Isolation of Fish Muscle cell

Summary of Project Jan – Mar 2020

Cellcibus/CELLINK

OVERVIEW

Aim: Isolate, Culture and bioprint Salmon muscle cell.

Background: With the increasing demand for seafood and growing environmental concerns, “cellular aquaculture™” is becoming an essential solution of the problem rather than an alternative way to produce seafood. The main challenge is to make competitive end products with taste and design characteristics that resemble currently existing ones. This creates the necessity in reproducible cell models that can be used for both long-term research projects and for making competitive and sustainable food products in the near future. The best way to deliver such models is to implement 3D bioprinting techniques. In this study, CELLINK will focus on 3D bioprinting of fish muscle cells models in collaboration with an industrial partner (Christer Lagnell, the founder of Laxbutiken).

Project Overview:

- Part 1 covers the isolation of salmon muscle cells and requires close cooperation with Christer who will supply fresh fish for the experiment.
- Part 2 is dedicated to the culturing of successfully isolated fish muscle cells in one of the CELLINK’s incubators.
- Part 3 includes mixing the cultured cells with CELLINK’s bioinks and bioprinting of fish cells models.
- Part 4 focuses on the evaluation of the bioprinted models including shape fidelity and cell analysis.
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Current Status: We have encountered issues at the transition from step 2 to 3. Cells have successfully been isolated and cultured but the yield is low, leading to a limited number of cells. Isolation method have been optimized, however without increased yield. Literature tells that we might need to use younger fish with an age of less than 6 months.

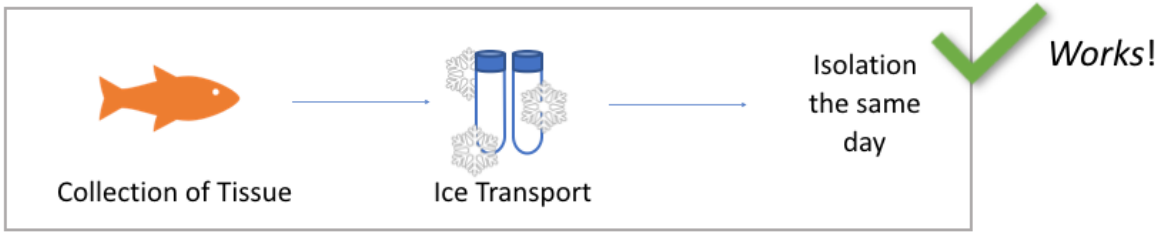
Overview of Isolation Attempts

ISOLATION 1, 200115 – FISH BROUGHT TO CELLINK'S LAB

- Mass: 18g
- Yield: 1x T25 (25cm2) after10 weeks of culture.
- Conclusions:
- L2020 coating of culture vessel works.
- 20°C culture on bench top works.
- Extra protein content in medium at isolation (20% instead of 10%) helps in attachment and survival of the cells.
- Cell culture medium support cell growth and proliferation.

ISOLATION 2, 200219 – FISH COLLECTED AT CHRISTER'S FARM

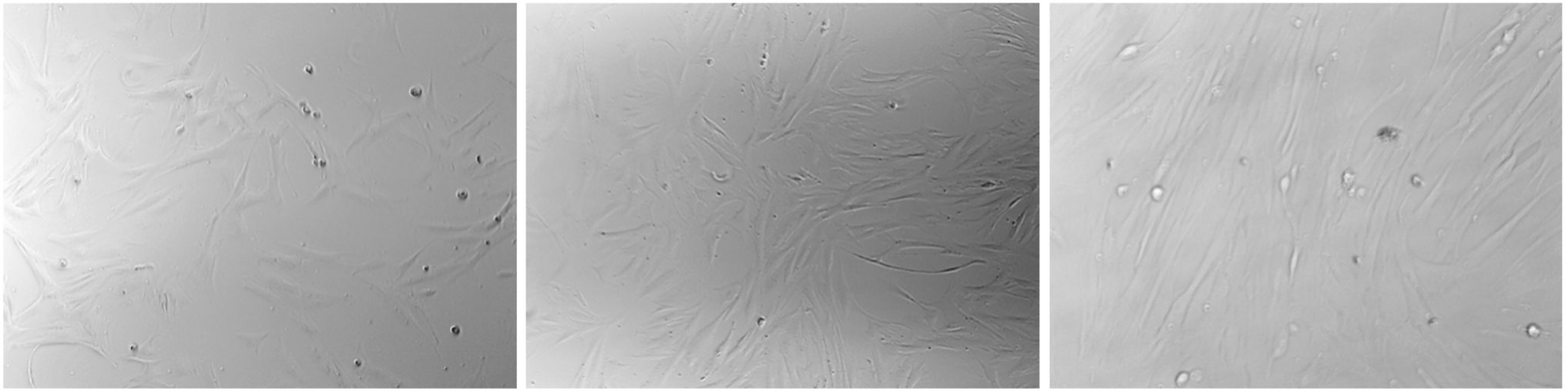
- Mass: 2x 8g transferred on ice in high serum growth medium and 12x 1.5g transferred on dry ice in freezing medium (10% DMSO) and stored in -80°C for future sampling.
- Yield:
- The 16g processed 19/2: 1x 24 well (1.9cm2) after 5 weeks of culture.
- 2x 1.5g thawed 3/3: No cells, same isolation method as previous but refined to collect even more cell suspension.
- 2x 1.5g thawed 10/3: No cells, new isolation method without enzymatic treatment.
- 1x 1.5g thawed 17/3: No cells, also new isolation method without enzymatic treatment but other coatings.
- Conclusions:
- The fish tissue used still provide a low yield of cells.
- Frozen samples not successful source of cells, however bringing tissue on ice from farm to lab works.



