



## Cellular Agriculture

### Meat in a lab




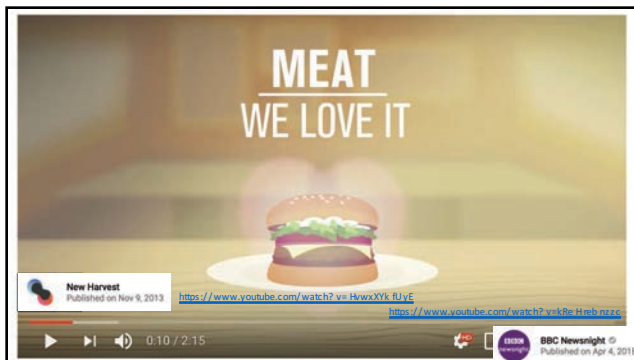
**Lucy Lee, PhD**  
 Dean, Faculty of Science



Dec 5, 2018

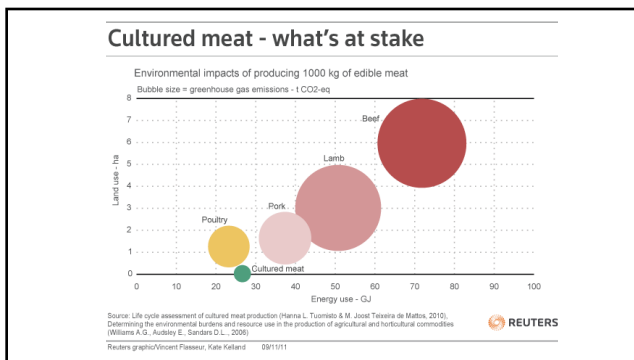
IMPOSSIBLE ?





news  
 Dec 4, 2018  
**Carbon emissions from food**  
 Equivalent kilometers driven per kilogram of food\*

Eating more locally produced and organic foods — advice given by some environmental groups — won't necessarily make a difference, studies show.



PRODUCT	COMPANY NAME	YEAR FOUNDED	LOCATION	LATEST FUNDS RAISED	INVESTORS & PARTNERS
Animal fat	Mission Barns	2018	Berkeley, Calif.	Undisclosed	Undisclosed
Beef	Aleph Farms	2017	Ashdod, Israel	Undisclosed	Strauss Group, Technion—Israel Institute of Technology, New Crop Capital
	Mosa Meat	2016	Maastricht, Netherlands	\$8.8 million series A round	M Ventures, Bell Food Group
Poultry	Memphis Meats	2015	Berkeley, Calif.	\$17.0 million series A round	Cargill, Tyson Ventures, Richard Branson, Bill Gates
	SuperMeat	2015	Rehovot, Israel	\$3.0 million seed round	New Crop Capital, PHW
Seafood	BlueNalu	2017	San Diego	\$4.5 million seed round	New Crop Capital
	Finless Foods	2017	Emeryville, Calif.	\$3.5 million seed round	Draper Associates
	Wild Type	2016	San Francisco	\$3.5 million seed round	Spark Capital
Undisclosed	Future Meat Technologies	2018	Jerusalem	\$2.2 million seed round	Tyson Ventures, Bits x Bites
	Integriculture	2015	Tokyo	\$2.7 million seed round	Real Tech Fund

<https://cen.acs.org/business/food-ng-ndi-emb/listicle/ean-mea-196/142> Oct 2018

**Clean meat:** genuine meat made by farming cells, rather than farming animals

Myocytes (muscle cells)  
Fibroblasts, chondrocytes (connective tissue)  
Adipocytes (fat cells)

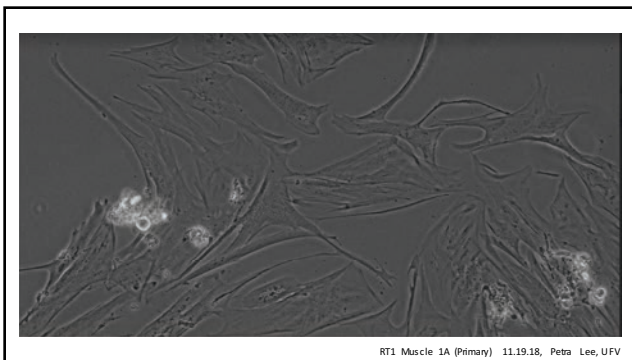
Endothelial cells  
Blood cells  
Bone cells

For over 100 years, scientists have been able to culture cells outside an organisms' body. Scientific advancements for last 60 years have made it feasible to grow generations of cells in vitro, as cell lines.

