Prospects for the Use of Induced Pluripotent Stem Cells in Animal Conservation and Environmental Protection

MORGAN M. STANTON,a EVANGELINE TZATZALOS,a MATTHEW DONNE,a NIKOLA KOLUNDZIC,b INGVAR HELGASON,a DUSKO ILIC a,b

aVitroLabs Inc., South San Francisco, California, USA; bDepartment of Women and Children’s Health, Faculty of Science and Medicine, King’s College London, School of Life Course Sciences, London, United Kingdom

SUMMARY

Stem cells are unique cell populations able to copy themselves exactly as well as specialize into new cell types. Stem cells isolated from early stages of embryo development are pluripotent, i.e., can be differentiated into multiple different cell types. In addition, scientists have found a way of reverting specialized cells from an adult into an embryonic-like state. These cells, that are as effective as cells isolated from early embryos, are termed induced pluripotent stem cells (iPSCs). The potency of iPSC technology is recently being employed by researchers aimed at helping wildlife and environmental conservation efforts. Ambitious attempts using iPSCs are being made to preserve endangered animals as well as reanimate extinct species, merging science fiction with reality. Other research to sustain natural resources and promote animal welfare are exploring iPSCs for laboratory grown animal products without harm to animals offering unorthodox options for creating meat, leather, and fur. There is great potential in iPSC technology and what can be achieved in consumerism, animal welfare, and environmental protection and conservation. Here, we discuss current research in the field of iPSCs and how these research groups are attempting to achieve their goals.

INTRODUCTION

Reprogramming of adult somatic cells into induced pluripotent stem cells (iPSCs) has massive potential to revolutionize personalized medicine, drug discovery, and cell therapy, but this represents only a portion of iPSC technology’s capabilities. iPSCs also have great promise to aid endangered animal species and native habitat preservation [1], revive several extinct species [2], and reduce consumer dependence on animal products [3]. Similar to embryonic stem cells (ESCs), iPSCs can expand indefinitely and are capable of differentiating into all three germ layers [4, 5]. However, unlike ESCs, iPSCs do not require embryonic tissues or oocytes for harvesting. For endangered species, the supply of embryos is often restricted and iPSCs from somatic tissue offer a more practical source of stem cells with less moral and ethical dilemmas. This also offers significant advantages for using iPSCs from domestic animals to reduce animal death for commercially produced animal products. Domestic farm animals are a large drain on natural resources, requiring deforestation for pasture, significant water consumption, and produce considerable greenhouse gas emissions. Alternative animal products derived from iPSCs have the potential to reduce the environmental damage caused by large-scale farming and present eco-friendly commercial applications. Here, we discuss past and current research utilizing iPSCs and how they could benefit wildlife conservation and animal welfare as well as their limitations and future avenues for iPSC technology.

IPSC LINES FOR REVIVAL OF ENDANGERED OR EXTINCT WILD ANIMAL SPECIES

The ability to readily make iPSC lines from adult tissue raises the possibility of adding a critically important safety net in the preservation of endangered animal species. However, iPSCs must prove they can aid in animal assisted reproductive technologies (ART), where gametes can be developed in vitro. The
first reported example of fully functional animals derived from iPSCs was in mice using the tetraploid complementation assay [6, 7], where iPSCs are injected into an in vitro cultured tetraploid blastocyst and transferred to a surrogate female for gestation [8]. Since then, iPSC lines have been derived from an extremely diverse group of wild species including birds, primates, bovids, and large cats, as seen in greater detail in Table 1. The majority of the iPSC lines were created using Yamanaka reprogramming factors (OCT4, SOX2, KLF4, and c-MYC) and almost all these cell lines have been reported to be derived from samples taken from adult tissue. Some of the samples used in this research were made possible through use of the San Diego Zoo Institute of Conservation Research’s Frozen Zoo center, which currently has over 10,000 samples from approximately 1,000 species in a reference bank known as Frozen Zoo that began collecting samples in 1976 [9]. Another reference base, The Genome 10K project also provides vital biological data for creating iPSCs and generating viable offspring from endangered animals.

