger*. Potato protein for example makes the
145 burger softer whilst coconut fat causes it to sizzle when cooked. With the production of their
146 *Impossible Burger*, Impossible Foods has demonstrated how biochemical research can be used
147 to develop a huge step forward in meat alternatives.

148

149 **Cellular techniques to make clean meat**

150

151 Many still believe that the production of meat substitutes must be done with animal
152 tissue so as to move away from the minced-meat alternatives that are the only ones available
153 so far, and to develop more accurate meat mimics. This would offer enhancements in
154 appearance, smell, texture and taste over conventional meat alternatives.

155 Clean meat exploits animal cells, obtained with biopsies, to grow animal tissues in the
156 laboratory in a controlled fashion. Because the tissue would be made from animal cells, it
157 would theoretically be identical to meat from slaughtered animals. The work of Dr Mark Post
158 is an example of these so called cellular methods, but there are now around 18 start-ups using
159 similar techniques [11].

160 As shown in the process flowchart in Figure 1, cellular methods generally begin with
161 a minimally invasive muscle biopsy [10] to extract myosatellite stem cells from the animal (the
162 cells that build and repair muscle tissue in healthy animals [12]). The stem cells are then grown
163 in culture inside a laboratory using well-developed techniques that are already widely used
164 in research. Once there a sufficient number of these stem cells in the culture, certain conditions
165 and chemicals are used to make them differentiate into cells that produce muscle fibres –
166 known as myoblast cells. Correct synthesis of muscle fibres is a crucial step for tissue
167 production in living organisms, and a variety of external cues are required for this, including
168 the presence of growth factors and mechanical stimulation. Successful clean meat production
169 must therefore replicate these processes. To achieve this, electric impulses are applied whilst
170 the cells develop as fibres. Growth nutrients are provided in the form of foetal bovine serum.
171 Further research is undergoing to identify additional components needed for muscle tissue
172 production such as fat-providing cells (adipocytes) [13]. In order to produce different forms

173 of meat (e.g. steaks rather than burgers) a synthetic skeleton called a scaffold is provided for
174 the fibres to grow on. The scaffold gives shape to the clean meat, and also provides routes for
175 nutrient entry into the centre of the tissue structure.
176

