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Expansion of skeletal muscle cells in lab-bench bioreactor – One step closer to cultured meat (#616)

Dimitrios Tzimorotas[2], Randi C. Andreassen[1], Torill Hagen[1], Ragnhild S. Berg[1], Mona E. Pedersen[1], Eva Veiseth-Kent[1], Nina Solberg[1], Sissel B. Rønning[1]

[1] Nofima AS, Raw Materials and Optimisation, Ås, Norway; [2] Nofima AS, Food and Health, Ås, Norway

Introduction

There is an increasing pressure on the world's livestock sector to meet the growing demand for high-value animal protein. A revolutionary new alternative to the traditional way of producing animal protein is cultivation of muscle cells outside the living animal in a bioreactor, thus bypassing animal production. Optimistic estimations suggest that using this modern technology, 10 000 kg cultured meat can be generated from as little as 1 g of beef muscle. The methodology for culturing meat is inspired by techniques used for medical purposes such as tissue reconstruction of damaged muscle tissue and large-scale production of biopharmaceuticals using mammalian cells. Although bioreactors are widely used for large-scale production of biopharmaceuticals using mammalian cells, this technology must be modified before it can be used for edible animal protein production. The basic technology includes the following steps: 1) Sample and harvesting of the muscle stem cells, 2) multiplying the number of cells in a bioreactor (expansion), and differentiation of the satellite cells into muscle cells and fibers, before 3) assembly into a final food product (See figure for overview). However, large-scale bio-production of animal proteins needs to become more efficient than the 2D standard technique used for culturing cells in the laboratory. It is difficult to grow enough amounts of viable muscle cells, since the muscle cells are adherent, and efficient muscle growth depend on optimal growth conditions including enough nutrients and an appropriate microenvironment

Our aim is to expand skeletal muscle cells in a lab-bench bioreactor for 21 days to get one step closer to cultured meat.

Methods

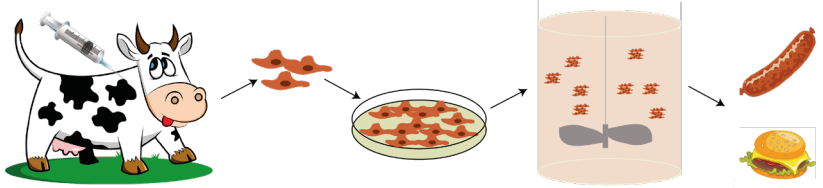
We isolated bovine skeletal muscle cells from newly slaughtered cattle at an industrial abattoir using a well-established method. Previous work demonstrate that it is possible to culture bovine muscle cells in small bioreactors (up to 250 ml) using microcarriers to achieve high surface area to voulme ratio. We have up-scaled this culturing to bench bioreactors (up to 700 ml), using commercial Cytodex®1 microcarrier beads from Sigma-Aldrich, using two seeding conditions: 1) 650 cells/cm^2 microcarriers. The pH was set to 7.8, DO to 35%, sparging with a combination of gases (CO_2, NO_2 and compressed air), temperature 37°C and agitation 40 RPM. 2) 1800 cells/cm^2 microcarriers. The pH was set to 7.3, no spargin, but using a set combination of overhead gases (CO_2, and compressed air), temperature to 38°C and agitation 40 RPM. Parameters analyzed included glucose consumption, lactate production as well as DNA and cell growth.

Results

We demonstrated that the bovine skeletal muscle cells were able to attach to the beads and that the cells were multiplying in both seeding conditions. We also observed that not only did the number of empty beads drop, also the number of beads with more than 3 cells attached increased. The cells consumed glucose and produced lactate demonstrating cell activity after as long as 20 days. Although we did observe initial cell growth and live cells during the whole period for the first alternative seeding condtions, the cells reached senescence, and we had a challenge with foaming. Foaming was produced from day 1. The seeding conditions were important for cell expansion as the cell growth and DNA amount increased more using the second alternative, and no foaming was produced.

Conclusion

One of the main challenges during our experiments using the first seeding alternative was the foaming, leading to microcarrier losses from the bioreactor. This probably also had impact on the medium composition. Secondly, a major challenge with these cells, is how to achieve a longer and optimised expansion period. Our preliminary results show that although the cells did multiply, they reached senescence and started dying after some time. The reason for this could be to low seeding densities, the microcarrier losses or medium composition changes due to foaming. The second seeding alternative, using higher amount of cells pr cm^2 microcarrier, lower pH and no sparging overcame the foaming problems and we did observe higher cell growth. Overcoming these optimisation challenges is vital in order to efficiently produce animal proteins as feeding strategy.



Workflow Sustainable production of animal proteins

Notes