Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary Material**

Supporting Text

*Metabolic analysis*

Metabolism of the cells was assessed to determine substrates being used for energy, and to understand the extended survival of the insect cells *in vitro*, even in the absence of media replenishment. During embryonic development, maternally-derived yolk cells provide fuel to the developing embryo. In later stages of development, the yolk cells are incorporated into the gut or ingested by the immature larva; however, fuel delivery in these systems is not well understood in the early stages of development *(S1)*. In the larval stage, the animal's diet is primarily responsible for supplying carbohydrates to provide energy for growth and foraging activity, while in the adult, fat cells may deliver fuel to the flight muscles in one of three forms, depending on the species. These forms include carbohydrates as the sugar trehalose, which is converted to glucose; and proline, which is metabolized to alanine *(S3, S4)*. The third source, triglycerides, are metabolized to diacylglycerol, which is shuttled through the hemolymph to the muscle, where further metabolism leads to free fatty acids for energy in the mitochondria. The extracellular levels of glucose, protein, and triglycerides/diacylglycerol were assessed in the media of insect muscle cell cultures over time to identify the fuel source utilized in the tissues.

Figures S1-S4

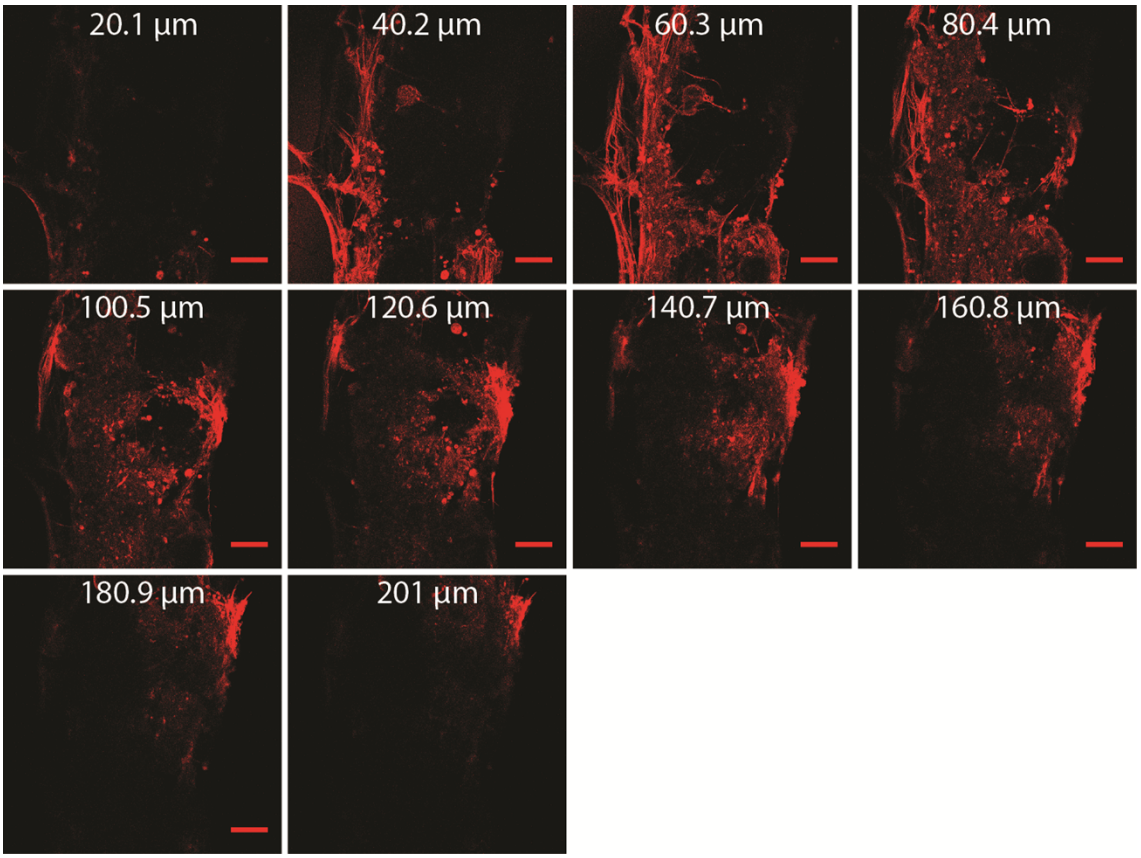

**Figure S1. Consecutive images taken from confocal Z-stack.** Confocal microscopy images showing the 3D nature of actin stained (red) engineered insect muscle tissue. Scale bars are 2000 µm.