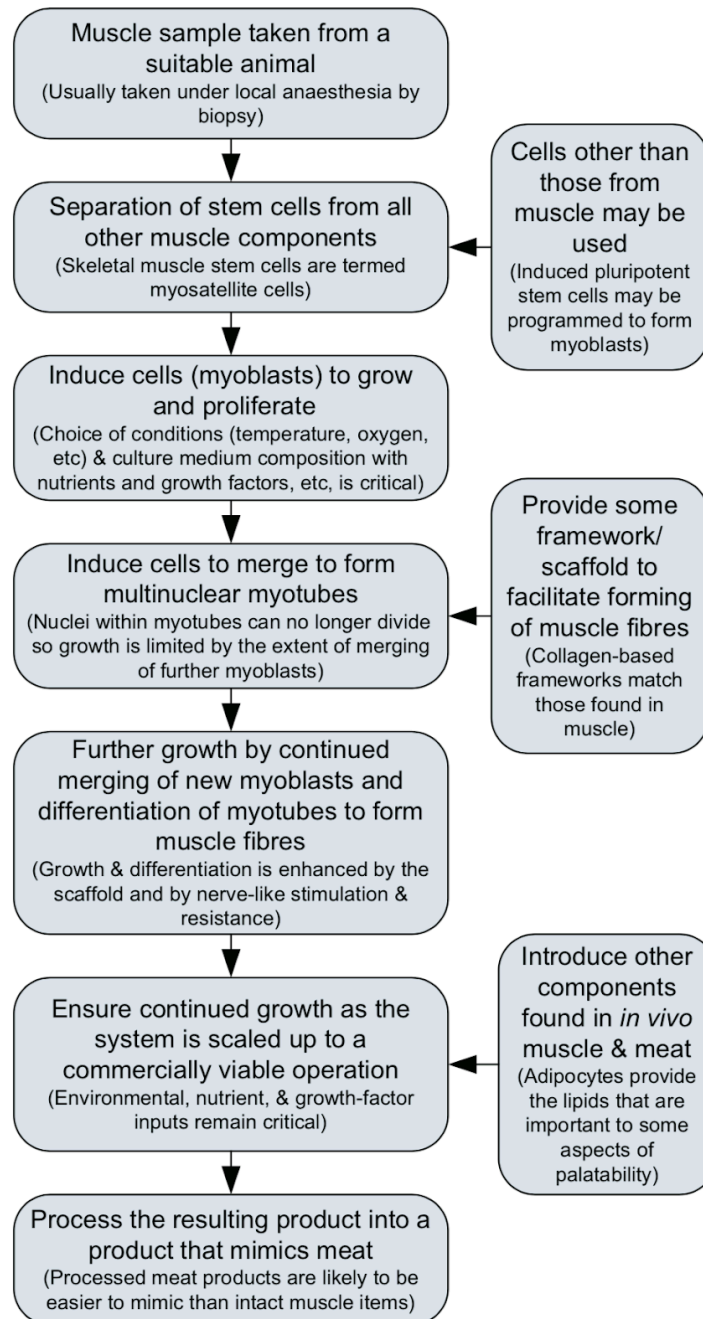


Figure 1: A flowchart to show the main steps required in the production of a clean meat product

177

178 Two huge advantages of clean meat can be made obvious. Firstly, the ability to grow
179 cells in sterile culture conditions allows for a huge reduction in the amount of antibiotics used

180 in food production as previously mentioned. Secondly, the control over which chemicals and
181 nutrients are added to the cell culture permits alteration in the fats, proteins and other
182 biomolecules produced by the cells. This creates the potential for cultured meat to be more
183 nutritional than traditional meat as it could be possible, for example, to decrease the content
184 of cholesterol and increase the protein content.

185

186 **The current commercial climate of clean meat research**

187

188 The scientific developments and breakthroughs currently being made in the clean
189 meat field are primarily in the laboratories of start-ups developed and funded through
190 incubators, investment funds and private investors. Impossible Foods, for example, has raised
191 over \$180 million in funding from sources including Google Ventures and Bill Gates [14]. The
192 latter has also invested in Memphis Meats, a clean meat company that relies on cellular
193 systems and which expects to release products onto the market in 2021. Other clean meat
194 companies are still in early development stages and have only demonstrated products at
195 publicity events. Impossible Foods, on the other hand, has already released products
196 throughout America, from chain stores to Michelin Star restaurants [14]. While there are many
197 obstacles which still need to be overcome by cellular based techniques for clean meat
198 production, at least there has been a precedent set for consumer adoption of acellular meat
199 substitutes like the *Impossible Burger*. We will later discuss these obstacles in more detail.

200 Traditionally, incubators have focussed on technology start-ups but they are now
201 transitioning to ventures founded on deep science. An example is IndieBio, a US-based
202 incubator that is tailored to the biological sciences and to scientists that want to commercialise
203 their research. They provide funding of \$250,000 as well as laboratory space to help scientists
204 create viable products from their initial research in the space of four months. Finless Foods is
205 the most prominent clean meat company to have gone through the IndieBio accelerator; as
206 their name suggests, they are attempting to produce lab-grown fish meat using stem cells
207 derived from fish tissue [15]. Their aim is to bypass current fish farming methods to make

208 clean fish meat that is healthier, cheaper, more environmentally friendly and produced with
209 more sustainable methods.

210 Alongside this, scientific research into clean meat in the for-profit world is also
211 supported in academia. The Oxford Martin School is currently working to tackle global
212 problems using interdisciplinary research. As it is stated on its website, the school
213 acknowledges food production and sustainability as a great global challenge of this century:
214 “Without radical change to the way we produce and consume food [...] there is a substantial
215 risk of significant increases in food prices with major political, environmental and
216 humanitarian consequences.” [16]. Its specific interdisciplinary food research programme
217 brings together the private sector, academia and government to solve the global food crisis.
218 One of the aspects of the programme includes the development of clean meat production. This
219 interdisciplinary approach is extremely important because the obstacles which face clean meat
220 production are not purely scientific, as the next section shows.

221

222 **Obstacles to the production and adoption of clean meat**

223

224 There are three main technological considerations that are presently withholding the
225 large-scale production of clean meat: the type of cell line used for maximal meat production
226 efficiency and for different types of meat, improvements in cell culture media used to grow
227 cells and finally the scaffold that is used for the fibres to assemble on [17].

