Decellularized Mycelium as An Edible 3D Scaffold for Cultured Insect Fat

Sophia Letcher, Natalie Rubio, Adham Ali, Aidan McCreary, Olivia Calkins, David L. Kaplan
Tufts University Department of Biomedical Engineering

Statement of Purpose: The present study explores the use of decellularized mycelium as a scaffold to improve scalable three-dimensional culture of insect cells for food purposes. **Background**: As the ethical, environmental, and public health concerns of industrial livestock farming become clear, there is significant interest in the development of animal-free meat alternatives. One promising option is cellular agriculture ("cultured meat"), or growing edible products from cells. The majority of cellular agriculture research focuses on recreating traditional livestock species (e.g., beef, pork). However, many challenges still exist in creating scalable products using these cell types. *Entomoculture* is a nascent but growing field exploring the use of insect cells as a sustainable food source. Insect cells are favorable because they: (1) can be grown near room temperature, reducing the energy needed for heating incubators and media, (2) are more resilient and adaptable to changing conditions when compared with mammalian/avian cells, thus avoiding tight process control requirements, (3) some cell lines can transition between adherent and suspension cultures to support scale up options, and (4) they have simpler media requirements when compared with traditional cell sources, likely resulting in lower costs (Rubio et al. 2019). Our laboratory previously established methods for isolating embryonic *Manduca sexta* (also known as the Tobacco Hornworm) primary cells (Baryshyan et al., 2012), and then optimized procedures to develop proliferative embryonic cells, termed "Ms PCs." Because meat is primarily comprised of muscle and fat, research efforts have focused on engineering Ms PCs to differentiate into muscle or accumulate lipids. Efforts have also focused on using various scaffolds to create meat-like texture with insect cells, including in 3D scaffolds and on films (Rubio et al., 2019). The present work explores decellularized mycelium scaffolds for 3D insect cultured fat using Ms PCs that have been stimulated to produce lipids. Decellularization of plants has been identified as a promising approach to generate animal-free scaffolds for cultured meat (Jones et al., 2021), however to our knowledge decellularized fungi/mycelium has not been previously explored for this utility. **Methods**: Excell™ Mycelium Scaffolds were kindly donated by Ecovative Design (Green Island, NY). Decellularization involved soaking 8 mm biopsy-punched scaffolds in 70% ethanol, deionized water, 1% sodium dodecyl sulfate, 0.1% Tween 20 in 10% sodium hypochlorite bleach, deionized water, and finally 10 mM Tris buffer. Next, samples were frozen at -20 °C, lyophilized for 48 hours, and sterilized via ethylene oxide treatment. Following decellularization and sterilization, pore size, pore density, zeta potential, and mechanical properties of scaffolds were analyzed. Ms PC isolation was performed as previously described (Baryshyan et al., 2012). Briefly, *Manduca sexta* eggs were collected and incubated until myogenesis occurred (19h), sterilized, homogenized to release cells from eggs, centrifuged, and plated in media that promotes proliferation. Cells were expanded and seeded via pipetting 6 million cells directly onto each decellularized mycelium scaffold. Lipid accumulation was induced by addition of Intralipid (a soybean oil emulsion) to the culture media. Cells were allowed to proliferate and accumulate lipids for up to two weeks. Intralipid treated scaffolds were compared with control scaffolds through imaging (BODIPY neutral lipid staining) or mechanical testing. **Results & Conclusions**: The decellularization process resulted in complete removal of all fungal DNA as well as compatible pore size with Ms PCs. Seeding of Ms PCs onto decellularized scaffolds resulted in cells that remained healthy for at least two weeks (later timepoints were not tested). Treatment of cell-laden mycelium scaffolds with 0.1 mM Intralipid resulted in robust lipid accumulation as demonstrated by BODIPY staining (Figure 1). Mechanical properties of scaffolds showed moduli that were comparable to animal-sourced fat. Based on these results, decellularized mycelium is a promising candidate for the production of 3D cultured fat, with potential for scale up and further comparisons to animal-derived fat.

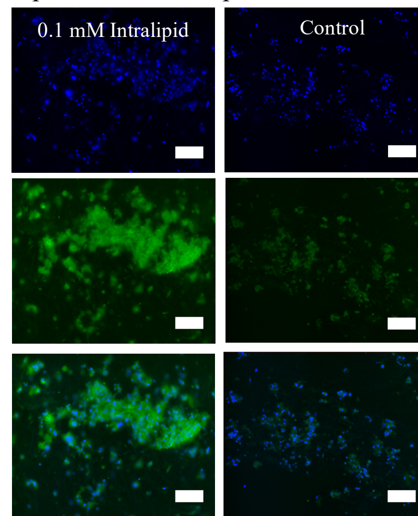

Figure 1*: Ms PC lipid accumulation in cryosectioned decellularized mycelium scaffolds. Right represents cell-laden scaffold in control media, and left represents cell-laden scaffolds treated with 0.1 mM Intralipid (soybean oil emulsion) for 2 weeks. Green = BODIPY stain for neutral lipids, Blue=DAPI, SB=100 uM.*

References: Rubio NR, Fish KD, Trimmer BA, Kaplan DL. ACS Biomaterials Science & Engineering. 2019 Jan 2;5(2):1071-82.; Rubio NR, Fish KD, Trimmer BA, Kaplan DL. Frontiers in Sustainable Food Systems. 2019 Apr 17;3:24.; Jones JD, Rebello AS, Gaudette GR. Food Bioscience. 2021;41:100986.Baryshyan AL, Woods W, Trimmer BA, Kaplan DL. PloS one. 2012 Feb 15;7(2):e31598.