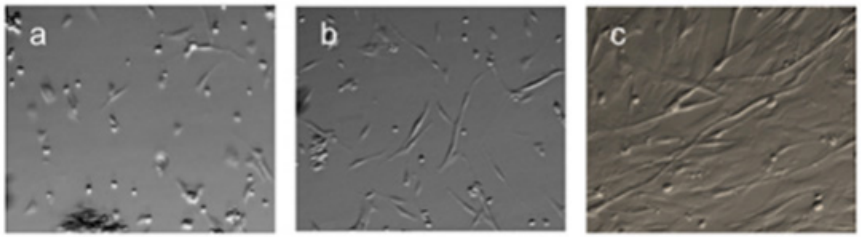
IMAGES

Cell Culture

Bright field images in 5x magnification of isolated, proliferative Rainbow Trout cells. Middle image is taken 3/2, 3 days after passaging from 24 well to 2x 12 well, and right image is taken 5/2 in same well. Showing good proliferation. Right image is from 31/1 and show how the cells align to each other.

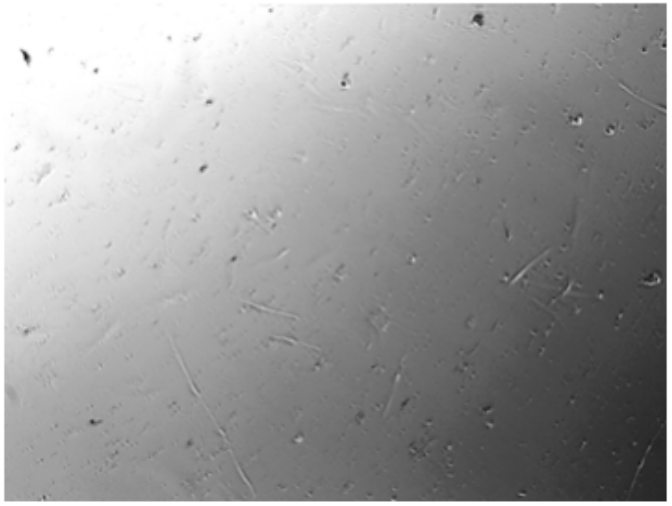


Rainbow Trout, *Oncorhynchus mykiss*

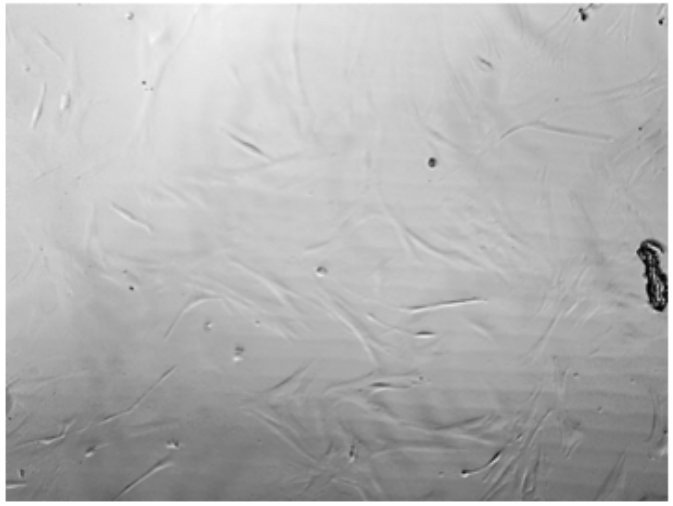


Comparision to Litterature

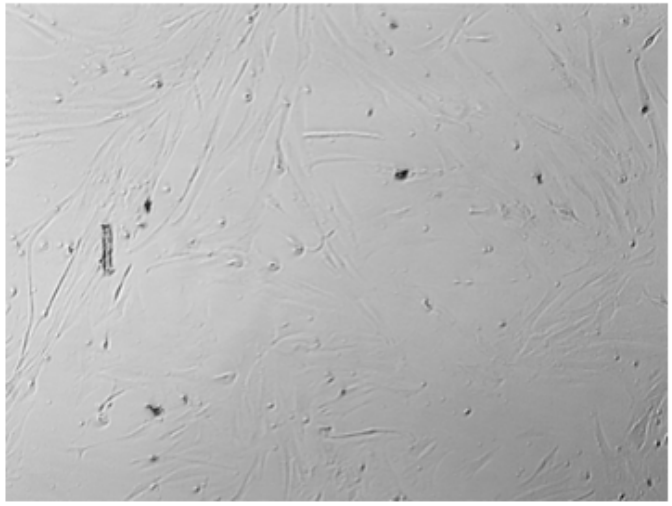
Froehlich, J. M., Seiliez, I., Gabillard, J. C., & Biga, P. R. (2014). Preparation of primary myogenic precursor cell/myoblast cultures from basal vertebrate lineages. Journal of Visualized Experiments, 86, 1–11. https://doi.org/10.3791/51354