228 Cell lines (for example, the myosatellite stem cells), must firstly be derived from an
229 appropriate animal species because the replacement of current farming methods will require
230 successful production of replacements for all types of meat e.g. clean chicken, clean lamb etc.
231 Cell lines must also have stable genetics for consistent long-term production of clean meat.
232 Finally, they must be optimised for large scale culture as cells usually behave differently when
233 grown in industrial scales.

234 The culture medium in which the cells are grown will need to be rapidly produced on
235 a large scale and at low costs. It will also need to have an optimal combination of synthetic
236 growth factors to support cell growth and, importantly, it should not contain products

237 derived from animals (as is the current gold-standard media component foetal bovine serum).
238 This last constraint is required to make clean meat truly clean since the production of foetal
239 bovine serum requires animal slaughter. As was mentioned earlier, the main aim of the clean
240 meat field is to ensure global meat demand can be met without the rearing and slaughter of
241 animals. As decades of cell culture in research has required foetal bovine serum, its
242 replacement will need a huge paradigm shift and so this particular aspect of clean meat
243 production represents a particularly challenging obstacle for the clean meat industry.

244 Another massive hurdle is the development of the scaffolds that the cells are grown
245 on because they must allow cell adhesion to their surface and also support the growth of blood
246 vessels whilst being fit for human consumption. Moreover, different scaffolds will be needed
247 for different types of clean meat because the 3D shapes, nutrient requirements and number of
248 blood vessels varies significantly between different tissues. As an example, a fish fillet and
249 steak are constituted from tremendously different types of tissue as the former is white meat
250 with little fat and no blood vessels whilst the latter has a high amount of blood vessels as well
251 as a high protein and fat content. This would need scaffolds that would allow physical
252 stimulation of the muscle fibres as they produce tissue. In order for the development of
253 suitable scaffold, extensive scientific collaboration has already been required and will need to
254 continue. For all the requirements above to be met, the skills of material scientists need to be
255 combined with those of biochemists and tissue scientists.

256 There are also considerable non-scientific obstacles before clean meat can become a
257 viable consumer product. It is currently unclear which organisations would actually regulate
258 clean meat production. In the United States, the safety and quality of conventional livestock
259 meat is under the jurisdiction of the US Department of Agriculture (USDA), whereas cell
260 cultures and biomedicine are regulated by the Food and Drug Agency (FDA) [18].

261 Another obstacle may be the divisions within the industry about what clean meat
262 should actually be. Some of the most influential names in the industry, including Bruce
263 Friedrich of The Good Food Institute, think that it would be bad to deviate from the normal
264 composition of meat and change its fat and protein content, as this may be preferable from a
265 consumer standpoint. However, others such as the CEO of Memphis Meats, Uma Valeti, think

266 that a precision-engineered product will enhance consumer uptake—for example low fat
267 versions of clean meat for health conscious buyers. Although both versions of livestock meat
268 currently exist, it is unclear which marketing tactic will be optimal for the adoption of clean
269 meat.

270 Following from this, a market for clean meat must be created so that the scientific and
271 technological investment is not in vain. Scientists insist that the product is biologically
272 identical to meat grown on animals, but the consumer opinion does not seem to agree (as
273 attested by several polls). A 2016 survey carried out in America revealed that most of the
274 participants were willing to try clean meat but only one third of the total was ‘definitely or
275 probably willing’ to eat clean meat regularly in place of farmed meat [19]. The survey notes
276 that the positive attitudes towards clean meat arise from the potential environmental and
277 public health benefits of product, while negative attitudes come from reservations about the
278 feasibility of industrial scaling and overtones of the ‘unnaturalness’ of meat grown in the
279 laboratory (although this last query is incorrect because clean meat will be grown in
280 bioreactors). This disgust factor may be the biggest hurdle that proponents of clean meat may
281 face, perhaps even more so than the technological barriers.

282

283 **Summary and our proposals for the future of clean meat**

284

285 In summary, we believe that the current state of clean meat research looks set to
286 overcome some of the world’s greatest problems. These include the uncertainty of supplying
287 meat to the world’s growing wealthy population; the ethical considerations regarding the
288 suffering of animals on factory farms; and the potential danger to human populations from
289 factory farming practices such as zoonotic pathogen escape and emerging antibiotic
290 resistance. We discussed the technological hurdles of upscaling cellular growth, efficient
291 growth in serum free media and the availability of edible scaffolds. Non-technical challenges
292 like the public perception of clean meat may prove to be the biggest barriers.

