Nutritionally Tuning Cultured Meat via Arachidonic Acid Accumulation for Pet Food Purposes

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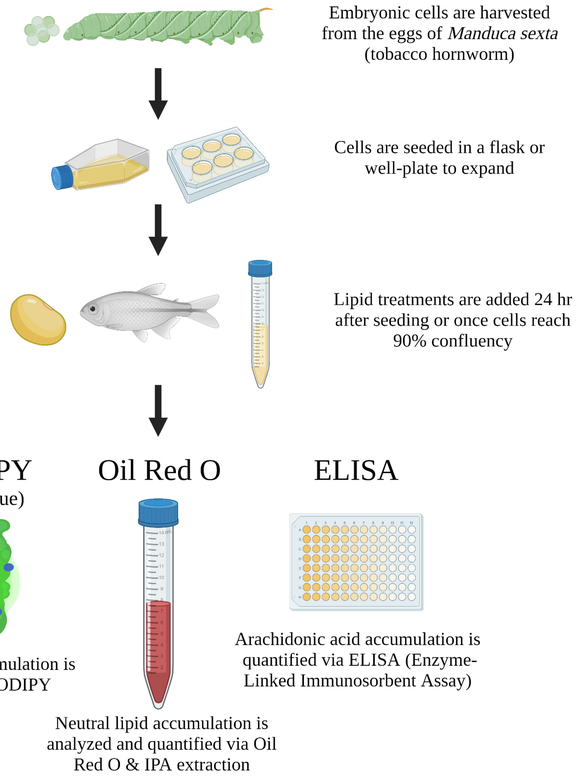
Background

- Rise in controversy surrounding conventional meat industry
  - Animal-welfare, sustainability, human health, population growth
- **Cellular agriculture: the process of creating animal sourced products through cell culture**
  - Similar textural & flavor profiles to traditional meat products
  - Unique control *in vitro* that is not possible harvesting meat from livestock (e.g. nutritional customization).
- Insect cells provided benefits compared to mammalian cells
  - Do not need CO_2, adaptable to suspension culture, accommodating of serum-free culture media, etc. [1]
  - Can accumulate lipids [2]
- Human neophobia and feelings of disgust towards edible insects
  - May be easier to enter market with cultured-insect pet food
- Felines are 'obligate carnivores' → can we make a nutritionally complete cultured product for cats?
  - Arachidonic acid is an essential component of cat food

Purpose

This present study explores the use of media supplementation to alter the cellular arachidonic acid content in *Manduca sexta* embryonic precursor cells (Ms PCs). The overall goal was to explore the possibility of nutritionally enhancing cultured meat products through exogenous factors.

Methods



Results

Lipid Accumulation

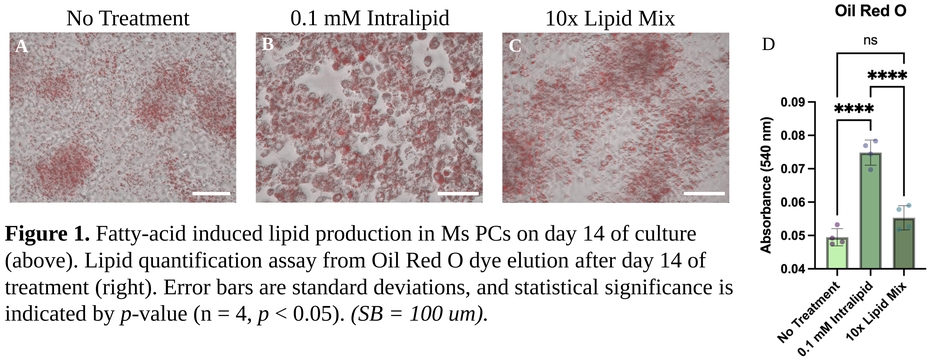


Figure 1. Fatty-acid induced lipid production in Ms PCs on day 14 of culture (above). Lipid quantification assay from Oil Red O dye elution after day 14 of treatment (right). Error bars are standard deviations, and statistical significance is indicated by *p*-value (n = 4, $p < 0.05$). *(SB = 100 um).*