The next successful attempt at creating live animals from iPSCs was achieved in 2013 with viable piglets [12]. These piglets were achieved using iPSCs as nuclei donors for nuclear transfer into an enucleated donor egg (somatic cell nuclear transfer). It was reported that viable offspring were created from both the tetraploid complementation and intracellular nuclear injection methods. However, these methods have not yet transferred to any endangered species even though they have successfully derived iPSCs (Table 1). It should be noted that donor embryos or eggs are likely to be unavailable when working with endangered, extinct, or nearly extinct species. Therefore, the only way forward would be creation of embryos from iPSC-derived gametes. Fully functional spermatozoïds and oocytes derived from iPSCs have been reported in mice but required injection of these immature gametes into an adult mouse testis or ovary to become fully functional [13, 14]. Generation of fully functional oocytes from mouse iPSCs was reported again in 2016, but this was achieved through ex vivo coculture with female gonadal somatic cells [15].

In a more recent development, ESCs and embryos have been created from the critically endangered northern white rhinoceros (NWR, *Ceratotherium simum cottonii*) [16]. This represents a meaningful step in bridging ESCs and iPSCs research in domestic and laboratory animals to assist with endangered species preservation. In March 2018, the last male NWR died in captivity, leaving only two remaining females for the species and they are both infertile. Hil-derbrandt et al. recovered oocytes from the southern white rhinoceros (SWR, *Ceratotherium simum simum*), a related subspecies of the NWR and not currently endangered. The oocytes were matured in vitro, fertilized, and the resulting embryos were implanted into a female SWR and carried to term, preserving NWR genes through ART. The next step is to attempt to develop artificially generated NWR oocytes from cryopreserved NWR somatic tissue using iPSCs. With such a limited supply of NWR genetic material, iPSCs offer the ability to develop the genetic diversity and increase the population size of a critically endangered species [1].

| Year | Event | Citation |
|------|-------|----------|
| 1976 | Frozen ZOO | [9] |
| 2006 | Derived first iPSC line | [4] |
| 2008 | Derived iPSC from rhesus macaque (Macaca mulatta, O235#) | [17] |
| 2009 | Initiation of the Genome 10K Project | [10] |
| 2009 | Adult mice generated from iPSCs | [6, 7] |
| 2011, 2012 | Functional mouse gametes, in vitro | [13, 14] |
| 2011 | Derived iPSCs from drill (Mandrillus leucophaeus) | [1] |
| 2011 | Derived iPSCs from bovine | [18] |
| 2011 | Derived iPSC from equine fibroblasts | [19] |
| 2012 | Derived iPSC from water buffalo (Bubalus bubalis) | [20] |
| 2012 | Derived iPSC from snow leopard (Panthera uncia) | [21] |
| 2012 | Derived iPSC from quail | [22] |
| 2013 | Piglets generated from iPSCs | [12] |
| 2013 | Derived iPSC from Bengal tiger (Panthera tigris), serval (Leptailurus serval), and jaguar (Panthera onca) | [23] |
| 2015 | Derived iPSC from orangutan (Pongo abelii) | [24] |
| 2015 | "Conservation by Cellular Technologies" meeting to discuss the potential for iPSCs to preserve the nearly extinct northern white rhinoceros (Ceratotherium simum cottonii) | [25] |
| 2015 | Report of editing Asian elephant cells to contain gene sequences of the Woolly mammoth (Mammuthus primigenius) | [26] |
| 2016 | Functional mouse oocytes from iPSCs, ex vivo. | [15] |
| 2018 | Development of hybrid northern white rhinoceros (Ceratotherium simum cottonii) and southern white rhinoceros (Ceratotherium simum simum) embryos in vitro with potential for implantation in surrogate | [16] |
sequences with the goal of creating an elephant–mammoth hybrid that would be able to survive to colder climates. Genome editing has numerous applications in editing living organisms with extinct animal sequences, but extensive knowledge of the genetic differences between the two species is needed. Comparisons between Asian elephants and woolly mammoths found 1.5 million nucleotide differences [28], making it difficult to gauge if an elephant–mammoth hybrid would be more similar to one animal or the other in physicality or behavioral traits. Analogous to the NWR, to create a viable mammoth embryo, the potency of iPSCs could be enhanced or extinct animals can be generated in lab. The majority of animal reproductive research using stem cells has only a 5%–13% efficacy rate for viable animals [8], and the mechanisms that control the generation of fully pluripotent iPSCs have not been sufficiently investigated. More research into understanding iPSC reprogramming factors needs to be explored in order to aid in wildlife preservation and restoration. The main goal of conservation is to prevent an extinction event for any animal, and genetic engineering and iPSCs have the potential to reverse what was once thought to be irreparable damage. However, animals developed through these processes, (endangered, extinct, or hybrids) will be raised in captivity which may make these species ineligible for release into the wild. It is also unclear once a species is resurrected if its behavior will help or harm its previous native habitat or help reestablish previous ecosystem balance. Reintroduction of endangered animals into their original habitats has had success stories for ecosystem recovery, including the reintroduction of gray wolves (Canis lupus) to Yellowstone National Park through the U.S. Endangered Species Act of 1973. Bringing back wolves into the park reduced the excess elk population and consequently increased the populations of various trees and plants that the elk fed upon. Reintroduction of wolves also brought surges in population growth of other endangered species in the park including the beaver (Castor canadensis) and bison (Bison bison) [33]. However, some efforts to restore animal populations hurt the species more than help. In July 2018, wildlife workers in Kenya tried relocating 11 black rhinoceroses (Diceros bicornis) to Tsavo East National Park to help restore their population, but 10 died due to contaminated drinking water at their new location [34]. These events indicate ongoing work is needed, to ensure after species resurrection, that the newly revived species will be able to exist and live a healthy life outside of captivity and survive in a new habitat.