293 In light of the current state of the field of clean meat field and its main obstacles, we
294 consider that there are four key areas where there is room for improvement: there is a need

295 of better scientific collaboration; alternatives to foetal bovine serum for media should be
296 produce; public engagement with clean meat research should be increased; a policy
297 infrastructure governing the sale, distribution and regulation of clean meat for the relevant
298 authorities should be brainstormed. We believe the first of these is important as developing
299 clean meat requires work from a wide range of scientific disciplines such as tissue
300 engineering, materials science and stem cell research. Following on from this, the
301 development of non-animal derived culture media will also be necessary for clean meat to be
302 truly free of animal slaughter, without which clean meat will achieve the goal of replacing
303 livestock animals from the human diet. This is in development already but will require further
304 interdisciplinary research to determine the specific molecules a growth medium requires and
305 mass produce them in commercial quantities.

306 Finally, increased public and authority engagement is a necessity to educate the public
307 on the problems of current meat production techniques as well as ensuring the proper
308 publicity and regulation of clean meat production. Without this there may be a chance that a
309 sizeable market for clean meat will not exist at the right time and the project discontinued,
310 when in other circumstances there might have already been a willing consumer base—
311 nurtured through careful marketing and tactful public education about the failures of factory
312 farming and the viability of clean meat. Appropriate development of the clean meat industry
313 also relies on the imposition of regulatory bodies and distribution networks poised to turn the
314 product into a widely available commodity.

315

316

317 **References:**

318

319 1. J. Bunge, "Cargill Invests in Startup That Grows 'Clean Meat' From Cells". *Wall Street*
320 *Journal*, Aug. 23, 2017.

321 2. FAO, "Livestock's long shadow—Environmental issues and options.". *FAO*
322 *publications*, 2006.

323 3. FAO, "World Livestock 2011. Livestock in food security.". *FAO publications*, 2011.

- 324 4. M. J. Post, "Cultured meat from stem cells: Challenges and prospects". *Meat Science*,
325 vol 92, pp 297–301, 2012.
- 326 5. H. L. Tuomisto and M. Joost Teixeira de Mattos, "Environmental Impacts of Cultured
327 Meat Production", *Environ. Sci. Technol.* vol 45, pp 6117–6123, 2011.
- 328 6. Y. N. Harari, 'Industrial farming is one of the worst crimes in history', *The Guardian*,
329 Sept. 25, 2015.
- 330 7. G. Guglielmi, "Are antibiotics turning livestock into superbug factories?". *Science*
331 *Magazine*, Sept. 28, 2017.
- 332 8. M. E. J. Woolhouse and S. Gowtage-Sequeria, "Host Range and Emerging and
333 Reemerging Pathogens." *Emerging Infectious Diseases*, vol 11(12), pp 1842–1847, 2005.
- 334 9. T. Berg, "Sandwich of the Week". *USA Today*, Sept. 4, 2016.
- 335 10. I. Datar, "Possibilities for an In Vitro Meat Production System". *Innovative Food Science*
336 *and Technology*, Volume 11(1), pp 13-22, Jan, 2010.
- 337 11. "Cell-Ag 101", *Cleanmeat.info*, 2018 [Online]. Available: <https://www.cleanmeat.info>
338 [Accesses: 18-Jun-2018].
- 339 12. Z. F. Bhat and H. Bhat, "Animal-free Meat Biofabrication". *The American Journal of Food*
340 *Technology*, vol 6(6), pp 441-459, 2011.
- 341 13. I. T. Kadim et al., "Cultured meat from muscle stem cells: A review of challenges and
342 prospects". *Journal of Integrative Agriculture*, vol 14(2), pp 222–233, 2015
- 343 14. "Impossible Foods," *Wikipedia*, 19-Apr-2018. [Online]. Available:
344 https://en.wikipedia.org/wiki/Impossible_Foods. [Accessed: 22-Apr-2018].
- 345 15. "Sustainable Seafood, Without the Catch," *Finless Foods*. [Online]. Available:
346 <http://finlessfoods.com/>. [Accessed: 22-Apr-2018].
- 347 16. "About | Oxford Martin Programme on the Future of Food | Programmes," *Oxford*
348 *Martin School*. [Online]. Available:
349 <https://www.oxfordmartin.ox.ac.uk/research/programmes/future-food/about>. [Accessed:
350 22-Apr-2018].