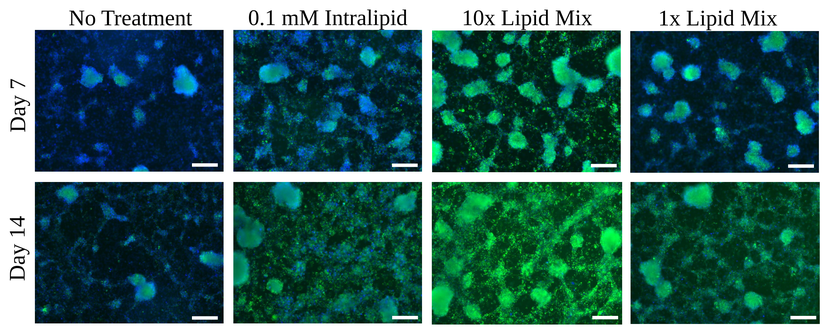


Figure 2. Fatty-acid induced lipid accumulation in Ms PCs. Fluorescence microscopy images of Ms PCs stained with BODIPY (green) and Hoechst 33342 (blue) on day 7 and 14 of treatment. *(SB = 100 um).*

Arachidonic Acid Accumulation

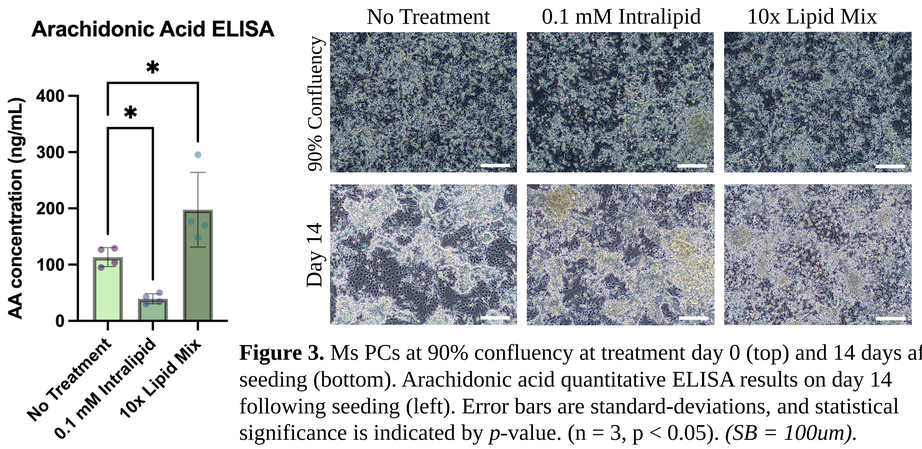


Figure 3. Ms PCs at 90% confluency at treatment day 0 (top) and 14 days after seeding (bottom). Arachidonic acid quantitative ELISA results on day 14 following seeding (left). Error bars are standard-deviations, and statistical significance is indicated by *p*-value. (n = 3, $p < 0.05$). *(SB = 100um).*

Conclusions

- **My work demonstrated the possibility of nutritionally customizing a cultured meat product *in vitro* through lipid supplementation in culture media**
- 10x Lipid Mix resulted in significantly more cellular arachidonic acid content when compared to the control
- Intralipid resulted in significantly less cellular arachidonic acid content when compared to the control
- Although the mechanism of altered arachidonic acid content via lipid supplementation is unclear, clues from *in vivo* data suggest:
  - Supplementation of dietary linoleic acid (found in Intralipid, or soybean oil emulsion) has been seen to correlate with a decrease in arachidonic acid [3]
  - Fish oils, containing eicosapentaenoic acid, cause an increase in arachidonic acid accumulation [4]

Future Directions

- Lipid treatment effect on cell growth
  - CyQuant growth curves
- Animal-free alternatives
  - eliminate cod liver oil – can other lipid sources result in similar outcomes?
  - serum-free media – is arachidonic acid accumulation possible?
- **Explore other nutrients for enhancement**
  - high-density lipoprotein (HDL) cholesterol, iron, etc.

Acknowledgements

I would like to sincerely thank Sophie Letcher, Michael Saad, Adham Ali, Aidan McCreary, and all the other members of the Kaplan Lab Cellular Agriculture Group for their scientific input and guidance. I would also like to express gratitude to the Laidlaw Foundation for their support.

References

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