**What are cell lines?**  
Cell cultures that have been maintained outside an organism for several generations

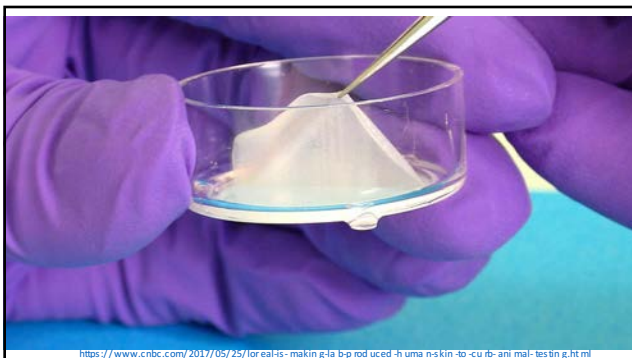
- **Continuous** – permanent, eg. HeLa (since 1951)
  - Available from 'Repositories' eg. ATCC or scientific colleagues
  - Relatively uniform cellular composition
  - Relatively easy to maintain
  - Highly replicable
- **Finite** – limited life span – limited use
  - Many available from commercial sources or prepared 'in-house'

<https://www.youtube.com/watch?v=FaFjya7FVSM>



**Some of my fish cell lines**

- RTL-W1** from trout liver (Lee *et al.* 1993, Cell Biol Toxicol 9:279) -200 citations
- RTgill-W1** from trout gills (Bols *et al.* 1994, J Fish Dis 17:601) in ATCC: ORL2523
- RTP-2** from trout pituitary (Bols *et al.* 1995, Mol Mar Biol Biotech 4:154)
- RTg-GC** from rainbow trout gut (Kawano *et al.* 2011, Aquacult Nut 17: e241)
- GFSK-S1** from goldfish skin (Lee *et al.* 1997, Histochem J 29:31)
- HEW** from haddock embryos (Byson *et al.* 2006, Marine Biotech 8: 641)
- PBLE** from peripheral blood of eel (DeWitte-Or *et al.* 2006, In Vitro Cell Dev Biol Anim 42:263)
- EelB** from eel brain (Boch *et al.* 2016, In Vitro Cell Dev Biol Anim 52:395)
- ZEB2J** from zebrafish embryos (Xing *et al.* 2008, Zebrafish 5:49)
- ZSSJ** from zebrafish spleen (Xing *et al.* 2008, In Vitro Cell Dev Biol Anim 45:163)
- SBB4** from seabass brain (Servili *et al.* 2009, Comp Biochem Phys A 152:245)
- KFE-5** from Killifish embryos (Gignac *et al.* 2014, Comp Biochem Physiol A 175:15)
- GML-5** from cod larvae (MacLeod *et al.* 2018, J Fish Dis 41:1359)



**BIOPRINTING 3D STRUCTURES WITH CELL LINES**

Print me baby, one more time  
How bioprinting works

1. Stem cells, or cells taken from a biopsy of a patient, are put into a growth medium to multiply and are used to form a bioink made of cell aggregates.
2. The bioink is loaded into cartridges that consist of a syringe fitted with a long extrusion nozzle for printing.
3. Software drives the bioprinter to deposit a pattern of cell aggregates in precise layers, one on top of the other, and interspersed with layers of a water-based substance called a hydrogel, which is deposited by a separate nozzle and functions as a temporary mould around the cells.
4. The printed tissue is left to grow and mature and the hydrogel removed.
5. The printed tissue can then be used in medical research or as a transplant material.

Sources: Organisms; The Economist  
<https://www.economist.com/news/tec-hnol-org-qua-re-div/21598322-bioprinter-building-living-tiss-ue-3d-p-rint-en-beco-ming-one-w-b-usiness>

**Two-dimensional tissue**

- Skin
- Cartilage

**Hollow tubes**

- Trachea
- Heart valve
- Vasculature

**Solid organs**

- Kidney

Murphy & Atala (2014)  
Nature Biotech 32: 773-785

Dr. Lucy Lee & Tony Atala, June 2017

**CELLS SAMPLING**

- Stem Cells
- Egg Cells
- Blood Vessel Cells
- Muscle Cells

**3D TISSUE FORMATION**

3 WEEKS

MEAT

Grow cells in collagen scaffolds or bioprint

<https://firsttweeast.com/eat/2016/02/d-eubanking-wagyu-beef-myth>

<https://www.aleph-farms.com>

**OUR MEATLESS FUTURE**  
Traditional Meat Production vs. Lab-Grown Veal Chops

**What does it take to make a 1/4 lb. burger?**

Resource	Traditional Meat Production	Lab-Grown Veal Chops
Feed	6.7 Pounds of grain and forage	0.0001 Pounds of growth medium
Water	52.8 Gallons for drinking and irrigating feed crops	0.0001 Gallons of water
Land	74.5 Square feet for grazing and growing feed crops	0.0001 Square feet
Energy	1,036 BTUs for feed production and transport, enough to power 1 phone	0.0001 BTUs
GHG	13.4 Pounds of CO2 equivalent released	0.0001 Pounds