- 351 17. E. A. Specht, "Opportunities for applying biomedical production and manufacturing
352 methods to the development of the clean meat industry". *Biochemical Engineering Journal*, vol
353 132, pp 161–168, 2018.
- 354 18. E. Devitt, "Artificial chicken grown from cells gets a taste test—but who will regulate
355 it?" *Science Magazine*, May 15, 2017.
- 356 19. M. Wilks and C. J. C. Phillips, "Attitudes to in vitro meat: A survey of potential
357 consumers in the United States". *PLoS ONE*, vol 12(2), Feb. 16, 2017.

Preprint

Reviews for 'Meat without the animals: cleaning our conscience with clean meat' by Alex Norman and Pranay Shah (STAAR 8 - 2018)

Review 1 - Elsa Field – Major revision

1. Is the subject matter of the article suitable for an interdisciplinary audience? Yes. It is very much a popular science article. As a non-specialist in medical biology, I had no problem reading the article.

2. Does the title reflect the subject matter of the article? Yes

3. Does the article make a contribution to the discussion in its field? I would not say this is a particularly novel or thorough review. However, as an entry level review on clean meat for those unfamiliar with the issues, it is well written and fairly detailed.

4. Is the article clearly written? Yes, the article is generally written in very good English with just a few grammatical errors (pointed out in Notes on the PDF which I suggest you forward to the author).

5. Is the article well structured? Yes, the article structure is excellent. It is clearly laid out and easy to follow.

6. Are the references relevant and satisfactory? No – the referencing is generally very sparse and this is either due to lack of attention to detail or because the author has not read around the subject very widely. I am sure it is the former rather than the latter, but unless the article is properly referenced (I have given examples in the text where it could be better) then it is impossible to know. General point: beware generalising statements without references!

7. Do you feel the article appropriately uses figures, tables and appendices? There is no real need for further figures and tables and figure 1 is an excellent and clear overview (although, it is not clear if it is an original figure or if it has been taken from another article).

8. What is your recommendation? Major revision

Reviewer's comments to the author: This article is well structured and you have successfully synthesised the main issues at play. I am impressed at the summary of the medical biology – well explained for an interdisciplinary audience. However, the socioeconomic points in the article need to be much more comprehensively referenced with more reference to specific previous research to support your conclusions. I do not think there is a problem with the structure at all, but there are areas where much more detail is needed, such as:

- Environmental and ethical implications of animal farming under the current system. There are currently very few specific references when there are a plethora to choose from and many statistics you could include – including work done by the Oxford Martin School which

you later go on to mention but without any specifics (e.g., see the analysis of the environmental implications of vegan vs meat diets by Peter Scarborough and Marco Springmann, and this important recent publication from Charles Godfray and others as a starting point: DOI: 10.1126/science.aam5324). You suggest that meat consumption might grow in developing countries when the fact is it already has hugely – more specifics needed.

- Consumer perception of clean meat: what studies have been done and what do they show? Perhaps tabulate them to organise them by date and illustrate the main conclusions. You state this is a key challenge for clean meat research to overcome but then do not adequately summarise the research on this (or alternatively, state that little research has been done into this issue so it is a key knowledge gap rather than a barrier as of yet!).

- Who should fund research into clean meat in the future? Governments, NGOs, etc? Who has already funded it and is this funding adequate to meet research challenges ahead?

*

Review 2 - Elliot Swartz - Major

1. Is the subject matter of the article suitable for an interdisciplinary audience? The subject matter is suitable for an interdisciplinary audience

2. Does the title reflect the subject matter of the article? The title can potentially be adjusted. For instance, the title suggests a focus on clean meat, however the authors also discuss plant-based meats such as the Impossible Burger in the text. As described below, I suggest to remove this section altogether or to edit, as plant-based meat production is not 'acellular' (plants are made of cells)! Additionally, "cleansing" may be more appropriate than "cleaning" given the content of the article.