**Future Prospects and Challenges of iPSCs for Wildlife Conservation**

With the rapid ascent of ESCs and iPSC technology, it can be hypothesized that the iPSCs research will develop fully functioning, mature gametes without the use of extraneous tissue for other endangered or extinct species to form viable embryos. Nevertheless, despite the optimism for iPSC technology, there are practical and ethical obstacles to overcome before endangered or extinct animals can be generated in lab. The majority of animal reproductive research using stem cells has only a 5%–13% efficacy rate for viable animals [8], and the mechanisms that control the generation of fully pluripotent iPSCs have not been sufficiently investigated. More research into understanding iPSC reprogramming factors needs to be explored in order to aid in wildlife preservation and restoration. The main goal of cellular agriculture and iPSC—Laboratory Research to Commercial Aspects

Due to human expansion, mass extinctions of other species have become common; current research suggests that the planet is entering a sixth “mass extinction” event with a dozen of animal and plant species lost daily [35, 36]. In spite of...
conservation efforts, large-scale land clearing for livestock, overfishing, and environmentally harmful farming practices continue to reduce and irreparably damage native habitats and species. Animal welfare is a priority but equally as important is reducing the need for livestock animals and the land resources they require. Reports from the United Nations Food and Agriculture Organization (FAO) have shown that livestock is the world’s largest user of land resources, with 80% of all agricultural land dedicated to their development and feeding [37], leading to subsequent increased deforestation and greenhouse gas emissions [38]. Additionally, to sustain large quantities of farm animals in enclosed environments, livestock are often given significant antibiotics and growth hormones generating antibiotic resistance in consumers [39]. The knowledge of detrimental effects of industrial farming on the environment and consumer health has created a desire for more environmentally sustainable animal products. To reduce the impact of industrial farming and deforestation, the scientific community has established a new field of stem cell research deemed, cellular agriculture. Cellular agriculture seeks to create animal-based products in the lab, such as meat, eggs, leather, or fur without harming or killing live animals while simultaneously reducing land resources for farming. Stem cells from the animals are collected using a small biopsy, multiplied in the lab, and then engineered to imitate the desired animal products. Large numbers of domestic animals have already had protocols for their respective iPSC lineage derived (Table 1), making it an advantageous moment to explore laboratory grown animal products. Cellular agriculture offers a more environmentally friendly alternative to farming, and iPSC technology presents an exciting opportunity to create animal products at industrial scale [40].

