

Maintaining the stemness of satellite cells during long-term culture

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Valorization

Humans are omnivores, eating plants and animals. Meat remains important in the human diet for many consumers in both developed and developing countries. However, meat demand is predicted to increase substantially due to global population growth and meat consumption increase in many developing countries, including China, India and Brazil and South Africa.

If that prediction turns into reality, the production of meat will become an important concern as the environmental impact of meat production is high. Cultured meat is produced by obtaining stem cells from donor animals to culture muscle and fat tissue *in vitro* for meat consumption. It is a technology, which has the potential to partially solve major upcoming problems with traditional livestock. My thesis mainly focuses on characterization and isolation of highly purified porcine and bovine satellite cells as cell sources for cultured meat. Since satellite cells lose proliferation and differentiation ability during *in vitro* culture, the reasons and solutions for this loss were also assessed. In this chapter, I will discuss the social, economic, scientific relevance of the thesis.

Social and economic relevance

Expansion of livestock meat production causes problems such as deforestation and land degradation, greenhouse gas emission, fresh water use and loss of biodiversity. Animal welfare and public health are also societal concerns associated with traditional livestock. By 2020, the global market for protein meat analogues such plant-based substitutes and cultured meat, is estimated to \$46 billion (GBP£33 billion), with significant growth potential. The global cultured meat market for the normal scenario is expected to be valued at USD 15.5 Million in 2021 and is projected to grow at a CAGR of 4.0%, to reach USD 20.0 Million by 2027. The North American region is projected to account for the largest share in the global cultured meat market because of a large meat-eating population, growing investment and research on cultured meat, and the presence of cultured meat manufacturers. The markets in Europe, Middle East & Africa, South America and Asia Pacific region are also expected to grow at fastest growth rate. The growth in cultured meat market is also influenced by the presence of major players such as Mosameat (the Netherlands), Memphis Meat (United States), Just, Inc. (United States), Supermeat (Israel) Integriculture Inc. (Japan), etc. The numbers of these companies are also growing faster.

Cultured meat has social and economic benefits. Several life cycle analysis studies have estimated that cultured meat requires smaller quantities of agricultural inputs and land. Greenhouse gas emission (GHG) would be less in cultured meat compared with traditional livestock. However, the energy use would be more or less in cultured meat depending on different calculating methods. Those estimates are sometimes contradictory because industrialization of cultured meat has not occurred yet and assumptions need to be made. It is clear however that further technological development is still needed to realise the full benefits of cultured meat for society and environment.



It is prerequisite to characterize and isolate highly purified porcine and bovine satellite cells as cell sources for cultured meat. Muscle tissue has dozens of cell types. The satellite cell is the most important cell type for cultured meat since it can differentiate into muscle with high efficiency *in vitro*. By isolation of highly purified satellite cells, one avoids inefficient (co)culture of cells that do not have muscle formation abilities. Loss of stemness of satellite cells in culture was also investigated and we have established some solutions for maintaining stemness during culture. Maintaining stemness results in a higher yield of cells from a single biopsy and therefore reduces the number of biopsies from live animals needed. Increasing the number of cell doublings per batch also reduces the number of transfers from small culture systems to larger systems, making the whole process more resource efficient and cost-effective.

Scientific relevance

The work presented in this thesis was primarily focused on scientific understanding of pig and bovine satellite cells. We unveiled the full-length cDNA sequence of porcine Paired box 7 (PAX7), the most important satellite cell marker. We developed fluorescence-activated cell sorting (FACS) strategy for isolating highly purified satellite cells from farm animals (like pig and cow), thus promoting satellite cell research in farm animals. The cultured meat technology will also greatly benefit from the presence of highly purified satellite cells as the cell source.

Satellite cells from mouse, dog and human lose their stemness after culturing *in vitro*. We observed similar results in pig and bovine satellite cells. We found that interfering with the p38-MAPK signal supports long-term culture of bovine muscle stem cells. This example brings the future of culturing functional satellite in large scale closer. Western blots and especially proteomics results indicated that the subculture of myoblast cells resembles the effect of aging. The proteomics data increase understanding of the protein expression in satellite cells leading to aging upon long-term culture. This may lead to hitherto not explored intervention in this aging process.

The aging effects of long-term culture are similarly occurring in other mammalian cells, such as fibroblast cells, endothelial cells, vascular smooth muscle cells and mesenchymal stem cells. In the future, we can also draw lessons from those cell types to fully understand the aging process during long-term culture *in vitro*. Likewise, our results may in part be translated to aging in those cells.

Large animals (like pigs) have many advantages in modeling human diseases due to their similar anatomic and physiological features to human beings. The work in this thesis will facilitate the production of large animal muscle organoids for drug testing and for non-food tissue engineering, for instance to repair large muscle defects for medical applications. Careful characterization of the large animal skeletal muscle stem cells (satellite cells) will also facilitate the generation of large animal models of skeletal muscle disease. For example, the swine Duchenne muscular dystrophy (DMD) model

recapitulates human symptoms better than the mouse model.

Beyond the thesis

In this work we characterized and isolated highly purified satellite cells from livestock (pig and bovine) as cell sources for cultured meat. We also investigated the reasons for loss of stemness during long-term culture and solutions to prevent these defects. Future work may focus on aging pathways. Preventing aging will facilitate large scale production of satellite cells, thus allowing the development of a cultured meat product with a higher percentage of muscle proteins. These studies on satellite cells can also be translated to other cell types in meat such as adipocytes, which in turn will eventually lead to successful development of a “real steak”.