3. Does the article make a contribution to the discussion in its field? The article does a fair job at summarizing the context for which clean meat is to be developed and the current state of the industry. The article as a whole does little in terms of adding any novel insights, however.

4. Is the article clearly written? The article is clearly written. There are few typos and the text is easy to follow.

5. Is the article well structured? The article is structured appropriately but may benefit from deletion of some sections (those discussing plant-based meats) and insertion of additional sections (such as a section on Universities, non-profits, or other institutions currently supporting the industry). Some sections would benefit from clearly stated opening statements as well as a summarizing closing statement.

6. Are the references relevant and satisfactory? The article would benefit greatly from additional references which more thoroughly capture the current state of the literature and field as a whole. Some of these are listed in my comments to the author below.

7. Do you feel the article appropriately uses figures, tables and appendices? The figure used is appropriate. The reader may benefit from having a pictorial representation of the texts to go along with the text boxes of the figure.

8. What is your recommendation? Major revision

Reviewer's comments to the author: This article summarizes some of the history and reasons behind adopting clean meat technologies. The article also discusses some of the technical aspects of clean meat development as well as hurdles to reaching mainstream adoption. In general, the authors do a good job at assessing the overall state of clean meat, in a manner easy to read, although adjustments to the text are needed prior to acceptance. These include more citations which accurately portray the current state of the industry as well as removal of sections on plant-based meats, or otherwise re-framing these sections as appropriate (i.e. mentioning plant-based meats alongside cellular products but as "alternative" protein sources). Specific comments are mentioned below:

Title : "Cleansing" may be a more appropriate descriptor as compared to "cleaning" for the content of the article.

Abstract : The current abstract reads as more of an introduction. I would recommend re-writing the abstract to more accurately frame what is written in the article and the key takeaways.

Line 35 - Clean meat does not necessarily reproduce the complex 3D structure of animal fibers, and products first to market are likely not to recapitulate cuts of meat in their entirety.

Line 47 - may be worth mentioning the NASA study as the foundational work: <https://www.sciencedirect.com/science/article/pii/S0094576502000334>

Line 50 - 15 years is an ambiguous number. Suggest re-wording.

Line 56/57 cite

Paragraph starting on Line 69. Please give a range for CO2 emissions from various studies. There are several studies that have reached different conclusions, and these should be noted. For instance, 18% is a commonly cited reference: Herrero, Mario, Stefan Wirsenius, Benjamin Henderson, Cyrille Rigolot, Philip Thornton, Petr Havlík, Imke de Boer, and Pierre J. Gerber. "Livestock and the Environment: What Have We Learned in the Past Decade?". Annual Review of Environment and Resources 40, no. 1 (2015): 177-202.

How much land, energy, and water? A good source for some metrics: Godfray et al., Science 361, 243 (2018)

Line 76 : Citation number 5 has been updated. There are also additional studies that have been performed. Mention caveats... such as the studies relying on lots of speculation due to clean meat production at scale still not happening yet.

See: Tuomisto, Hanna L., Marianne J. Ellis, and Palle Haastrup. "Environmental Impacts of Cultured Meat: Alternative Production Scenarios." Proceedings of the 9th International Conference on Life Cycle Assessment in the Agri-Food Sector, San Francisco, 2014. <http://lcafood2014.org/papers/132.pdf>

Mattick, C. S., A. E. Landis, B. R. Allenby, and N. J. Genovese. "Anticipatory Life Cycle Analysis of in Vitro Biomass Cultivation for Cultured Meat Production in the United States." *Environ Sci Technol* 49, no. 19 (Oct 06 2015): 11941-9.

This study (Mattick et al.) is generally considered to be the most accurate, as it relies on production of CHO cells at scale, which is most similar to how clean meat production may occur.

Line 85 : Use actual numbers of chickens, fish, cattle, etc that are slaughtered and cite. Use the numbers to make the point, and then support this idea by citing the authors or documentaries who hold similar views.

Line 106 - the cells themselves usually aren't the hosts of contamination in cell culture (with exception being mycoplasma). Rather, bacterial or fungal contamination that grows off of the nutrients in the cell culture medium can be detected through routine quality control measures. If contamination is detected, then the container can be sterilized.