The first major cellular agriculture development occurred with Mark Post’s group in 2013, where he and his team showed the world that lab grown meat was feasible [41]. After thousands of muscle fibers were grown over three months, Post and his colleagues cooked and ate the cells in front of an
with higher dimensional (2D) plates, provide an environment to culture cells where cells can be grown in suspension instead of on two-dimensional (2D) plates. iPSCs, need to be engineered to reduce the cost. Bioreactors, consuming.

may initiate regulations on how iPSCs will be labeled for uses for when these products inevitably go to market and ble animal product alternatives. The petition will create dis-

sumers about their food [45], however research labs continue lowering the cost compared to traditional cell culture methods.

animal iPSCs from a bioreactor could be collected and mixed with future regulatory restrictions as well as have improved product-free conditions and these regulations could also apply to future iPSCs meats or products for consumers. Some recent cell culture methods have developed xeno-free and feeder-free methods of stem cell culture, reducing or completely eliminating animal products from their protocols to comply with future regulatory restrictions as well as have improved quality control processes [43, 44]. An advantage of having highly standardized media will not only be the elimination of animal proteins but also the elimination of hormones and anti-

biotics that are given to farm animals that can be transferred to consumers. The final laboratory meat product would offer a competitive alternative to traditional meat producers. The U.S. Cattlemen’s Association has taken notice and in February 2018 filed a petition with the U.S. Department of Agriculture to prevent laboratory meat product from being labeled with the words “beef” or “meat,” stating it might misinform con-

sumers about their food [45], however research labs continue to work on building and marketing their products as comparab-

le animal product alternatives. The petition will create dis-

putes for when these products inevitably go to market and may initiate regulations on how iPSCs will be labeled for consumption.

Another challenge for cellular agriculture is to address the high cost of its fabricated products. At ~$40,000 per kg, labora-

tory meat is currently not financially available for most con-

sumers. Improved methods of mass cell culture, in particular iPSCs, need to be engineered to reduce the cost. Bioreactors, where cells can be grown in suspension instead of on two-

dimensional (2D) plates, provide an environment to culture cells with higher efficiency producing billions of iPSCs and their deriv-

atives in only a few days. Shafa et al. had successful prolifera-
tion of mouse iPSCs in stirred bioreactors [46], and Abecasis et al. demonstrated human iPSCs scale up in bioreactors using xeno-free media [47]. To imitate meat like ground beef or pork, animal iPSCs from a bioreactor could be collected and mixed together to mimic the real processed meat product substantially lowering the cost compared to traditional cell culture methods.

For the formation of highly ordered complex tissue, such as skin, fur, or meat fillets, the collected cells must also be integrated into a scaffold with defined porosity and vasculari-

zation system. Fabrication of dense tissue with micro vasculari-

zation for nutrient exchange is exceedingly difficult to build, but there has been significant progress in the field of three-

dimensional (3D) bioprinting to overcome this issue. Bioprint-

ing uses living cells suspended in a hydrogel bioink that can be extruded or polymerized into a complex 3D structure with assistance of computer generated models [48]. Ma et al. were successful at printing 3D microscale hexagonal gelatin con-

structs for human iPSCs with improved phenotypic and func-

tional enhancements when compared to cells in 2D cultures [49]. Artificial skin constructs, also created using human iPSCs, were designed by Abaci et al. using a printed vasculature within an alginate hydrogel [50]. Skin and fur derived from ani-

mal iPSCs would offer a suitable leather and fur alternative, particularly for exotic animals such as crocodiles farmed for their pelts. Common leather types, such as bovine or porcine, are collected from domestic animals that are bred principally for meat, but iPSC-derived sources of this animal leather offer a first-step for consumers and industry to move away from industrial farming. As meat, leather, fur, and other animal derived products move closer to being released for market, public awareness to animal and environmental welfare becomes greater, creating more incentive for research in the cellular agriculture field and greater demand for more environ-

mentally sustainable products.

**FUTURE DIRECTIONS**

Looking forward, iPSC technology will continue to play a major role not only in the advancement of medical sciences, but also improving animal conservation and environmental protection. iPSCs, gene editing, and “biobanks” with tissues of all living and some extinct animals will allow us to save current and future endangered species through increasing genetic diversi-
ties, or in worst case scenarios, de-extinction events. Although this type of research awaits funding, we should keep in mind that the iPSC technology might have much broader potential. Cellular agriculture from iPSCs of pig, cow, and other domestic animals will create clean meat and reduce the impact on the environment caused by commercial-scale animal husbandry [51]. We can also imagine using iPSC technology to manufac-
ture and commercialize exotic animal products without killing animals. For example, high-quality ivory generated in vitro and made available commercially could compete with black market and would decrease poaching, indirectly protecting the species from extinction. There are boundless opportunities to use iPSCs to protect the environment. The future potentials of iPSC technology and what we can achieve in environmental protec-
tion and conversation are directed by our own creativity and ambition (Fig. 1).