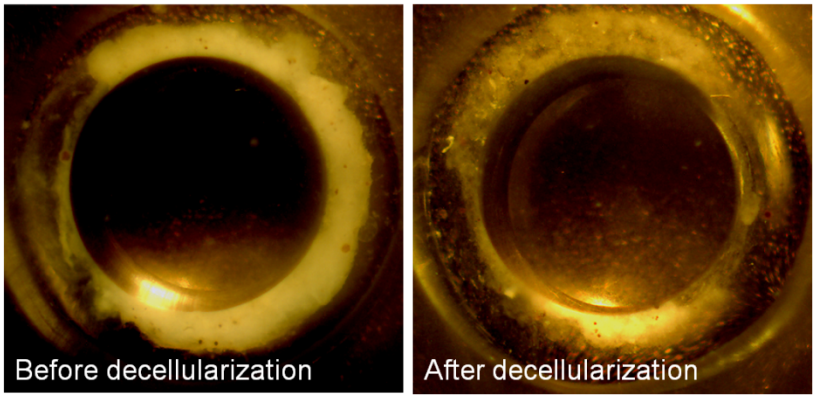


**Figure S2. Decellularization of insect muscle tissue** Insect muscle construct before (left) and after (right) decellularization with SDS. Remaining material after the decellularization process is hypothesized to be ECM material generated by epithelial cells present in the 3D culture.

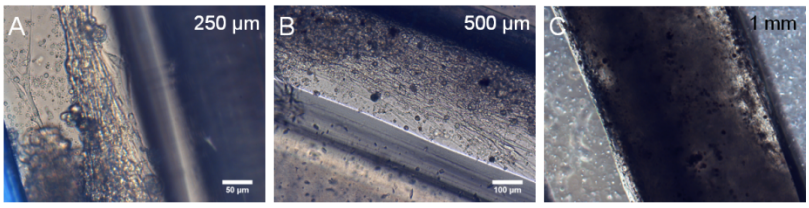


**Figure S3. Muscle tissue organization relative to chamber width.** Phase construct microscopy images showing muscle tissue organization relative to chambers of 250 µm (A), 500 µm (B), and 1 mm (C) wide. Scale bars are 100 µm.

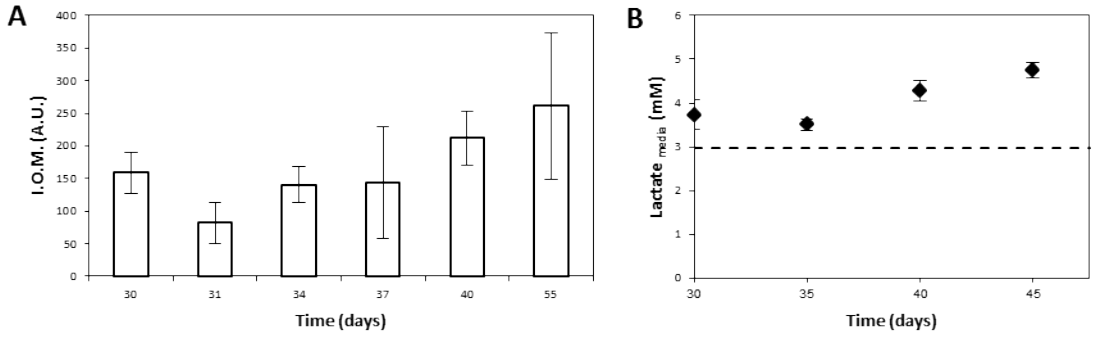


**Figure S4. Activity of muscle tissues used in metabolism studies.** Contractile activity and lactate production of constructs used in metabolic evaluations. (A) Index of movement (I.O.M.) analysis of contractile activity of constructs over the 20 day experimental period. (B) Extracellular levels of lactate over a 20 day contractile period of 30 day old mature tissue constructs. The dotted line represents the level present in the media at day 1.

## References (S1- S5)

S1. Y. Yamahama, K. Seno, T. Hariyama, Changes in lipid droplet localization during embryogenesis of the silkworm, *Bombyx mori*. *Zool. Sci.* **25** (6), 380 – 356 (2008).

S2. R. K. Suarez, C. A. Darveau, K. C. Welch, D. M. O'Brien, D. W. Roubick, *et. al.* Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J. Exp. Biol.* **208**, 3573 – 3579 (2005).

S3. L. Auerswald, G. Gade, Metabolic changes in the African fruit beetle, *Pachnoda sinuate,* during starvation. *J. Insect Physiol.* **46**, 343 – 351 (2000).

S4. N. H. Haunerland, Transport and utilization of lipids in insect flight muscle. *Comp. Biochem. Physiol.* **117B** (4), 475 – 482 (1997).