Section on Line 125 — plant-based meats are not acellular. Acellular agriculture would be recombinant protein production / harvesting using yeast as the source (e.g. companies such as Clara Foods, Perfect Day). It's unclear the necessity of this section, as plant-based meats are fundamentally different from clean meat. It also strays from the title of the article, which indicates a focus on clean meat. The hyphae of the fungus used in Quorn products is made up of cells.

Line 135 Impossible Foods commissioned a study looking at environmental impacts of the Impossible Burger: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189029>

Line 138-139. — be specific. 'Red substance' —> iron-containing heme?

Line 160: authors should note that other cell types can be used — MSCs, iPSCs, etc. in the text (this is partially mentioned in Figure 1).

Line 164: typo "Once there is a sufficient number...."

Line 166: The stem cells are myoblasts — the differentiated cells are myotubes or myofibers. The progression and terminology can be seen here: Myoblast —> myocyte —> myotube —> myofiber
Development (2017) 144, 2104-2122 doi:10.1242/dev.151035

Line 169 -170 : It may not be required to provide electrical stimulation in order to make a

clean meat product. Rather, stimulation may influence the maturity of the muscle cells (in terms of sarcomeric organization and hypertrophy) as well as texture of the final product.

Figure 1: The reasoning for 'merging' of myotubes needs to be re-written.

Overall it is fairly accurate. I'd put more emphasis on the separation of proliferative / differentiation phases, as these will occur in a spatiotemporal manner.

Refer again to Development (2017) 144, 2104-2122 doi:10.1242/dev.151035 and related citations within the document for understanding the developmental progression of skeletal muscle.

Line 179: avoid colloquialisms — "a huge reduction"

Line 180 - need citations for the claim that "control over which chemicals and nutrients are added to the cell culture permits alteration in the fats, proteins, and other biomolecules produced by the cells"

Line 190 - Impossible Foods is not clean meat. Subsequent mention of Impossible Foods in this section is not relevant.

Line 200 - "focussed" typo

You may want to make mention in this section of GFI's global map of incubators/accelerators as a

resource: <https://www.google.com/maps/d/u/0/edit?mid=1OWDz0tll-KiqT0aMRx55efkOoiY&ll=2.251337582717788%2C0&z=2>

204 - Both Memphis Meats and Finless Foods have gone through IndieBio, with Memphis being the most prominent in terms of funds raised and press attention.

Line 210 - Not sure if this paragraph belongs in this section. Maybe start a new paragraph discussing institutions (Universities, non-profits, etc) that are aligned in their interest for clean meat research.

Paragraph 234 - mention that serum-free alternatives exist and several papers show iPSC — > muscle differentiation in entirely serum-free conditions. Note that some formulations include bovine serum albumin, a protein derived from animals, which may also be considered for removal of future medium formulations. Some examples:

<https://www.nature.com/articles/srep41833>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4022691/>

<https://www.nature.com/articles/nbt.3297>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4266001/>

245 - don't necessarily need blood vessels specifically.. just need a way to oxygenate the tissues. There are strategies for this. A good recent review on the topic: <https://doi.org/10.1016/j.tibtech.2018.02.012>

252 - you don't need physical stimulation. Stimulation may result in muscle hypertrophy or influence the texture of the final product. It's unclear if it's necessary, however.

260 - state that FDA has asserted authority. Still unclear how the regulation will work out, however.

<http://www.sciencemag.org/news/2018/07/fda-tries-take-reins-regulating-cultured-meat>

Paragraph 261 - citation for the quotes from Uma Valeti and Bruce Friedrich

Paragraph 270 - GFI's new survey has more encouraging results when the question is framed differently. Would be worth discussing some of the various studies and how they compare.

<https://faunalytics.org/wp-content/uploads/2018/08/Clean-Meat-Acceptance-Primary-Findings.pdf>

Line 286 - appropriate to just label this section "Discussion"

Line 295 - note my previous comments on the current availability of serum-free alternatives

296 - maybe mention w/ public engagement so as to not replicate the disaster of GMOs. A good article is below on how public perception influenced GMO foods.

<https://www.sentienceinstitute.org/gm-foods>

302 - Importantly, also cost effective and reproducible

*

Reviewer 3 - Max Schwiening – Minor revision

1. Is the subject matter of the article suitable for an interdisciplinary audience? Yes

2. Does the title reflect the subject matter of the article?: Yes

3. Does the article make a contribution to the discussion in its field?: Yes

4. Is the article clearly written?: Yes on the whole it is very clearly written. The word zoonotic might need explaining. What is deep science? large NUMBER of blood vessels or highly vascularised rather than "high amount of blood vessels"

5. Is the article well structured?: Yes

6. Are the references relevant and satisfactory?: Possibly make the reference on line 72 more detailed as I tried to look up the exact information but the source is too big to look through.