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et al. Embryos and embryonic stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.

5. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–317.

6. Boland MJ, Hazen JL, Nazor KL et al. Adult mice generated from induced pluripotent stem cells. Nature 2009;461:91–94.

7. Zhao XY, Li W, Lv Z et al. IPS cells produce viable mice through tetraploid complementation. Nature 2009;461:86–90.

8. Boland MJ, Hazen JL, Nazor KL, Rodriguez A. R., Martin G, Kupriyanov S., Baldwin K. K. Generation of Mouse Derived from Induced Pluripotent Stem Cells. J Vis Exp 2012;(69):e40033.

9. Benirschke K. The frozen ZOO concept. Zoo Biol 1984;3:325–328.

10. Koepfl F, Paten B, Genome 10K Community of Scientists et al. The Genome 10K Project: A way forward. Annu Rev Anim Biosci 2015;5:375–117.

11. Du X, Feng T, Yu D et al. Barriers for deriving transgene-free IPS cells with epigenetic vectors. Stem Cells 2015;33:3228–3238.

12. Fan N, Chen J, Shang Z et al. Piglets cloned from induced pluripotent stem cells. Cell Res 2013;23:162–166.

13. Hayashi K, Ogushi S, Kurimoto K et al. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. Science 2012;338:971–975.

14. Hayashi K, Ohta H, Kurimoto K et al. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 2011;146:519–532.

15. Hayashi K, Hamazaki N, Nagamatsu G et al. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature 2016;539:299–303.

16. Hildebrandt TB, Hermes R, Colleoni S et al. Embryos and embryonic stem cells from the white rhinoceros. Nat Commun 2018;9:2589.

17. Liu H, Zhu F, Yong J et al. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 2008;3:587–590.

18. Han X, Han J, Ding F et al. Generation of induced pluripotent stem cells from bovine embryonic fibroblast cells. Cell Res 2011;21:1509–1512.

19. Nagy K, Sung HK, Zhang P et al. Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Rev 2011;7:693–702.

20. Deng Y, Liu Q, Luo C et al. Generation of induced pluripotent stem cells from buffalo (Bubalus bubalis) fetal fibroblasts with buffalo defined factors. Stem Cells Dev 2012;21:2485–2494.

21. Verma R, Holland MK, Temple-Smith P et al. Inducing pluripotency in somatic cells from the snow leopard (Panthera uncia), an endangered feld. Theriogenology 2012;77:220–228, e1–e.

22. Lu Y, West FD, Jordan BJ et al. Avian-induced pluripotent stem cells derived using human reprogramming factors. Stem Cells Dev 2012;21:394–403.

23. Verma R, Liu J, Holland MK et al. Nanog is an essential factor for induction of pluripotency in somatic cells from endangered felines. Biore Open Access 2013;2:72–76.

24. Ramaswamy K, Yik W, Wang XM et al. Derivation of induced pluripotent stem cells from orangutan skin fibroblasts. BMC Res Notes 2015;8:577.

25. Saragusti et al., Zoo Biol 2016;35:280–292, DOI: 10.1002/zoo.21284.

26. Leake J. Science close to creating a mammoth. The Times 2015. Available at https://www.thetimes.co.uk/article/science-close-to-creating-a-mammoth-z8zlvbgr9fl. Accessed on July 23, 2018.

27. Thomson C. The Lazarus Project—To Bring Back Australia’s Southern Gastro-Brooding Frog. 2017. Available at https://awpc.org.au/the-lazarus-project-to-bring-back-australias-southern-gastro-brooding-frog/. Accessed July 23, 2018.

28. Lynch VJ, Bedoya-Reina OC, Ratan A et al. Elephantid genomes reveal the molecular bases of woolly mammoth adaptations to the arctic. Cell Rep 2015;12:217–228.