Cost of producing 1 lb. of lab-grown meat for consumers at Missionary University in the Netherlands

2013: \$225,000 per pound  
2018: \$12 per pound

<https://www.cbinsights.com/research/future-of-meat-and-its-alternatives>

Nov 9, 2017

**Cellular Agriculture**

- Beef
  - Aleph-farms <https://www.aleph-farms.com>
  - Higher Steaks <https://www.highersteaks.com>
  - Meatable <https://www.meatable.com>
  - Memphis Meats <https://www.memphismeats.com>
  - Mission Barns <https://www.missionbarns.com>
  - Mosa Meat <https://www.mosameat.com>
- Chicken, avian products
  - Just <https://justforall.com/en-us/stories/clean-meat>
  - SuperMeat <https://www.supermeat.com>
  - Integrature <http://integrature.jp/?lang=en> Foie gras
- Pork
  - New Age Meats <https://www.newagemeat.com>
  - Kiran Meats <https://www.kiranmeats.com>

**Cellular Aquaculture**

- Fish:
  - BlueNalu <https://www.bluenalu.com>
  - Seafuture <http://seafuturebio.com>
  - Finless Foods <https://finlessfoods.com> (bluefin tuna) Sushi, Sashimi, Surimi
  - WildType <https://www.thewildtype.com> (salmon)

Finless Foods

**90%** of large predatory fish biomass has gone from the oceans

**TIME**

WORLD

**Japan: World's Most Expensive Fish Sold for \$1.8 Million**

A 222-kilogram bluefin tuna was sold at Tokyo's Tsukiji market for an all-time high of 155.4 million yen, or 1.8 million dollars.

By Patrick Boshier Jan. 07, 2013

**THE MOST EXPENSIVE FISH IN THE WORLD**

PRICE **\$1.8 MILLION**

222KG

~\$4,000/lb

58,000 POUNDS

BLUEFIN TUNA SOLD IN TSUKIJI FISH MARKET, TOKYO

**IN VITRO EDIBLE MUSCLE PROTEIN PRODUCTION SYSTEM (MPPS): STAGE 1, FISH**

M. A. BENJAMINSON<sup>1</sup>, J. A. GILCHRIST and M. LORENZ  
NSR/Touro Applied BioScience Research Consortium, 1700 Union Boulevard Bay Shore, NY 11706, USA

*(Received 20 November 2000; revised version received 5 December 2001)*

**Abstract**—The working efficiency and state-of-mind of a Space vehicle crew on long-term missions is dependent on the availability of living conditions including food. Our purpose was to establish the feasibility of an *in vitro* muscle protein production system (MPPS) for the fabrication of surrogate muscle protein constructs as food products for Space travelers. In the experimental treatments, we cultivated the adult dorsal abdominal skeletal muscle mass of *Carassius* (Gold fish). An ATCC fish fibroblast cell line was used for tissue engineering investigations. No antibiotics were used during any phase of the research. Our four treatments produced these results: a low contamination rate, self-healing, cell proliferation, a tissue engineered construct of non-homologous co-cultured cells with explants, an increase in tissue size in homologous co-cultures of explants with crude cell mixtures, maintenance of explants in media containing fetal bovine serum substitutes, and harvested explants which resembled fresh fish filets.

We feel that not only have we pointed the way to an innovative, viable means of supplying safe, healthy, nutritious food to Space voyagers on long journeys, but our research also points the way to means of alleviating food supply and safety problems both the public and private sectors worldwide. © 2002, Published by Elsevier Science Ltd.



Finless Foods

### Developing Continuous Cell Lines

### Fish Cell Lines can be readily obtained

## "Fishy"?

Indeterminate growth- abundant adult stem cells

High regeneration potential

### Immortalizing features of fish cells

#### High telomerase activity

Usually restricted to cancerous & stem cells in humans

High constitutive activity in adult organs [Klapper et al. 1998, FEBS Letters 434: 409-12]

activity present throughout the development of a trout fibroblast cell line [Ossum et al. 2004, J. Fish Biol. 64: 1103]

and other fish tissues and cell lines, but not all: [Bysson et al. 2006, Marine Biotech 8: 48-1]

#### Lack of inducible p53

key protein regulating apoptosis

[Rau Embry et al. 2006, Oncogene 25: 2004-10]

[Liu et al. 2011, Comp Biochem Physiol C Toxicol Pharmacol 154(4):328-32]