7. Do you feel the article appropriately uses figures, tables and appendices?: Yes.

8. What is your recommendation?: Minor revision

Reviewer's comments to the author: None

*

Reviewer 4 – Marie Gibbons – Minor revision

1. Is the subject matter of the article suitable for an interdisciplinary audience? yes-- this review is tailored to experts in the field and those who have little to no knowledge of clean meat. Biologists, social scientists, and those in non-scientific fields alike are able to gain insight and relate to the information.

2. Does the title reflect the subject matter of the article? Absolutely-- the authors do a great job of describing the process of clean meat production, as well as several different hurdles the field must face.

3. Does the article make a contribution to the discussion in its field? yes-- the authors bring novel insight to the field regarding the history of the product, as well as the seriousness of the hurdles faced by consumer acceptance

4. Is the article clearly written? yes! I caught a few typos with fresh eyes:

228— spelling is “myosatellite”

253— “a” suitable scaffold

296— produce”d”

5. Is the article well structured? yes

6. Are the references relevant and satisfactory? yes

7. Do you feel the article appropriately uses figures, tables and appendices? yes

8. What is your recommendation? Minor revision

Reviewer's comments to the author: This is a very well written and researched article. I would suggest making a few minor revisions:

The authors refer to the clean meat process as "lab-grown" and make note of being grown in the lab several times within the article (lines 37, 104, 113, 118, 156). However, it is only near the end (line 280) that the authors point out that final production will take place in food factories and not labs. I would suggest making this case sooner, and clarifying that, like many food products we consume today, the initial research into the production of clean meat is taking place in a lab. However, the final products will not be lab-sourced, but rather made in factories, local craft carneries, and even in table-top bioreactors in the home. I would also suggest differentiating between conventional meat and clean meat by pointing out that clean meat is made without animals, rather than pointing out that it is currently made in a lab. I would also substitute “chemicals” with “vitamins and minerals” in line 180 to educate rather than confuse or even put off readers.

Lines 121, 122, 125, 149, and 160 suggest that yeast/fungi/plant-based meat substitutes are

"acellular" however these organisms have cells too! I recommend changing the phrases from "acellular" to "animal-free" or even plant-based, to cut down on confusion. You may also want to rephrase starting at line 190 so as not to classify impossible foods as a clean meat company.

Regarding the scientific statements:

Line 39-- you should clarify that we only theoretically need a single isolation from an animal to produce unlimited meat production, thanks to exponential cell growth in optimum conditions. This is important to point out, otherwise readers may assume that animal farming would still continue for the purpose of routine isolation procedures, but in reality that will not be the case!

155-- I would suggest using the term "utilize" vs "exploit"

164-165— its actually just a decrease in nutrients and crowded conditions that elicits differentiation, if you wanted to get more technical than "certain conditions and chemicals"

168-170— I would argue that you may not need to stimulate muscles mechanically. This might be able to be replicated via increased calcium exposure, rather than electrical stimuli. Maybe phrase this as a possibility, rather than a necessary step?

243— I would recommend mentioning that several serum-free media formulations exist, and it is just a matter of applying them to clean meat cell lines and decreasing costs via scale-up. FBS is used because it is easy, not because it is necessary

250— I think fish have blood vessels in their "fillet"... just nowhere near as many as cows do! Maybe rephrase from "no blood vessels" to "fewer"? BUT I'm also not a fish cardiologist so I could be wrong :P

A few more random notes:

81— not sure if this last sentence is necessary—Don't want to appear bias towards either extreme!

105— may want to elaborate on where the majority of our food-borne illness-based food pathogens come from: animal guts/poop. And I'm pretty sure clean meat will be poop-free :P

115— it should actually end up being cheaper!

204— Memphis Meats went through Indiebio too

218— so excited to hear that Oxford Martin is focusing on clean meat!!!!!! Other academic institutions include schools at Harvard, Tufts, Bath, Ottawa, Technion, and Maastricht