2nd isolation, 5x brightfield image 2/3 after pooling of cells into a 48 well. Show early attachment.



1st isolation, 5x brightfield image 29/1.

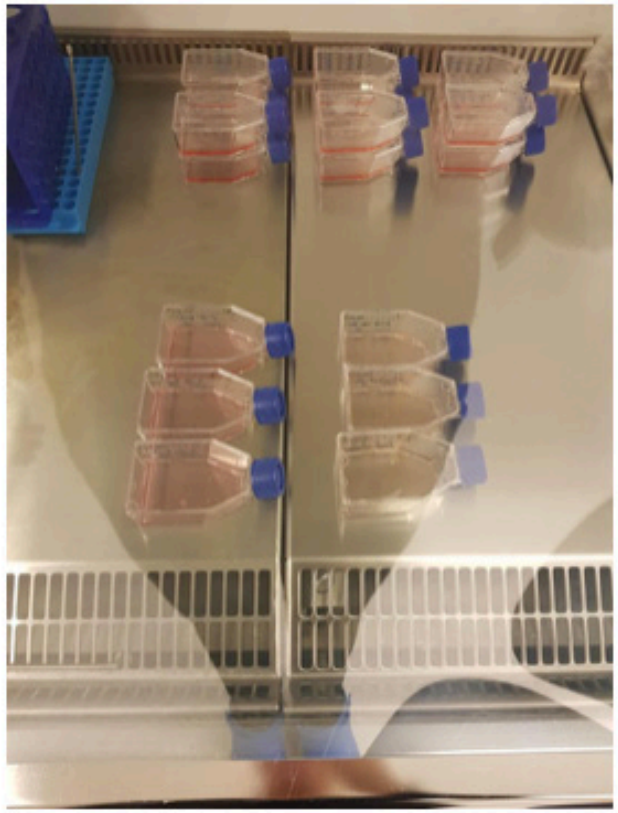


1st isolation, 5x brightfield image 31/1.

The proliferation over the two days in this culture show a positive pattern, the cells seems to be forming a similar structure as in literature.

Image from the lab

Top: 12 well plates.
Left: Rainbow Trout.
Middle: A T25 flask with fish cells.
Right: 18x T25, what we hope to see crowded with cells in future expansion.



Future

Cells currently in culture – Continue to step 3

- Current cell number is low but sufficient to mix with bioink and bioprint, estimated possible final volume of bioink with cells is ~100µL.
- Typically gives 4-6 droplets a la ca 20µL each.
- Pool cells from first and second isolation to maximize study.
- Aim: Observe cell to matrix interaction.
- Analysis: Viability analysis at two timepoints (see example results below).
- Suggestion: Analysis at day 3 and 10, this to get an initial view of how the cells have handled the transfer to 3D at day 3 and a view of the development after 7 days from first time point.
- Bioink: GelXA Laminin 521.
- Composed of GelMA (methacrylated gelatine), alginate, xanthan gum and laminin 521. In future bioink needs to be adapted to food grade, this formulation is not to far away from such a scenario.
- Laminin 521 is the most prominent laminin in muscle tissue for humans, it is also the most universal laminin found throughout tissues. Provides cell attachment sites and nich signals to cells.
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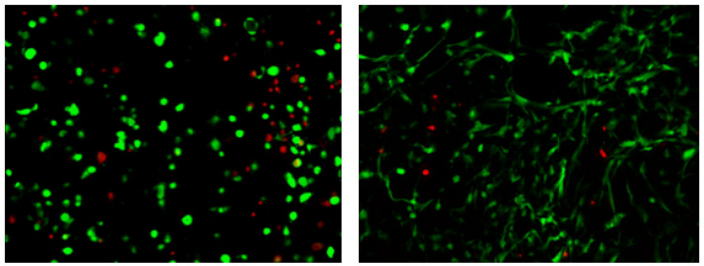
Future isolations – With younger fish

- Aim: Evaluate yield and proliferation rate of cells isolated from ≤6 months old Salmon/Rainbow Trout.
- Expected outcome: Higher cell yield and a more agile cell culture.

Example of viability stain result:
Left image – One day post bioprinting.
Rigth image – 14 days post bioprinting, elongated morphology of cells show good interaction with 3D matrix.
10x Magnification, Green = Viable cells, Red = dead cells.



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