29. Fisher DC, Tikhonov AN, Kosintsev PA et al. Anatomy, death, and preservation of a woolly mammoth (Mammuthus primigenius) calf, Yamal Peninsula, northwest Siberia. Quat Int 2012;255:94–105.

30. Progress to Date. Available at http://revivestorever.org/projects/woolly-mammoth/progress-to-date/. Accessed July 23, 2018.

31. Pimm SL, Alibhai S, Bergl R et al. Emerging technologies to conserve biodiversity. Trends Ecol Evol 2015;30:685–696.

32. Johnson JA, Altwegg R, Evans DM et al. Is there a future for genome-editing technologies in conservation? Anim Conserv 2016;19:97–101.

33. Mao JS, Boyce MS, Smith DW et al. Habitat selection by elk before and after wolf reintroduction in Yellowstone National Park. J Wildl Manage 2005;69:1691–1707.

34. Karimi F. CNN 2018. 11 endangered rhinos were moved to start a new population—10 died. Available at https://edition.cnn.com/2018/07/27/africa/black-rinos-dead-kenya-relocation/index.html. Accessed July 27, 2018.

35. Ceballos G, Uhrich PR, Barnosky AD et al. Accelerated modern human-induced species losses: Entering the sixth mass extinction. Sci Adv 2015;1:e1400253.

36. The Extinction Crisis. Available at www.biologicaldiversity.org/programs/biodiversity/elements_of_biodiversity/extinction_crisis/. Accessed July 23, 2018.

37. Steinfeld H, Gerber P, Wassenaar T et al. Livestock’s Long Shadow: Environmental Issues and Options. Food and Agriculture Organization of the United Nations. Rome, 2006. Available at http://www.fao.org/docrep/010/a0701e/a0701e.pdf. Accessed July 27, 2018.

38. Garnett T. Livestock-related greenhouse gas emissions: Impacts and options for policy makers. Environ Sci Policy 2009;12:491–503.

39. Ronquillo MG, Angeles Hernandez JC. Antibiotic and synthetic growth promoters in animal diets: Review of impact and analytical methods. Food Cont 2017;72:255–267.

40. Specht EA, Welch DR, Rees Clayton EM et al. Opportunities for applying biomedical production and manufacturing technologies to the development of the clean meat industry. Biochem Eng J 2018;132:161–168.

41. Post MJ. An alternative animal protein source: Cultured beef. Ann N Y Acad Sci 2014;1328:29–33.

42. Genovesi NJ, Domeier TL, Telugu BP et al. Enhanced development of skeletal myotubes from porcine induced pluripotent stem cells. Sci Rep 2017;7:41833.

43. Devito L, Petrova A, Miere C et al. Cost-effective master cell bank validation of multiple clinical-grade human pluripotent stem cell lines from a single donor. Stem Cell Transl Med 2014;3:1116–1124.

44. Stephenson E, Jacquet L, Miere C et al. Derivation and propagation of human embryonic stem cell lines from frozen embryos in animal product-free environment. Nat Prot 2012;7:1366–1381.

45. Rowland MP. Labeling Wars: The U.S. Cattlemen’s Association has beef with its competition. Forbes 2018. https://www.forbes.com/sites/michaelpowellmanrowland/2018/02/14/usda-labeling-laws-meat-beef/.

46. Shafa M, Day B, Yamashita A et al. Derivation of iPSCs in stirred
suspension bioreactors. Nat Methods 2012;9: 465–466.

47 Abecasis B, Aguiar T, Arnault É et al. Expansion of 3D human induced pluripotent stem cell aggregates in bioreactors: Bioprocess intensification and scaling-up approaches. J Biotechnol 2017; 246:81–93.

48 Stanton MM, Samitier J, Sánchez S. Bioprinting of 3D hydrogels. Lab Chip 2015; 15:3111–3115.

49 Ma X, Qu X, Zhu W et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proc Natl Acad Sci U S A 2016;113:2206–2211.

50 Abaci HE, Guo Z, Coffman A et al. Human skin constructs with spatially controlled vasculature using primary and iPSC-derived endothelial cells. Adv Healthc Mater 2016;5:1800–1807.

51 Pimentel D, Pimentel M. Sustainability of meat-based and plant-based diets and the environment. Am J Clin Nutr 2003;78:660S–663S.