### Fish cell lines have lots of uses

**Uses in parasitology:**  
*Loma morhua*, an obligate intracellular parasite grows in GMI-5

MacLeod *et al.* 2018, *J Fish Dis* 41: 1359

**Study differentiated characteristics**

1:1200 anti  $\alpha$ -actinin KFE-5

Killifish embryo (KFE-5) cell line  
 Gignac *et al.* (2014)  
*Comp Biochem Physiol A* 175: 15-27

Blue = cell nuclei  
 Green = striated muscle

*Histochemical Journal* 28, 31-43 (1997)

**Development of a cell line from skin of goldfish, *Carassius auratus*, and effects of ascorbic acid on collagen deposition**

**GFSk-S1**

L. E. J. LEE\*, S. J. CALDWELL and J. GIBBONS  
 Department of Veterinary Anatomy, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon

**YPF-5 Yellow Perch Fin cell line**

K. Spiteri, MSc 2014

Spiteri *et al.* in prep

Anti-salmon Collagen I (Cedarlane) @ 1:250; Alexa Fluor 488 2<sup>nd</sup> Ab @ 1:1000; nuclei counterstained w DAPI

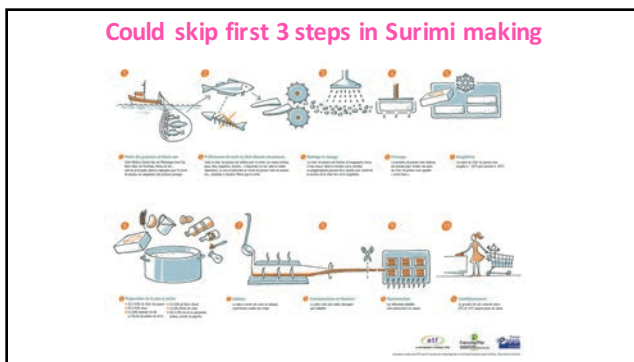
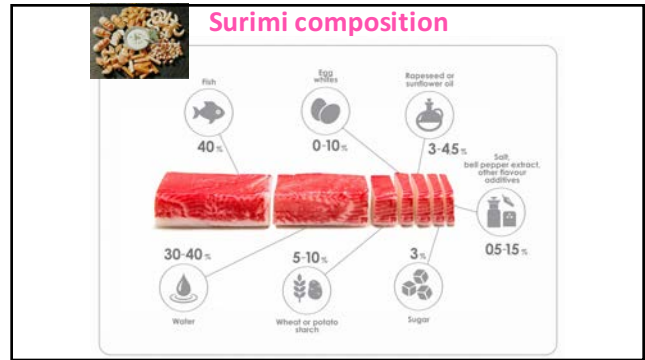
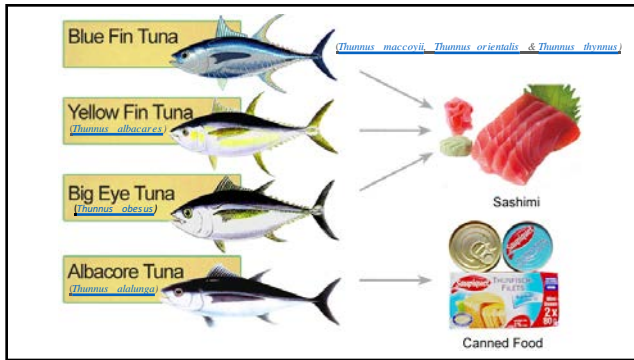
**The Solution: Finless Foods**  
<https://www.finlessfoods.com>  
 We make the healthy fish you deserve

<https://www.cnn.com/video/2017/09/02/indie-bio-to-ur-l-abs-p-awne-d-memphis-meats-fund-ed-by-ga-tes-b-rans-on-h.tml>

Finless Foods

January 2018

Finless Foods



- Advantages of cultured meat:**
- Reduction of energy to produce 1 unit of output by 35-60%.
  - Increased output with reduced waste output (no offal, no feces)
  - Reduction of the area of production by 98%.
  - No space constraints.
  - Reduction of harmful emissions by 90%.
  - Saving of fresh water used in production by 70%.
  - No need of antibiotics
  - Reduce level of animal fat in the meat
  - Humanity towards animals. No ethical dilemmas.
- THE NEW FOOD ECONOMY Published on Mar 6, 2018

- Disadvantages:**
- 
- Industrial production will require cellular growth factors. Unknown how these factors could affect humans.
  - Need to develop technologies for industrial production..
  - Cultured meat may not look like the natural meat.
  - Resistance from the existing industry.
  - Human muscle cell growth .... Cannibalism?

